Current Zoology 61 (1): 23–33, 2015

Rufous-and-white wrens *Thryophilus rufalbus* do not exhibit a dear enemy effects towards conspecific or heterospecific competitors

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Abstract Many territorial animals exhibit reduced aggression towards neighbours. Known as “the dear enemy effect”, this phenomenon has been documented among conspecific animals across a wide range of animal taxa. In theory, the dear enemy effect can also exist between individuals of different species, particularly when those species compete for shared resources. To date, a heterospecific dear enemy effects has only been documented in ants. In this study, we test for both a conspecific and heterospecific dear enemy effect in neotropical rufous-and-white wrens *Thryophilus rufalbus*. This species competes for resources with banded wrens *Thryophilus pleurostictus*, a closely related sympatric congener. We used acoustic playback to simulate rufous-and-white wren and banded wren neighbours and non-neighbours at the edges of rufous-and-white wren territories. Rufous-and-white wrens responded more strongly to signals from their own species, demonstrating that resident males discriminate between conspecific and heterospecific rivals. They did not, however, exhibit a conspecific dear enemy effect. Further, they did not exhibit a heterospecific dear enemy effect. This could be due to neighbours and non-neighbours posing similar levels of threat in this system, to the possibility that playback from the edges of the subjects’ large territories did not simulate a threatening signal, or to other factors. Our study provides the first test of a heterospecific dear enemy effect in vertebrates, and presents a valuable experimental approach for testing for a heterospecific dear enemy effect in other animals [*Current Zoology* 61 (1): 23–33, 2015].

Keywords Conspecific aggression, Dear enemy effect, Heterospecific aggression, Intra-specific interactions, Inter-specific interactions, Resource competition

Many territorial animals live in communication networks where they interact frequently with neighbours and less frequently with non-neighbours (McGregor, 2005). Whenever a territorial animal confronts a competitor, whether it is a neighbour or a non-neighbour, it must choose whether to avoid, tolerate, or fight the competitor (Tanner and Adler, 2009). Fights are costly to animals; they can result in serious injury to both the winner and loser, they require time and energy, and they increase the risk of predation (Huntingford and Turner, 1987; Neat et al. 1998). Therefore, fighting should be chosen as a strategy of last resort (Maynard Smith and Price, 1973).

Among many territorial animals, the identity of the opponent can influence the probability of fighting. In particular, territorial animals often show reduced aggression towards neighbours, especially when those neighbours remain within the boundaries of their own territories (Fisher, 1954). Known as the “dear enemy effect” (Fisher, 1954), this phenomenon has been documented in a wide range of species, including ants, crabs, fish, reptiles, amphibians, mammals, and birds (e.g. Jaeger, 1981; Heinze et al., 1996; Leiser and Itzkowitz, 1999; Langen et al., 2000; Bourne et al., 2001; Mackin, 2005; Leiser et al., 2006; Pratt and McLain, 2006; del Barco-Trillo et al., 2009; Booksmythe et al., 2010; Zenuto, 2010). In his review on the dear enemy effect, Temeles (1994) found that the Relative Threat Hypothesis provided the best explanation for the dear enemy effect. The Relative Threat Hypothesis proposes that territory owners recognize their neighbours and reduce aggression towards them while maintaining high levels of aggression in conflicts with non-neighbours (Temeles, 1994); familiar neighbours are less likely to take over an animal’s territory, or usurp their partner, than unfamiliar non-neighbours (Jaeger, 1981).

Most studies of the dear enemy effect have focused on within-species interactions. Yet animals may also benefit by reducing aggression to familiar heterospecific animals if both species compete for common resources,

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Received Feb. 4, 2014; accepted June 25, 2014.

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such as food or nesting sites. Aggressive interactions between sympatric, ecologically-similar species appear to be commonplace in songbirds (e.g. Martin and Martin, 2001, Freshwater et al., 2014), and therefore heterospecific interactions may also exhibit a dear enemy effect. As far as we are aware, there has been only one published study examining the dear enemy effect between different species. In a study of two species of competitive *Formica* ants, Tanner and Adler (2009) found that *F. xerophila* ants were less aggressive towards familiar versus unfamiliar *F. integroides* competitors. A heterospecific dear enemy effect has not been shown definitively in vertebrates, although it was suspected in a study on two species of cichlid fish in Lake Tanganyika (Ochi et al., 2012). In that study, *Variabilichromis moorii* males were found to be more tolerant of familiar versus unfamiliar *Neolamprologus mustax* males. It was unclear, however, if the differential tolerance constituted a dear enemy effect; an alternative explanation is that familiar and unfamiliar *N. mustax* males differed in some other way that evoked different responses from territorial *V. moorii* males.

Rufous-and-white wrens *Thryophilus rufalbus* and banded wrens *Thryophilus pleurostictus* are closely related congeners (Mann et al., 2006) that live in sympatry in parts of their range, including the neotropical dry forest of Santa Rosa, Costa Rica. Rufous-and-white wrens occupy mature semi-evergreen stands of forest that are dominated by guapinol trees *Hymenaea courbaril*, whereas banded wrens occupy drier secondary forests that are dominated by acacia (*Acacia* spp.) and oak (*Quercus* spp.; Molles and Vehrencamp, 2001; Mennill and Vehrencamp, 2005). Both species inhabit their territories year round and begin nesting at the onset of the rainy season in May (Molles and Vehrencamp, 1999; Topp and Mennill, 2008), and both species build their nests in acacia trees, using the trees' resident ants as a defense against predators. The two species of wren come into contact where the two types of habitat merge. In these transitional zones, rufous-and-white wrens and banded wrens often hold neighbouring territories and engage in aggressive heterospecific interactions; we have observed physical aggression between the two species at territory boundaries, including countersinging interactions that are sometimes followed by chases and physical aggression, and intense fights near nest sites in acacia trees. The two species sing remarkably different songs; although they exhibit superficial similarity in varied introductory notes, a trill, and emphatic terminal syllables, the length of songs and the frequency range of their songs show gross differences (Fig. 1; Molles and Vehrencamp, 1999; Mennill and Vehrencamp, 2005).

In this study, we tested whether rufous-and-white wrens respond to conspecific and heterospecific playback. We predicted that subjects would exhibit a strong territorial response towards simulated rufous-and-white wrens that threaten paternity and territory tenure, an intermediate response towards simulated banded wrens that threaten only territory tenure, and a weak response towards a simulated species that threatened neither paternity or territory tenure. We also tested whether they exhibit a dear enemy effect towards either conspecific rufous-and-white wrens or heterospecific banded wrens. Building on a classic experimental design (Falls and Brooks, 1975; Wiley and Wiley, 1977), we used acoustic playback to simulate neighbours and non-neighbours at the edges of rufous-and-white wren territories. We predicted that resident rufous-and-white wren males would exhibit greater aggression towards simulated non-neighbours versus neighbours, regardless of the species of the competitor. We predicted that rufous-and-white wrens would exhibit greater aggression towards neighbours when they were simulated from an unshared versus a shared territorial boundary (see Fig. 2

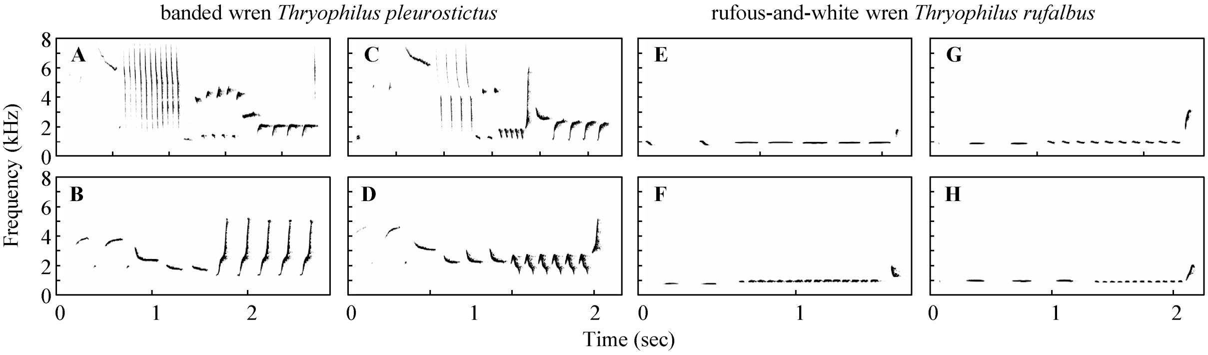

Fig. 1 Sound spectrograms depicting four male banded wren songs (A–D) and four male rufous-and-white wren songs (E-H) that were recorded in the Neotropical dry forest of northwestern Costa Rica and used as stimuli in the playback experiment

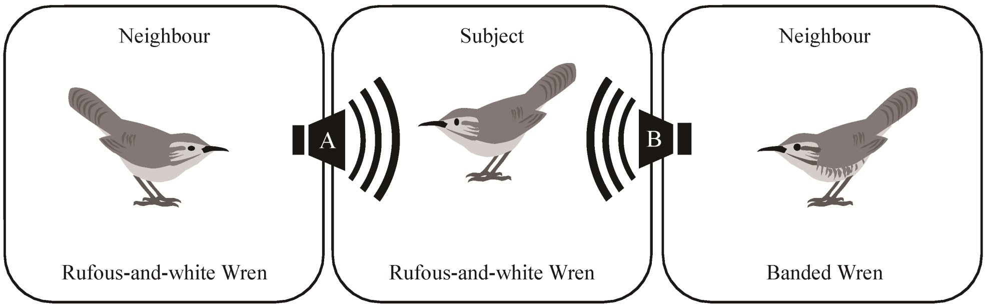

Fig. 2 Diagram depicting the experimental design used to test for a conspecific and heterospecific dear enemy effect in wrens Subjects were 15 male rufous-and-white wrens whose territories were bordered by both a neighbouring rufous-and-white wren and a neighbouring banded wren. We simulated territorial interactions by broadcasting pre-recorded songs of rival animals at two locations along the subjects' territory boundaries. Songs of the rufous-and-white wren neighbour were played back at a shared boundary (site A) and an unshared boundary (site B). Similarly, songs of the banded wren neighbour were played back at both a shared boundary (site B) and an unshared boundary (site A). Songs from non-neighbouring individuals of both wren species, as well as from a non-neighbouring, non-competing control species (Long-tailed Manakin), were played back from sites A or B based on a random selection.

for explanation of "shared" and "unshared" boundaries). Falls and Brooks (1975) postulated that if neighbours' songs were recognized as a class of familiar sounds, territory holders would respond to them in a similar fashion irrespective of playback location; however, if neighbours' songs are recognized individually, they should elicit a stronger response when simulated from an unusual position, since they would indicate that the neighbour is outside of his usual territory and potentially prospecting for extrapair copulations (i.e. an unshared versus a shared boundary; Falls and Brooks, 1975).

1 Materials and Methods

1.1 Study site and study species

We conducted our experiment in Sector Santa Rosa of the Guanacaste Conservation Area, Costa Rica (10°40' N, 85°30' W). Sector Santa Rosa features a mosaic of both secondary and primary forest, and these two habitats blend together in many regions of the park. This arrangement made it possible to find rufous-and-white wrens with both rufous-and-white wren and banded wren neighbours, particularly in zones of transition between the evergreen forest and dry forest. We conducted experiments between 0700 h and 1100 h from 4 to 28 June 2012, which coincided with the onset of the rainy season and the early breeding stages of both species (i.e. nest building, egg laying, and incubation of first nests of the year). The rufous-and-white wrens at this site are the subjects of a long-term investigation; annually, since 2003, we have captured the study birds in mist nets and fitted each animal with a unique combination of coloured leg bands, mapped their territories, recorded their vocal behaviour, and evaluated their breeding activities.

In studying wrens at this site since 2003, we have often observed countersinging interactions between conspecific birds in adjacent territories, and occasionally observed physical interactions near territory boundaries, particularly between conspecific animals but also between congeners. Numerous prior experiments confirm that both rufous-and-white wrens and banded wrens respond aggressively to playback of conspecific stimuli (e.g. Molles and Vehrencamp, 2001; Mennill, 2006). Territorial boundaries shift between years and even within breeding seasons, and countersinging interactions and physical aggression appear to be associated with changes in territory boundaries. Both species build nests in acacia trees *Acacia collinsii* that host acacia ants (*Pseudomyrmex* spp.), presumably so that the biting ants will deter predators from attacking nests. Acacia trees are sufficiently rare in the transitional zones between the evergreen and seasonally dry forests that wrens compete for nesting trees, especially if those trees contain wasp nests as a further defensive mechanism against predators (Joyce, 1993). We have observed many aggressive interactions between the two focal species (as well as with rufous-naped wrens; *Campylorhynchus rufinucha*) at acacia trees where one of the three species later built a nest. Extra-pair fertilizations occur in both species (2% of chicks and 6% of broods in rufous-and-white wrens; 4% of chicks and 10% of broods in banded wrens); in all instances males lost paternity to a neighbour in an adjacent territory (Cramer et al., 2011; Douglas et al., 2012). Non-territorial floaters appear to be rare in rufous-and-white wrens, although first-year adults will sometimes travel widely through the study site in the early part of the breeding season, moving into established territories. Therefore, we feel that our playback of the songs of neighbours and strangers at territory boundaries simulated a realistic situation. Further, the time of year when the playback study was conducted (early in the breeding season, shortly after first clutches were initiated) matches with the time of year when we have observed aggressive conspecific and heterospecific interactions.

We mapped territories of rufous-and-white wrens and banded wrens by following singing birds and recording the animals' positions with a handheld global positioning system (Garmin GPS-60SCx). We estimated the edges of territories by noting the maximum distance that birds travelled in each direction, paying special attention to the positions of birds during territorial disputes. Locations where neighbours interacted vocally or physically demarcated a territory boundary. We considered birds to be neighbours when their territory boundaries were less than 50 m apart; rufous-and-white wrens, like some other tropical bird species, often exhibit gaps between adjacent territories (Osmun and Mennill, 2011). In 2012 we mapped the territories of 51 Rufous-and-white wren pairs in this manner; 15 of them had both a rufous-and-white wren and a banded wren neighbour, and we conducted playback experiments to these 15 individuals.

1.2 Experimental protocol

We presented each subject with seven playback treatments in a repeated measures design (Fig. 2): (1) the neighbouring rufous-and-white wren at a shared territory boundary; (2) the same neighbouring rufous-and-white wren at an unshared territory boundary; (3) a non-neighbouring rufous-and-white wren; (4) the neighbouring banded wren at a shared boundary; (5) the same neighbouring banded wren at an unshared boundary; (6) a non-neighbouring banded wren; and (7) the duet of a long-tailed manakin *Chiroxiphia linearis* as a control stimulus. Long-tailed manakins are sympatric, frugivorous, non-territorial species that do not compete with either wren species. We defined a "shared boundary" as any location on the subject's territorial boundary that abutted the territory edge of the neighbour being simulated, or that directly faced the neighbour's territory when the two were separated by a slight gap. We defined an "unshared boundary" as any location on the subject's territorial boundary that did not abut or face the territory edge of the neighbour being simulated. Non-neighbours and control stimuli were broadcast from a location on the subject's territorial boundary that was always considered to be unshared.

We broadcast the seven treatments to each subject from two different positions on their territory boundaries (Fig. 1). One location was the shared boundary between the subject and the neighbouring rufous-and-white wren; this location was used for the conspecific neighbour at a shared boundary and the heterospecific neighbour at an unshared boundary treatments. The second location was the shared boundary between the subject and the neighbouring banded wren; it was used for the heterospecific neighbour at a shared boundary and the conspecific neighbour at an unshared boundary treatments. The control treatment and the two non-neighbour treatments were assigned at random to one of the two playback locations. The treatments at a given location were broadcast for 5 minutes each in a random order, and were each separated by 5 minutes of silence. In 23 trials, the neighbour that we were simulating approached the loudspeaker, so we aborted these trials and repeated them after 24 hours. Playbacks at the two locations were separated by 24 or 48 hours, though for one subject the sessions were separated by 96 hours because of inclement weather.

We conducted playbacks using a wireless speaker (Scorpion X1B, FOXPRO Inc., Lewistown, PA, USA) placed in vegetation 1 meter above the ground, and oriented towards the subject's territory. The speaker was calibrated so that all treatments were broadcast at 85 dB sound pressure level, as measured with an analogue sound level meter (RadioShack 33-4050; C-weighting, fast response) held 1.0 m in front of the speaker. Based on our assessment in the field, banded wrens sing at a higher amplitude than rufous-and-white wrens. It was important to use a standard playback amplitude across treatments, so that amplitude did not confound playback treatment. We chose 85 dB because it is the average sound pressure level used in previous studies to broadcast songs at realistic levels to rufous-and-white wrens (80 dB; Mennill and Vehrencamp, 2008) and banded wrens (90 dB; Molles and Vehrencamp, 2001).

During each trial, the observer sat concealed in the vegetation an average of 10 m (range 8–15 m) behind the speaker. The observer recorded the subject's vocal response during the entire 5-minute playback period and during the 3-minute period of silence immediately following the playback using a directional microphone (Sennheiser MKH416) and a solid state recorder (Marantz PMD 660, 44.1kHz sampling rate, 16-bit encoding, WAVE format). During the trial, the observer confirmed the identity of any responding animals and estimated their distance from the speaker throughout the trial. We placed flags 1 m to the right and left of the speaker before playback trials began, to facilitate distance estimates.

1.3 Playback stimuli

Prior to the experiment, we collected recordings of wrens' songs to use as playback stimuli. We followed wrens on their territories between 0500 h and 1100 h and collected recordings using a Sennheiser MKH416 shotgun microphone and a Marantz PMD660 solid-state digital recorder (44.1 kHz sampling rate, 16-bit amplitude encoding; WAVE format). We recorded 18 male banded wrens and 51 male rufous-and-white wrens. From these recordings, we used Syrinx-PC Sound Analysis Software (J. Burt, Seattle, WA) to extract one song from each male, choosing the song with the lowest level of background noise, as assessed visually from spectrograms. We then used Audition software (Adobe, San Jose, CA) to filter songs with an 800-Hz high-pass filter (800-Hz is less than the minimum frequency of any of the songs in this dataset) and to normalize their amplitude to -1 dB so they would be broadcast at the same level. We created each playback stimulus by repeating the selected song every 20 seconds for 5 minutes (a repetition pattern not atypical for these species; Mennill and Vehrencamp, 2005). This song rate is within the natural range of singing behaviour for both species (Molles and Vehrencamp, 1999; Mennill and Vehrencamp, 2005).

For each subject, we used the same stimulus for the neighbour at a shared boundary and the neighbour at an unshared boundary treatments, but a different stimulus for the non-neighbour treatment. For non-neighbour stimuli, we selected recordings of birds that were at least 2 km from the subject's territory center. This distance minimized the probability that the subject was familiar with the simulated non-neighbour, since wrens rarely travel outside of their territories (average rufous-and-white wren territory size is 1.35 ± 0.10 Ha; Mennill and Vehrencamp, 2008). Each stimulus was used to test at least two subjects; it was used to simulate a neighbour for one subject and a non-neighbour for another. In addition, to maintain our rule of selecting non-neighbour stimuli from at least 2 km from the subject, we allowed three of our conspecific stimuli to simulate non-neighbours for two different subjects, although we selected a different recording from each of these individuals to create the second stimulus. To create control stimuli we used recordings of the *toledo* duets of 15 different male-male pairs of Long-tailed Manakins from a different experiment conducted at the same study site (Maynard et al., 2013).

1.4 Quantifying subjects' responses

We focused our behavioural observations on territorial males. Both male and female rufous-and-white wrens defend territories, but males defend territories more aggressively; they sing more often and they approach rivals more closely than do females (the sexes appear similar, but can be distinguished on the basis of song; Mennill and Vehrencamp, 2005; Mennill, 2006; Mennill and Vehrencamp, 2008). We assessed subjects' behavioural responses to playback by examining the recordings from all 105 trials (7 treatments to each of 15 males). From each 8-min trial recording, we extracted the following responses: (1) number of songs; (2) number of song-type changes; (3) latency from the start of playback to the subject's first song (s); (4) distance of closest approach to the loudspeaker (m); (5) latency to approach to within 10 m of the loudspeaker (s); and (6) time spent within 10 m of the speaker (s). For treatments where a bird did not sing or approach to within 10 meters of the loudspeaker, we assigned values of 480 seconds (the full observation period) to the two latency measures. In cases where the observer heard the subject sing, but did not see him approach the speaker, we assigned a value of 20 meters as the distance of closest approach, since it was unlikely that the bird could have been closer without the observer's knowledge. We extracted these six response measurements by visualizing the recordings of the playback trials as spectrograms in SyrinxPC. From each recording, we annotated the vocal responses of the subject, as well as the observer's dictations, creating a time-stamped list of all behaviours exhibited during the trial.

1.5 Statistical analysis

Number of songs, number of song-type changes, and latency to sing were highly intercorrelated (Spearman's rank correlations: minimum Spearman's rho = 0.341, all $P < 0.001$), as were distance of closest approach, latency to approach to within 10 m of the loudspeaker, and time spent within 10 m of the speaker (Spearman's rank correlations: minimum Spearman's rho = 0.714, all $P < 0.001$). To avoid conducting independent statistical tests on non-independent response variables, we selected two representative variables for further analysis. We selected the number of songs as our primary song-based measure of response, and the distance of closest approach as our primary movement-based measure of response. These two variables were not correlated (Spearman's Rank Correlations: $\rho = -0.167$, $P = 0.09$), and thus provided two independent tests of our hypotheses. Distance of closest approach was not normally distributed, but was corrected prior to analysis by applying a square-root-transformation. We conducted statistical analyses on the transformed data, and present the non-transformed data in figures.

For both of our response variables, we conducted a repeated measures ANOVA, with the seven treatments constituting the within-subject factor. We tested for an effect of playback order by conducting repeated measures ANOVA for both of our response variables, with playback order as the within-subject factor. We assessed overall model significance using the Wilks' Lambda multivariate test, since the univariate assumption of sphericity was often violated. Where an overall model was significant, we conducted nine *a priori* pairwise comparisons to test our three hypotheses. We tested the hypothesis that individuals can discriminate between species, with three comparisons: (1) control *vs*. conspecific non-neighbour; (2) control *vs*. heterospecific non-neighbour; and (3) conspecific non-neighbour *vs*. heterospecific non-neighbour (we used non-neighbour treatments for all three of these comparisons because the control stimulus was always a non-neighbour). We tested the conspecific dear enemy hypothesis with three comparisons: (4) conspecific non-neighbour *vs*. conspecific neighbour at a shared boundary; (5) conspecific non-neighbour *vs*. conspecific neighbour at an unshared boundary; and (6) conspecific neighbour at a shared boundary *vs*. conspecific neighbour at an unshared boundary. We tested the heterospecific dear enemy hypothesis with three comparisons: (7) heterospecific non-neighbour *vs*. heterospecific neighbour at a shared boundary; (8) heterospecific non-neighbour *vs*. heterospecific neighbour at an unshared boundary; and (9) heterospecific neighbour at a shared boundary *vs*. heterospecific neighbour at an unshared boundary. We did not apply a correction for multiple comparisons because such corrections have been criticized for inflating the risk of Type II error when sample sizes are small (Nakagawa 2004). Instead, we described treatment effects using estimated marginal means (± standard error) and partial eta-squared (η^2_{partial}; Cohen, 1973).

We conducted retrospective power analysis so that we could evaluate whether our analysis was adequate to detect an effect of playback treatment if one existed. Following the suggestion of Thomas and Juanes (1996), we calculated power using the effect size from an independent investigation. Specifically, we focused on the results of a neighbour recognition playback study on banded wrens by Molles and Vehrencamp (2001). Based on the values from Table 1 of Molles and Vehrencamp (2001) for responses to neighbours versus unfamiliar birds, we calculated an effect size of 0.835 for distance of closest approach and an effect size 0.139 for number of songs. We used these effect sizes to calculate the power of our analyses of closest approach and number of songs. These two experiments were quite similar, conducted at the same time of year at the same study site; Molles and Vehrencamp (2001) broadcast one song approximately every 11 s for 3 minutes (approximately 16 songs in total) whereas we broadcast one song every 20 seconds for 5 minutes (exactly 15 songs in total).

All tests were two-tailed, and we considered results to be significant when $P \leq 0.05$. We conducted our statistical analyses in the statistical package PASW (version 18.0.3 for Mac; SPSS Inc, Chicago, IL), except for the power analysis which we conducted in G*Power (v 3.1.9; Faul et al., 2009).

2 Results

Male rufous-and-white wrens responded to the vast majority of our playback simulations (104 of 105) by singing, approaching the loudspeaker, or both. Subjects often appeared agitated, approaching the loudspeaker with frequent small perch changes, performing wing quivering displays, and cocking their tails, consistent with the idea that the birds were responding aggressively to playback. Although males were the primary responders, females responded in 14 of 105 trials, and joined males in duets in 8 of 105 trials.

The number of songs produced by males in response to playback did not differ significantly among experimental treatments (repeated measures ANOVA: Wilks' Lambda = 0.46, $F_{6,9} = 1.77$, $P = 0.21$, $\eta^2_{\text{partial}} = 0.54$). The number of songs did not vary with playback order (repeated measures ANOVA: Wilks' Lambda = 0.36, $F_{6,9} = 2.66$, $P = 0.09$, $\eta^2_{\text{partial}} = 0.64$).

The distance of closest approach varied significantly among experimental treatments (repeated measures ANOVA: Wilks' Lambda = 0.22, $F_{6,9} = 5.19$, $P = 0.01$, $\eta^2_{\text{partial}} = 0.78$); details of pairwise comparisons are given below. The distance of closest approach did not vary with playback order (repeated measures ANOVA: Wilks' Lambda = 0.39, $F_{6,9} = 2.32$, $P = 0.12$, $\eta^2_{\text{partial}} = 0.61$).

2.1 Conspecific versus heterospecific discrimination

With respect to the hypothesis that individuals can discriminate between species, pair-wise comparisons showed that rufous-and-white wrens responded more strongly to conspecific stimuli, approaching the loudspeaker more closely in response to the conspecific non-neighbour treatment than in response to the heterospecific non-neighbour treatment ($F_{1,14}$ = 7.23, P = 0.02, η^2_{partial} = 0.34) or the control treatment ($F_{1,14}$ = 11.28, P = 0.01, η^2_{partial} = 0.45; Fig. 3). Distance of closest approach did not differ significantly between the control and heterospecific non-neighbour treatments ($F_{1,14}$ < 0.01, P > 0.99, η^2_{partial} = 0.00).

2.2 Conspecific dear enemy effect

Rufous-and-white wrens did not exhibit a conspecific dear enemy effect; responses to the conspecific neighbour at a shared boundary, conspecific neighbour at an unshared boundary, and conspecific non-neighbour were statistically indistinguishable (pair-wise comparisons: all $F_{1,14} \leq 0.14$, all $P \geq 0.71$, all $\eta^2_{\text{partial}} \leq 0.01$; Fig. 4). Power analysis, using the effect size from an independent neighbour recognition study (Molles and Vehrencamp, 2001), revealed that we had high statistical power (0.85) to detect a significant difference in distance of closest approach, but low statistical power (0.08) to detect a significant difference in number of songs.

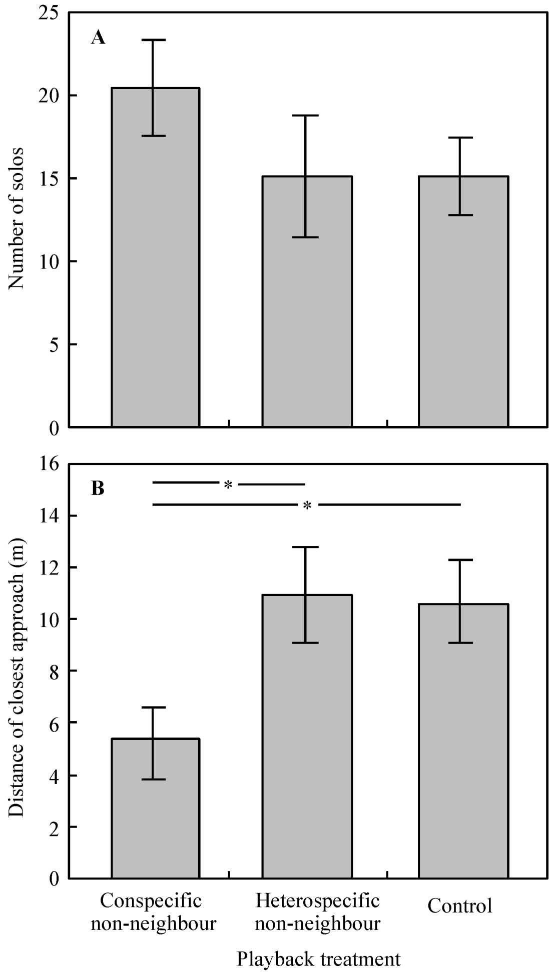

Fig. 3 Responses of rufous-and-white wren males to playback at their territory boundaries

Playback simulated three species: (1) other rufous-and-white wrens; (2) banded wrens, a sympatric congener that competes with rufous-and-white wrens; and (3) long-tailed manakins, a sympatric, non-territorial species that does not compete with rufous-and-white wrens, as a control. All simulated animals were non-neighbours to the subject and were recorded at least 2 km away from the subject. **A.** Subjects sang more songs in response to conspecific treatments than heterospecific or control treatments, but not significantly so. **B.** Subjects approached to significantly closer distances from the loudspeaker in response to conspecific treatments versus heterospecific or control treatments. Means and standard errors are shown. Asterisks denote statistically significant differences between treatments.

2.3 Heterospecific dear enemy effect

Rufous-and-white wrens did not exhibit a heterospecific dear enemy effect; responses to the heterospecific

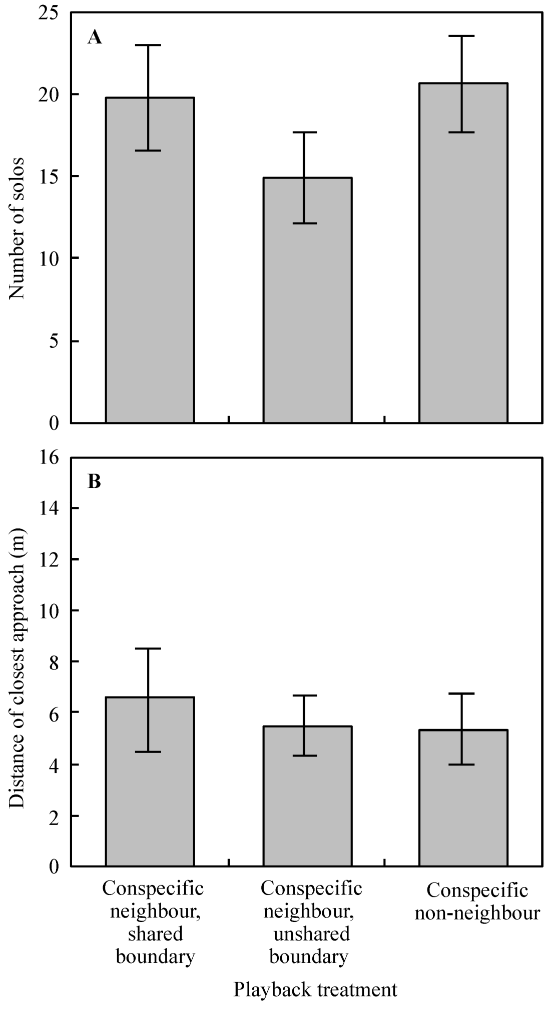

Fig. 4 Rufous-and-white wrens do not exhibit a conspecific dear enemy effect

Subjects were presented with three conspecific rivals simulated through playback, including a neighbour at a shared boundary, a neighbour at an unshared boundary, and a non-neighbour that had been recorded at least 2 km away from the subject. **A.** Subjects sang an equivalent number of songs in response to the three conspecific treatments. **B.** Subjects showed an equivalent distance of closest approach to the loudspeaker across the three treatments. Means and standard errors are shown.

neighbour at a shared boundary, heterospecific neighbour at an unshared boundary, and heterospecific non-neighbour were statistically indistinguishable (pair-wise comparisons: all $F_{1,14} \leq 3.31$, all $P \geq 0.09$, all $\eta^2_{\text{partial}} \leq 0.19$; Fig. 5).

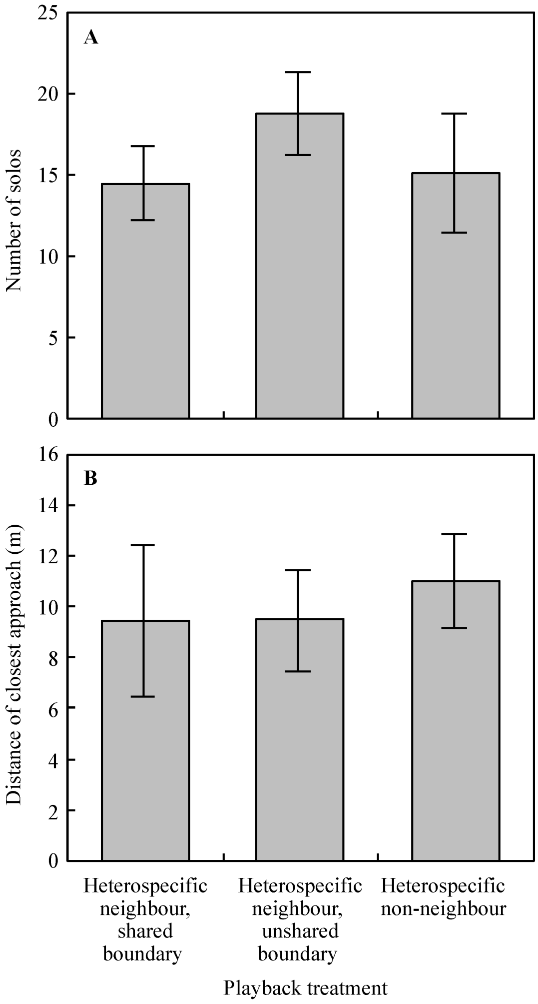

Fig. 5 Rufous-and-white wren males do not exhibit a heterospecific dear enemy effect

Subjects were presented with three banded wren rivals, including a neighbour at a shared boundary, a neighbour at an unshared boundary, and a non-neighbour that had been recorded at least 2 km away from the subject. **A.** Subjects sang an equivalent number of songs in response to the three heterospecific treatments. **B.** Subjects showed an equivalent distance of closest approach across the three heterospecific treatments. Means and standard errors are shown.

3 Discussion

We used playback to simulate both conspecific and heterospecific rivals along the edges of rufous-and-white wren territories to determine whether male rufous-and-white wrens exhibit dear enemy effects. Simulated rivals included other rufous-and-white wrens, which allowed us to test for a conspecific dear enemy effect; banded wrens, which allowed us to test for a heterospecific dear enemy effect; and long-tailed manakins, which served as a control. Our results show that rufous-and-white wrens discriminate conspecific from heterospecific rivals. Male rufous-and-white wrens responded more strongly to playback of their own species than to banded wrens or long-tailed manakins, approaching significantly closer to the loudspeaker in response to conspecific song. Yet rufous-and-white wrens did not exhibit any differences in response to playback of songs of neighbours versus non-neighbours of either wren species, or to neighbours' songs played back from a shared versus unshared territory boundary. Therefore our results do not support the hypothesis that rufous-and-white wrens exhibit a dear enemy effect towards conspecific or heterospecific neighbours. This study provides one of the only tests of a heterospecific dear enemy effect in vertebrates.

Rufous-and-white wrens approached the loudspeaker more closely during playback of conspecific rivals than during playback of banded wrens or long-tailed manakins. In other bird species, close approach is understood to constitute an aggressive response (reviewed in Searcy and Beecher, 2009). The close approaches exhibited by rufous-and-white wrens towards conspecific rivals suggest these rivals were perceived as the greatest threat among the three simulated species. This finding comes as little surprise; conspecific rivals threaten the resident male's paternity, his partnership, and the resources on his territory, including nesting sites and feeding sites. In contrast, banded wrens threaten only the resident male's territorial resources, whereas long-tailed manakins pose no threat at all. We were surprised that simulated banded wrens did not evoke a stronger response than simulated long-tailed manakins, since we often observe aggressive encounters between the two wren species, but never between wrens and manakins. One possible explanation is that our experiment involved only acoustic signals, whereas natural encounters involve both acoustic and visual signals when the rivals meet one another. Future experiments involving taxidermic models would help to evaluate the importance of both acoustic and visual signals during conspecific and heterospecific interactions. Alternatively, rufous-and-white wrens may only exhibit aggressive responses to heterospecific competitors when they intrude inside their territory, rather than when they sing from territory boundaries.

Rufous-and-white wrens did not respond differently to conspecific neighbours versus non-neighbours, or neighbours simulated from an unusual position at an unshared territory boundary, and thus they did not exhibit a conspecific dear enemy effect. It is possible that subjects were unable to discriminate between neighbours and non-neighbours on the basis of acoustic cues alone. This seems unlikely, however, for several reasons. The ability of songbirds to discriminate between different conspecific individuals on the basis of vocal characteristics is widespread (e.g. Falls and Brooks, 1975; Wiley and Wiley, 1977; Godard, 1991; Wilson and Mennill, 2010). Notably, experimental playback to banded wrens reveals a conspecific dear enemy effect in spite of a much larger vocal repertoire in that species (Molles and Vehrencamp, 1999). Furthermore, rufous-and-white wrens are known to respond specifically to the songs of their partners when performing vocal duets (Mennill and Vehrencamp, 2005; Osmun, 2010), a task that requires individual-level discrimination from acoustic cues. Consequently, we feel it is unlikely that rufous-and-white wrens are incapable of individual discrimination between conspecific rivals.

A conspecific dear enemy effect is widespread among vertebrates (although see Muller and Manser, 2007) and has been demonstrated in many investigations of wild birds on the basis of mating and territory defense signals (reviewed in Temeles, 1994; Stoddard, 1996), as well as other types of signals (e.g. threat calls, Masco, 2013; flight calls, Keen et al., 2013). A dear enemy effect has been suggested to be most prominent in animals that have multi-purpose breeding territories (Temeles, 1994), as in rufous-and-white wrens. Further, there exists significant variation between the songs of neighbouring rufous-and-white wrens (Mennill and Vehrencamp, 2005), which should facilitate neighbour-stranger discrimination. Why, then, did we fail to detect a conspecific dear enemy effect? Several explanations are possible: (1) Neighbouring rivals and non-neighbouring rivals may pose similar levels of threat in this species. Indeed, such an argument has been made for other species that fail to show a dear enemy effect. In tropical mockingbirds *Mimus gilvus*, for example, Botero et al. (2007) argued that neighbours and non-neighbours posed similar levels of threat to a male's territory tenure and paternity. We have observed male rufous-and-white wrens attempting insertions into the territories of both neighbours and more distant individuals, and we have observed females divorcing their breeding partners for both neighbours and more distant males (unpublished data); consequently, both neighbours and non-neighbours could pose similar threats. (2) Rufous-and-white wren territories in our study population are very large (Mennill and Vehrencamp, 2008; Osmun and Mennill, 2011), often with significant areas of unoccupied habitat between adjacent territories, and this may reflect a relaxed degree of competition between conspecific animals for territories. Many tropical birds are found at low breeding densities (Thiollay, 1994) whereas most studies of the dear enemy effect have been conducted in the temperate zone where breeding density is high (Temeles, 1994). More studies of the dear enemy effect in tropical animals are warranted to evaluate whether the pattern we describe here is widespread, and whether the dear enemy effect varies with population density and the intensity of competition for resources. (3) Rufous-and-white wrens may not exhibit graded levels of territorial aggression according to the perceived risk of a rival, but, rather, may exhibit an all-or-nothing response according to whether a conspecific rival is detected. This would account for a similar response to conspecific rivals whether they are familiar neighbours or not. (4) Our sample size may have been too small to detect a dear enemy effect in rufous-and-white wrens, particularly if the differences in intensity of response to neighbours and non-neighbours are subtle. Power analysis for distance of closest approach, however, reveals that we had high power to detect a difference (i.e. a difference of the magnitude seen in a neighbour-stranger experiment with banded wrens; Molles and Vehrencamp, 2001) demonstrating that this result was not a Type II error. We note that our sample size (n = 15) is not atypical of published studies of the dear enemy effect, including the significant demonstration of a dear enemy effect in banded wrens (n = 17; Molles and Vehrencamp, 1999). (5) The dear enemy effect might vary seasonally. We conducted playback early in the breeding season, but competition with neighbours might change as the breeding season progresses, leading to a stronger dear enemy effect at other times in the year. Skylarks *Alauda arvensis*, for example, exhibit the dear enemy effect in the middle of the breeding season, but not early or late in the breeding season (Briefer et al., 2008). (6) Our playback design, involving multiple playback treatments presented during a short interval on the same day, may have influenced the birds' responses through priming or habituation effects. Our analyses of playback order did not indicate the presence of such effects, but they may have influenced our data in subtle ways. We conducted our playback over a short time period in order to minimize changes in the birds' breeding status that might occur if each treatment was presented on a separate day. Yet given our unexpected finding of no demonstrated dear enemy effect, we caution others against this type of design and encourage the presentation of playback stimuli on successive days.

Whereas a conspecific dear enemy effect has been explored in diverse animal taxa (Temeles, 1994; Stoddard, 1996), a conspecific dear enemy effect is rarely investigated. If two species compete for common resources and occupy adjacent habitat for extended time periods, and if one or both of the species produces complex acoustic signals that facilitate individual discrimination, then a dear enemy effect is likely to be present; it would benefit animals to discriminate heterospecific rivals that pose an imminent threat (e.g. a strange heterospecific rival prospecting for territories) from heterospecific rivals that already possess an established adjacent territory. Nevertheless, in our investigation we failed to detect a heterospecific dear enemy effect in rufous-and-white wrens. We have often observed physical aggressive interactions between these two species over a decade of studying the ecology of wrens at this study site, and therefore we are confident that these two species are ecological competitors. These species may only engage in aggressive encounters when one animal forays beyond established territory boundaries, which may account for the lack of heterospecific dear enemy effect in our data. Alternatively, both vocal and visual cues may be important for rufous-and-white wrens to recognize a banded wren competitor; the presentation of visual models would help to understand inter-specific recognition signals and aggressive signals in this system.

In conclusion, this study of the dear enemy effect provides important insight into the evolution of territoriality, since it identifies specific social and ecological factors that can mediate or escalate aggressive behaviour during territorial encounters. In our study, we showed that territorial rufous-and-white wrens discriminate between conspecific and heterospecific rivals, but do not discriminate between neighbouring and non-neighbouring rivals. Although we failed to detect it in rufous-and-white wrens, we suggest that our experimental design will be useful for future investigations of a heterospecific dear enemy effect. We recommend that future experiments should begin with paired presentations of heterospecific stimuli versus control stimuli, to first confirm that subjects respond aggressively to heterospecific playback before the neighbour and non-neighbour stimuli are presented.

Acknowledgements We thank the staff of Sector Santa Rosa of the Guanacaste Conservation Area for logistical support, particularly R. Blanco. We thank P. Bitton for advice and assistance in the field. We thank three anonymous referees for helpful feedback. This research was supported by the Natural Sciences and Engineering Research Council of Canada (NSERC) through an Undergraduate Summer Research Award to MMB, a Postdoctoral Fellowship to DRW, and a Discovery Grant and Research Tools and Equipment Grants to DJM. Further support was provided by Frank M. Chapman Memorial Fund Awards from the American Museum of Natural History to BAG and KAK; from a Student Research Award from the American Ornithologists' Union, a Student Research Award from the Animal Behaviour Society, and a Paul A. Stewart Award from the Wilson Ornithological Society's to KAK; and from the Canada Foundation for Innovation, the Government of Ontario, and the University of Windsor to DJM.

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