universität wien

DIPLOMARBEIT

Titel der Diplomarbeit

Function of coordinated vocal displays as response to simulated territory intrusion in the group-living plain-tailed wren (*Pheugopedius euophrys*).

verfasst von

Christine Wu

angestrebter akademischer Grad

Magistra der Naturwissenschaften (Mag.rer.nat.)

Wien, 2013

Studienkennzahl lt. Studienblatt:	A 439
Studienrichtung lt. Studienblatt:	Zoologie
Betreut von:	Univ.-Prof. W. Tecumseh Fitch, PhD

Contents

Photo: Dr Chris N. Templeton

A group of plain-tailed wrens *(Pheugopedius euophrys),* during fieldwork in November 2011 in Pasochoa Volcano Reserve, Ecuador

1. Introduction

Coordinated vocalisation is a special form of communication and is known for a wide range of taxa including mammals, birds, frogs and insects (Langmore 2002). Bird song has proven to be a great model to address questions about social behaviour such as mate attraction, mating behaviour and resource defence. In contrast to northern temperate zones, where with a few exceptions only males sing, song behaviour in the tropics can be quite different. The tropic environment is quite stable, leading to year-round territoriality, long-term monogamy and a convergence in sex roles. This may induce individuals to join together and combine their songs to form vocal 'duets' (Langmore 1998, Mann et al. 2003, Hall 2004, Gill et al. 2005).

Duets occur in more than 220 bird species (Hall 2004) and are usually produced by a mated pair (Thorpe 1972, Catchpole & Slater 2008), but there are exceptions to this rule. For example, the duets of eastern whipbirds (*Psophodes olivaceus*) are often produced by females and males, which are not social partners (Rogers 2005, Mennill & Rogers 2006). Furthermore, lance-tailed manakins (*Chiroxiphia lanceolata*) and other *Chiroxiphia* species are known for their courtship dances and song displays, and these duets are produced by two unrelated males. Thereby, 'alpha'- and subordinate 'beta'-males cooperate to attract females and produce synchronized duets (DuVal 2007). Hence, the best definition to work with should concentrate on acoustic and temporal properties, rather than on the participants of a duet (Langmore 2002).

At their simplest, duets can be very loose songs, were both partners sing roughly at the same time, but in other cases, these can be highly coordinated antiphonal songs with a complex structure of sex specific phrases that are matched together and sound as if they were produced by a single bird, implying a very high level of coordination between the partners (Mann et al. 2003, Levin 1996a). Different selective pressures on song coordination may have led, depending on the taxonomic group, to the evolution of two different forms of duets: (a) antiphonal songs, that are precisely alternated and (b) synchronized, unison songs.
The song of the plain-tailed wren incorporates both these forms. And to add even more complexity, uniquely for its genus, this species lives in social groups and several individuals of both sexes join together to form highly synchronized choruses (Mann et al. 2006).

As a result of the historical focus of studying birds of the northern temperate zones, where songs are usually produced only by males, the function of duetting is still not completely understood (Langmore 1998, Slater & Mann 2004), but it has received considerable research attention in the last decades. There are numerous hypotheses for the function of duetting, the most widely supported concern joint resource defence (Logue 2005, Logue & Gammon 2004, Rogers et al. 2004), mate guarding (Sonnenschein & Reyer 1983, Seddon et al. 2002, Rogers et al. 2007) and pair bond (Hall 2004, Marshall-Ball et al. 2006). Duetting is mostly viewed as a cooperative activity between the partners and the pay offs are assumed to be the same for both sexes, but it can also be viewed as a conflict, whether duetting is used for within-pair communication or as a signal to other birds and also according to the type of information conveyed by the song (Langmore 2002, Levin 1996a). There is evidence in favour of all of these hypotheses, but some studies support and others reject each of them. Therefore it is not possible to define an overall theory. It is rather suggested that duetting can serve several different functions, even within a species (Mennill & Vehrencamp 2008, Marshall-Ball et al. 2006, Catchpole & Slater 2008, Templeton et al. 2011, Topp & Mennill 2008).

Choruses are much harder to analyse, since they are more complex than duets (Catchpole & Slater 2008). They are especially interesting from an evolutionary point of view. More individuals are involved, each potentially having a different degree of motivation for conflict or cooperation. Hypotheses explaining the adaptive significance of chorusing are suggested to be generally similar to duets. They may represent a cooperative signalling system, playing a role in joint resource defence (e.g., rufous-naped wren, *Campylorhynchus rufinucha*: Bradley & Mennill 2009b, black-breasted wood-quail, *Odontophorus leucolaemus*: Hale 2006, laughing kookaburra, *Dacelo novaeguineae*: Baker 2004, subdesert mesite, *Monias benschi*: Seddon 2002, white-crowned sparrow-weaver, *Plocepasser mahali*: Wingfield & Lewis 1993, Australian magpie, *Gymnorhina tibicen*: Brown & Farabaugh 1991) and in affiliation of social bonds within a group (e.g., rufous-naped wren: Bradley & Mennill 2009a, Australian magpie: Brown et al. 1988). Alternatively choruses may represent a conflict, in establishment and maintenance of social hierarchies between members of a group (e.g., laughing kookaburra: Reyer & Schmidl 1988).

Communal vocalizations are very likely to contain information about group size and the ability to assess this may play an important role in group-living species. Efficient assessors should consider the number of individuals that they can expect in a direct encounter. Group size may outweigh inter-individual differences in resource-holding

potential (RHP) in determining the outcome of a contest (McComb et al. 1994, Seddon & Tobias 2003). Therefore, it is possible that the advertising and assessment of group size mediates social interactions in group-living species.

The ability of numerical assessment to avoid costs of fights when outnumbered was first demonstrated in female lions, *Panthera leo* (McComb et al. 1994). Recordings of roars of a single female and a group of three females were played back to lion prides. Defending females were less likely to approach playback of three intruders than of one. They also adjusted their decision to approach according to the size of their own group. Since then, many studies provided evidence that group living species are able to assess rival group size by communal vocal displays and adjust the intensity of their response (e.g., chimpanzee, *Pan troglodytes*: Wilson et al. 2001, green woodhoopoe, *Phoeniculus purpureus*: Radford 2003, subdesert mesite: Seddon & Tobias 2003, black howler monkey, *Alouatta pigra*: Kitchen 2004, black-breasted wood-quail, *Odontophorus leucolaemus*: Hale 2006). For example, Hale (2006) suggested, that choruses in black-breasted wood-quail could function in relative assessment of group size, after finding that groups were less likely to approach the loudspeaker when the simulated intruding group was the same size or larger. Seddon & Tobias (2003) studied the effect of number of intruders on the group response in subdesert mesites and presented acoustic playbacks of different group sizes to territorial groups. Responses to the stimuli were significantly influenced by the number of simulated intruders, with increasing numbers of intruders causing territory holders to sing longer and approach the speakers more slowly. Radford (2003) used natural observations and playback broadcasts of varying group sizes to investigate the territorial vocal rallying display of the green woodhoopoe. Individuals and groups showed a more aggressive response to actual and simulated intrusions of larger groups than smaller groups by increasing the length of their song response.
All of these studies demonstrate that it is very likely that animals are able to assess the number of rivals based on the length and the intensity of vocal signals. Groups respond more collectively and are less likely to approach playback as the relative number of intruders increases.

We investigated the territorial behaviour of a group living bird, the plain-tailed wren, *Pheugopedius euophrys.* This species is widespread and a common songbird that inhabits the bamboo forests of Ecuador and Peru. It is unknown whether they are cooperative breeders, family groups, or groups composed of unrelated individuals. Understanding the relatedness patterns within a group is important for testing questions about the complex chorusing behaviour. If we assume that plain-tailed wrens do indeed reproduce cooperatively, the chorus song could have a synchronizing effect on

reproductive efforts by members of a group to help coordinate the timing of breeding (Mann et al. 2006).
Further, the competition for reproductive opportunities among non-breeders in a group should be very high if cooperative breeding is facilitated by ecological constraints (Curry 1988). If plain-tailed wrens do not breed cooperatively and instead just live in social groups in 'all-purpose' territories, chorusing may primarily function in communal territory defence and intergroup spacing.
Plain-tailed wren choruses likely contain information about the number and the sex of the birds contributing to them. In this species, both sexes vocalize with independent solo songs and produce precisely coordinated antiphonal duets and choruses, during which each individual sings the same song type at the exactly same time as others of their sex (Mann et al. 2006). Every group has a repertoire of combined song types, which can be rapidly alternated during a song, thus carrying the potential for group-specific recognition as well as numerical assessment. These songs can carry long distances and are likely to signal group characteristics such as territory occupancy, group size, group composition, or group identity.

We investigated whether plain-tailed wrens perceive and respond to such information using audio playback to simulate varying levels of threat (2 bird duets and 4 bird choruses) to differently sized groups. First, if coordinated songs provide groups with information to assess each other's size and RHP, we predict that the intensity of their response to increasing numbers of simulated intruders would therefore also increase. Second, given the possibility that there are strong within-group conflicts over reproduction, the composition of groups should also have an influence on the intensity of response. In groups where males outnumber females or vice versa, strong male-male competition or female-female competition might increase the intensity of response. Last, song initiation and termination might also be influenced by group composition. We examined which sex was more likely to initiate and terminate communal songs during territory defence. Mann et al. (2006) found that coordinated songs in plain-tailed wrens usually started and ended with female phrases. However, we predict that groups with more males show a higher male initiation / termination rate and vice versa.

2. Materials and Methods

2.1. Study site

Data collection took place in November 2011 in Pasochoa Volcano Reserve, which is located in the highland of Pichincha, on the east side of the Andean slopes in Ecuador (0.46°S, 78.45°W). The thick *Chusquea* bamboo forest (2000 – 3000 m) in the collapsed crater of the Pasochoa Volcano supports a large population of plain-tailed wrens (*Pheugopedius euophrys*). This cloud forest is one of the last relicts in the inter-Andean valley of Ecuador and is today surrounded by agricultural fields and dairy farms (Stern 1995).

2.2. Study species

In this study, 54 plain-tailed wrens of 21 different territory-holding groups were caught using mist nets and song playback on their territories. We marked birds individually with a unique combination of three plastic colour rings and one numbered metal ring for future identification in the field. Standard morphometric measurements were taken, including mass and the lengths of tarsus, tail, wing chord, and culmen. Groups ranged in size from pairs to 7 individuals (all adults - with one exception).

All members of each sex participated in territory defence and vocal displays. A single chorus can last up to 2 minutes (Mann et al. 2006). Males and females have different repertoires and sing in a cycle during which the two sexes alternate with little or no overlap between them. A cycle has 4 components (ABCD), which are repeated to form a song (ABCDABCDABC…), with males singing A and C and females singing B and D phrases (Mann et al. 2006, Catchpole & Slater 2008, Mann et al. 2008) (Figure 1).

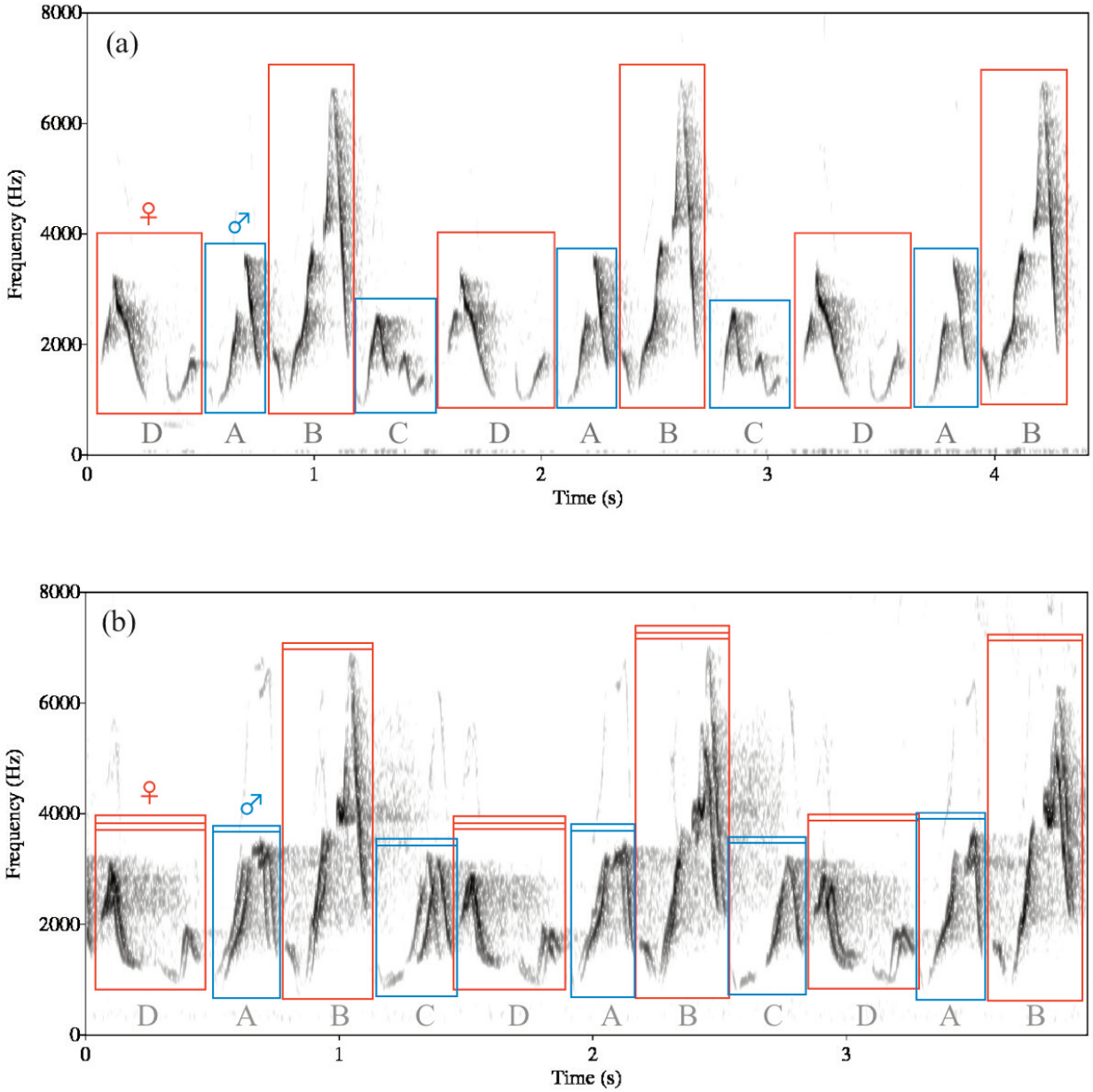

Figure 1. Spectrograms of plain-tailed wren songs. Female contributions are labelled in red, male contributions in blue, with the number of individuals singing each phrase denoted by the number of horizontal lines. The first example (a) shows a duet (1 male and 1 female), the second one (b) a chorus with 2 males and 2-3 females singing together in almost perfect synchrony.

One feature of the singing behaviour of this species that is particularly interesting is that each bird has a repertoire of about 20 phrases of each type and they choose the same type at the same time as others of their sex, leading to near perfect synchronized choruses (Mann et al. 2006; Figure 1b). When group members chorus, they often perch

close to each other, but also produce calls, when separated from the others by longer distances. These calls differ from song phrases and consist of two short (50–70 ms) subsequent notes, with the second one showing a significant higher mean fundamental frequency F0 (Hz, mean ± SD; first note: 2234.2 ± 93.2; second note: 2762.2 ± 105.3; paired samples t-test: $t(24) = -22.41$, $p < 0.001$), but both descending in pitch (Figure 2).

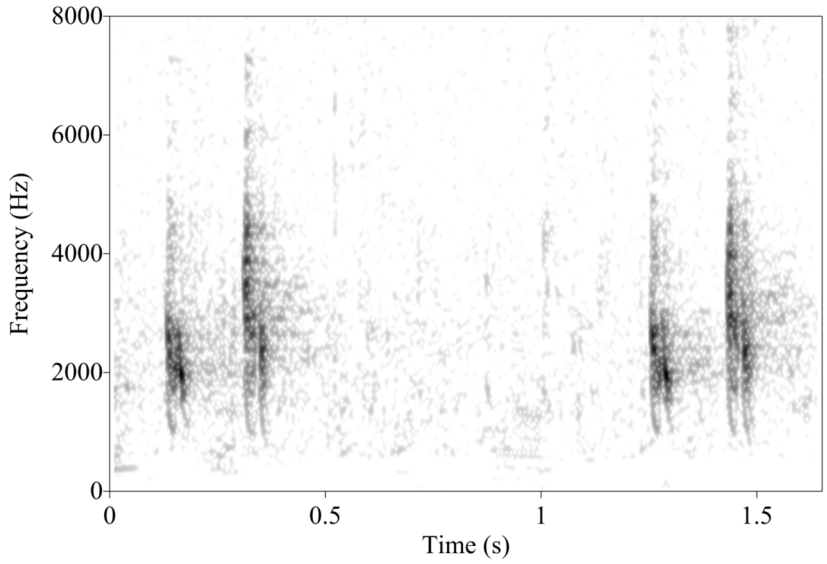

Figure 2. Spectrogram of two calls produced by the same plain-tailed wren. One call consists of two subsequent notes, both descending in pitch. The second one shows a higher mean pitch than the first (see text for details). This was the only call type recorded during the field study.

2.3. Playback stimuli

To investigate if plain-tailed wren groups distinguish between song playback involving different numbers of birds, each of our study groups received two different playback treatments that simulated different types of territorial intruders: duet (1 female and 1 male) and chorus (2 females and 2 males).

Playback stimuli were generated in Audacity® 1.3.13–beta for Macintosh (Audacity Team 2011) using clear, high-quality (low levels of background noise) single-channel recordings of plain-tailed wren songs, that were made on a previous field trip to Pasochoa in autumn 2002 (Mann et al. 2006). Hence, our playback stimuli should be true to population-specific dialects that might occur. As we did not recapture any ringed birds from 2002, it can be assumed that none of these individuals were still alive. This

also ensured that our focal groups were unfamiliar with the specific songs we used for playback, and therefore it should avoid potential associations with any particular plain-tailed wren group living at the study site during the time of the experiment (focal groups or neighbours).

Stereo duet playback stimuli were created, looping a single song cycle (ABCD) in immediate succession for a total duration of approximately 30 s (19 cycles, starting with the female B phrase) with the female contribution in the right and the male contribution in the left channel. Soundfiles were cleaned using the frequency curser filter function in Syrinx® 2.6h (Burt 2006) to remove background noise and the contribution of one sex from each channel. The exact timing of the original duet remained unchanged (Figure 3). We generated stereo chorus playback stimuli by overlapping 2 identical duets (one on the right and one on the left channel), with one duet being offset by 0.2 s from the other, to simulate naturally occurring choruses.

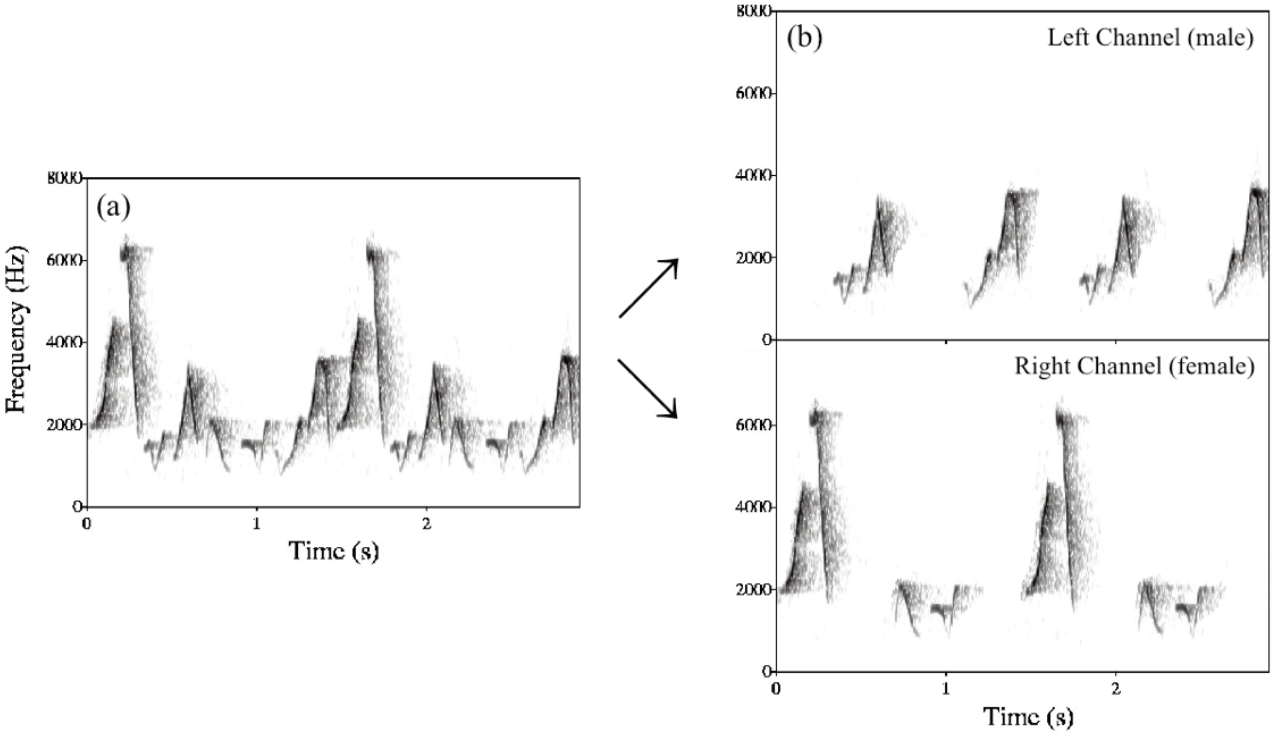

Figure 3. Creation of a two-channel duet playback stimulus from a single-channel recording (a). This particular example is just a small segment of the actual stimulus, which lasted 30 s. It shows 2 duet song cycles, which were initiated by the female (B phrase) and terminated by the male (A phrase). To create a stereo recording, male and female song contributions were separated into 2 channels without altering their temporal relationship. When broadcasted, the male and female contributions originated from two different point sources (b).

In total, 8 stimuli sets (duet and corresponding chorus) were created from field recordings of 8 different groups to reduce the amount of pseudoreplication (McGregor 2000). We normalized all stimuli to a standard amplitude of - 3 dB to remove differences in broadcast volume. They were then saved as digital sound files (.wav files, 16-bit accuracy, sampling rate: 44.1 kHz).

2.4. Experimental design and procedure

16 colour ringed plain-tailed wren groups received each of the two playback treatments on two consecutive days in a randomized order (Table 1). The large majority of our subject groups turned out to be groups, which consisted of 3 to 7 individuals. Only 2 focal territories were occupied by pairs.

Playback experiments were conducted between 07:00 and 12:00 h, the time when birds have the highest levels of activity and singing. Time of day was taken into account and therefore both treatments were presented to the same group at about the same time of day. We ensured that focal groups studied on the same day did not have adjacent territory boundaries.

Table 1. The order in which playback treatments (D = duet, C = chorus) were presented to subject groups.

	Subject Groups															
	1A	**5**	**6**	**9**	**15**	**16A**	**B1**	**B2**	**G1**	**M1**	**M2**	**S1**	**S2**	**S4**	**S5**	**Y3**
21.11.11	C					D		C	D			D				
22.11.11	D					C		D	C			C				
23.11.11			D		C		C			D			C			
24.11.11			C		D		D			C			D			
25.11.11		D		C							D			C	D	D
26.11.11		C		D							C			D	C	C

As a more realistic experimental approach, dual-speaker playback was used during the experiments for simulating coordinated songs with male and female contributions originating from two different point sources (Douglas & Mennill 2010, Langmore 2002, Logue & Gammon 2004, Molles & Waas 2006, Rogers et al. 2004).

For all treatments we set up a pair of speakers (FoxPro® SP-55; frequency response 40 Hz – 22 kHz). The speakers were connected to a digital playback device (FoxPro® Fury, digital game caller, FOXPRO Inc. Lewistown, USA), which was operated by a remote control. The distance between the 2 speakers was 6 m. Flagging tape was used to mark 3 and 6 m radiuses around each speaker to aid judgment of distance. Because of the dense bamboo thicket, we usually placed the speakers on branches next to a narrow hiking trail, about 1.5 m above the ground. The exact locations along the trail were chosen depending on the ability to view through the dense vegetation. We did not control for the exact territory size and location, but as plain-tailed wren territories were quite large the likelihood of the experiment taking place somewhere well within a territory was relatively high. However, sometimes, neighbouring birds responded to the playback and the focal group began to interact with them. This was the case in two territories (15 and B1), where we presented the playback unknowingly close to the territory border. Therefore these groups were excluded from analysis.

Two observers monitored playback responses (number of birds, distance from each playback speaker), with one on either side of the speaker (> 6 m distance to influence the approach responses of the focal birds as less as possible) (Figure 4). A third person, standing next to one of the observers, recorded all vocalizations of the focal group as a response to the presented stimulus.

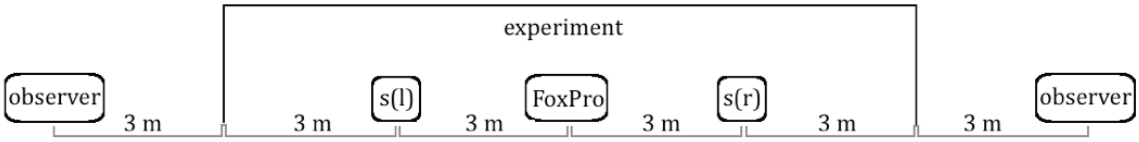

Figure 4. Simplified schematic of the experimental setup, showing the distances between left (l) and right (r) speaker (s) and the observers.

We adjusted the speaker volume by ear to a similar amplitude as natural songs, and used the same settings for all playbacks so that sound amplitude was standardized across all trials.

Prior to all playback trials, we had a settling period of at least 20 min, to allow the birds to resume normal behaviour.

Each playback trial lasted for 22 min and consisted of a 3-min pre-playback control period (in which focal groups were required to be silent), followed by a 30-s playback period (continuous singing); 30 s being close to the mean duration of natural occurring duets

and choruses. The same playback stimulus was repeated 3 min later, followed by a 15-min post-playback period, to allow the birds to respond and then return to normal behaviour.

2.5. Data collection

Every 30 s, from the start of the first playback until the end of the 15-min silent period, we noted the number of birds that were inside a 1 and 3 m radius of the two speakers. All vocal and behavioural responses were recorded with a Sennheiser ME66 directional microphone connected to a portable Marantz PMD660 digital recorder (sampling rate: 44.1 kHz, amplitude resolution: 16 bits). As the vegetation was very dense and plain-tailed wrens tended to stay very close to other group members while singing together, we did not track responses of individuals. Instead, responses of the entire group as a unit were recorded.

2.6. Song analyses

All experimental recordings were analysed using PRAAT software (Boersma & Weenink 2011). Measurements were taken from spectrograms generated, using the following settings: Fast Fourier Transform (FFT) method, window length = 0.015 s, time steps = 1000, frequency steps = 250, dynamic range= 45-55 dB, Hanning (sine-squared) window shape.

A song was defined as a continuous series of notes separated from previous vocalisation by an interval ≥ 2 s and can be in a form of a solo, a duet or a chorus. We defined duets and choruses as temporal associations of alternating song components produced by at least one male and one female, with the distinction that duets were produced by only 2 individuals and choruses by more than 2. Female and male plain-tailed wrens combine their sex-specific song types to create distinct duet/chorus types. As within a group a certain male song type is always combined with a certain female song type, we referred to the resulting duet/chorus types as ‘song types’ in general. Song types could be easily distinguished by comparing sound spectrograms.

The following 10 variables were measured to quantify the vocal and behavioural responses to both treatments, as we considered them to be good indicators of territorial

aggression: (1) duration of the first song given in response to playback, (2) total duration of vocal response, (3) duration of the longest song, (4) number of changes in song types, (5) number of different song types, (6) proportion of maximum number of group members simultaneously contributing to a song (singing at least one cycle together), (7) minimum distance of any group member to a speaker, (8) maximum number of birds within 3m to either of the speakers, (9) mean values of the maximum frequency of the female B phrase, which is the highest pitched element in a song cycle, (10) mean values of max pitch of the most frequent female B phrase.
In addition, the following parameters were extracted: proportions of initiations and terminations of each sex (the terms 'initiation' and 'termination' denote here the first and the last phrase of a song) as well as the proportion of types of songs (solo, duet and chorus) given as response to duet and chorus song playback.

2.7. Molecular sexing

It was not possible to sex the birds by visual inspection in the hand, as males and females are monomorphic. Therefore blood samples (50µl) were taken by puncturing the brachial vein. Samples were collected on filter paper (FTA® cards, Whatman, United Kingdom) to protect the DNA from degradation in the field. At the Molecular Ecology Laboratory of the University of St Andrews, the DNA was extracted following a modified version of the NH4Ac DNA extraction protocol found on www.parisveltsos.com/research (see *Appendix I*). Sex was determined by using the P2 and P8 method, which amplifies an intron of the CHD1 gene on the sex chromosomes of birds. The CHD1-Z gene occurs on the Z chromosome of both sexes, while the CHD1-W gene is only found in females (Griffiths et al. 1996, 1998). Sequences were analysed by electrophoresis on 2.5 % agarose gels at 120 volts. As the introns in CHD1-Z and CHD1-W differ in length, a male (ZZ) results in a single band and a female (ZW) in two bands (see *Appendix II*).

2.8. Kinship analysis

54 microsatellite primers that were known for their high cross-species utility (Dawson et al. 2010, 2006, 2005) were tested on a number of plain-tailed wren individuals as an attempt to estimate kinship relationship of group members (see *Appendix III*). As almost all microsatellite loci were monomorphic (except for two, which showed a low rate of

polymorphism), it was not possible to genotype families (see *Appendix III).* All molecular analyses were conducted at the Molecular Ecology Lab of the University of St Andrews, UK.

2.9. Statistical analysis

Statistical tests were carried out using SPSS for Macintosh Version 20.0 (SPSS, 2011).

2.9.1. Intensity of response

To measure the "intensity of response" to the two playback treatments (duet and chorus) a principal component analysis (PCA) was carried out on the variables by the use of a correlation matrix, to reduce them to a single composite response measure. 7 out of 10 above response variables were used for the PCA, after excluding the following 3 variables because of low correlation ($r \leq 0.35$) with others: (1) duration of the first song, (2) proportion of maximum number of group members contributing to a song, (3) mean values of max pitch of the most frequent female B phrase (these 3 variables were analysed separately). A Kaiser-Meyer-Olkin test and a Bartlett's test indicated that our data set was suited for a PCA (KMO measure of sampling adequacy = 0.576, Bartlett's test of sphericity: $X^2 = 98.39$, degrees of freedom = 21, $p < 0.001$). Based on a Parallel Analysis (Monte Carlo Simulation) to estimate the statistically significant versus the non-statistically significant eigenvalues (Franklin et al. 1995, O'Connor 2000), one factor (PC1) was extracted with an eigenvalue greater than 1.0.

PC1 accounted for 46.45 % of the total variance amongst the 7 variables and was referred to as 'intensity of response', where high positive PC1 scores indicated a strong aggressive response. 'Number of different song types', 'total duration of vocal response', 'number of changes in song types' and 'maximum pitch of female B phrase' were the 4 response variables with the highest weights in PC1, all of them showing strong positive factor loadings (>0.6) and high communalities (>0.4), which represented the proportion of each variable's variance that was explained by the PCA solution (Table 2). Therefore PC1 can be related to 'intensity of response', with high PC1 scores indicating strong combative responses (e.g. a high number of different song types, many changes in song types, a long vocal response).

Table 2. Principal component analysis (PCA) of response variables.

	PC1 factor loadings	Communalities
Number of different song types	0.81	0.66
Total duration of vocal response	0.77	0.59
Number of changes in song types	0.76	0.58
Maximum pitch of female B phrase	0.69	0.48
Number of birds within 3m	0.58	0.34
Minimum distance of any group member to the speaker	-0.58	0.34
Duration of the longest song	0.51	0.26
Initial Eigenvalue	**3.25**	
Total variance explained (%)	**46.45**	

To compare between the PC1 scores of the two different treatments (duet and chorus) we used a Wilcoxon signed-rank test for matched pairs. To determine if the numeric odds could affect the 'intensity of response', a one-way analysis of covariance (ANCOVA) was conducted with the PC1 scores as a dependent variable and the 'odds ratio' as a fixed factor. Group ID and playback treatment were included as covariates. The 'odds ratio' was calculated by dividing the number of territory defenders by the number of simulated intruders. For example, a pair encountering duet playback, faced the same odds as a group of 4 plain-tailed wrens that were confronted with chorus playback (Table 3).

Numeric odds where also classified into 3 categories: 'In favour', 'Even', 'Outnumbered'. To analyse differences between PC1 scores across these categories, we used a Wilcoxon signed-rank test for matched pairs.

We also performed ANCOVAs to assess the effects of group size, the number of males, the number of females and the relative number of males and females ('sex ratio') on the 'intensity of response' using the same covariates as above. The 'sex ratio' for each focal group was calculated by dividing the number of males by the number of females. Subject groups were classified into 3 sets, representing on the number of males in a group or the number of females in a group, respectively: '1', '2' and '3'. Additionally we classified subject groups into 3 categories, depending on the number of males and females: 'even', 'more females', 'more males'. Differences between the categories were analysed using Kruskal-Wallis H tests and Mann-Whitney U post hoc tests with Bonferroni corrections.

Table 3. Numerical odds ratios for each of the 14 groups of plain-tailed wrens. Each group was subjected to duet and chorus playback, resulting in different odds ratios (number of group members divided by the number of simulated intruders).

		Odds ratio		PC1 scores	
Subject group	**Sex ratio**	**Duet**	**Chorus**	**Duet**	**Chorus**
G1	1	1	0.50	0.33	0.00
M1	1	1	0.50	-1.00	-1.16
Y3	2	1.50	0.75	-1.42	-1.22
S1	0.33	2	1	1.12	0.85
6	0.33	2	1	-0.12	-0.37
S2	1	2	1	-1.91	0.30
S5	1	2	1	-0.18	-0.45
5	1	2	1	-0.11	2.06
M2	0.33	2	1	1.39	-1.01
S4	1	2	1	-0.05	1.14
16A	1.50	2.50	1.25	-1.24	0.49
1A	0.67	2.5	1.25	0.84	1.75
B2	1.50	2.5	1.25	-0.29	-0.61
9	1.33	3.5	1.75	0.31	0.56

2.9.2. Song initiation and termination

Wilcoxon signed-rank tests were used for comparisons between the proportions of males' and females' song initiations and terminations during the playback response of each focal group, depending on their group composition. Friedman tests and post-hoc Wilcoxon signed-rank tests with Bonferroni corrections were used to analyse differences in the proportions of the sex-specific song phrases A, B, C and D used to initiate and terminate songs.

2.9.3. Proportion of types of vocalisation as response to duet and chorus song playback

To investigate the motivation of pairs and groups to join in communal defence, we determined song output by calculating the proportion of each type of vocalisation (solo,

duet, chorus) that was produced as response to duet and chorus song playback. Wilcoxon-signed rank tests were used to compare the proportions of solo, duet and chorus songs depending on the playback treatment. To analyse differences between the proportions of solo, duet and chorus songs, Friedman tests and post-hoc Wilcoxon signed-rank tests with Bonferroni corrections were applied. Differences in song output between duet and chorus playback treatment were also analysed using a Wilcoxon signed-rank test for matched pairs.

3. Results

Playback experiment – Response to simulated intrusion

After the start of the playback, group members came together from scattered positions across the territory. All 14 subject groups responded vocally either during or shortly after both the duet and chorus playback. All group members participated in the chorus, with individuals joining in and dropping out at various points during a song. Individuals singing the same part in a chorus were not always perfectly synchronized in time.

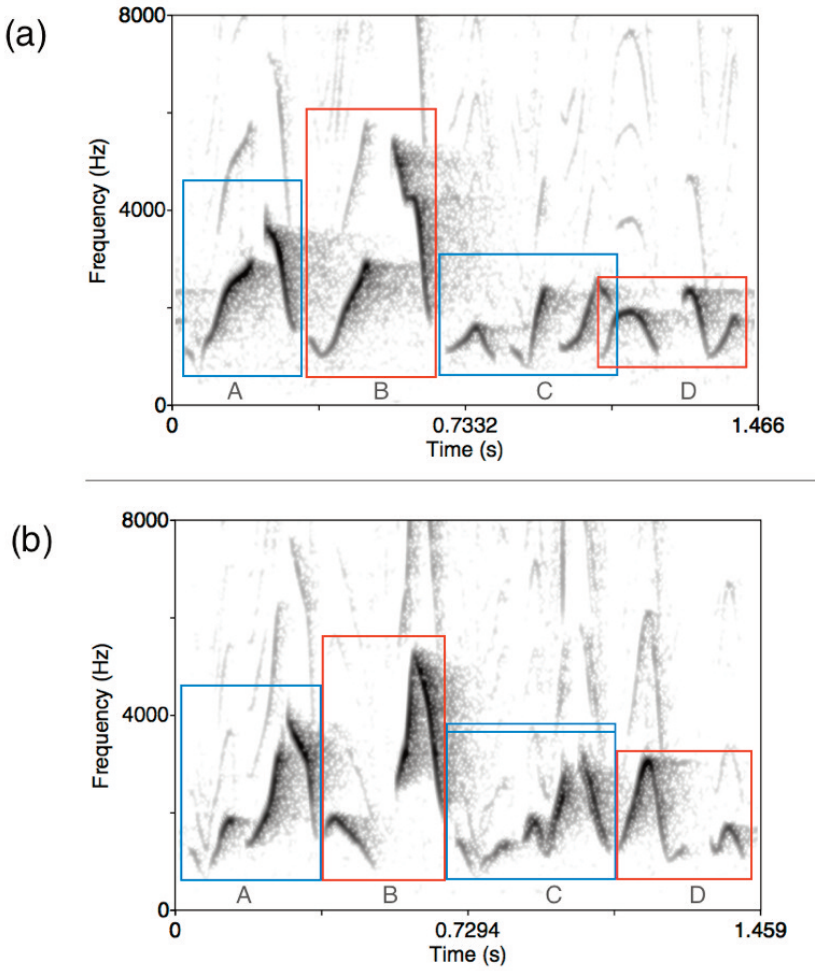

Figure 5. Example spectrograms of two different song types from two different focal groups. Each is showing one cycle of ABCD. (a) was recorded from group S5, (b) from S2. As visible, there is a variation in the male C. Both were recorded from group S1.

3.1. Vocal distinctiveness – Group identity

All 14 subject groups had their own vocal signature (Figure 5 and 6). They produced distinct song types without any exceptions during the experiment as they responded to the two playback treatments. A few male and female song phrases were shared across groups, but the combination of male notes (A and C) and the combination female notes (B and D) were always group specific. During a song, groups regularly switched between song types and occasionally quadruplets (ABCD) were a hybrid between the previous and subsequent one.

There was no correlation between group size and number of song types used (Pearson's correlation, $p > 0.05$ for both cases, duet and chorus playback) (Table 4).

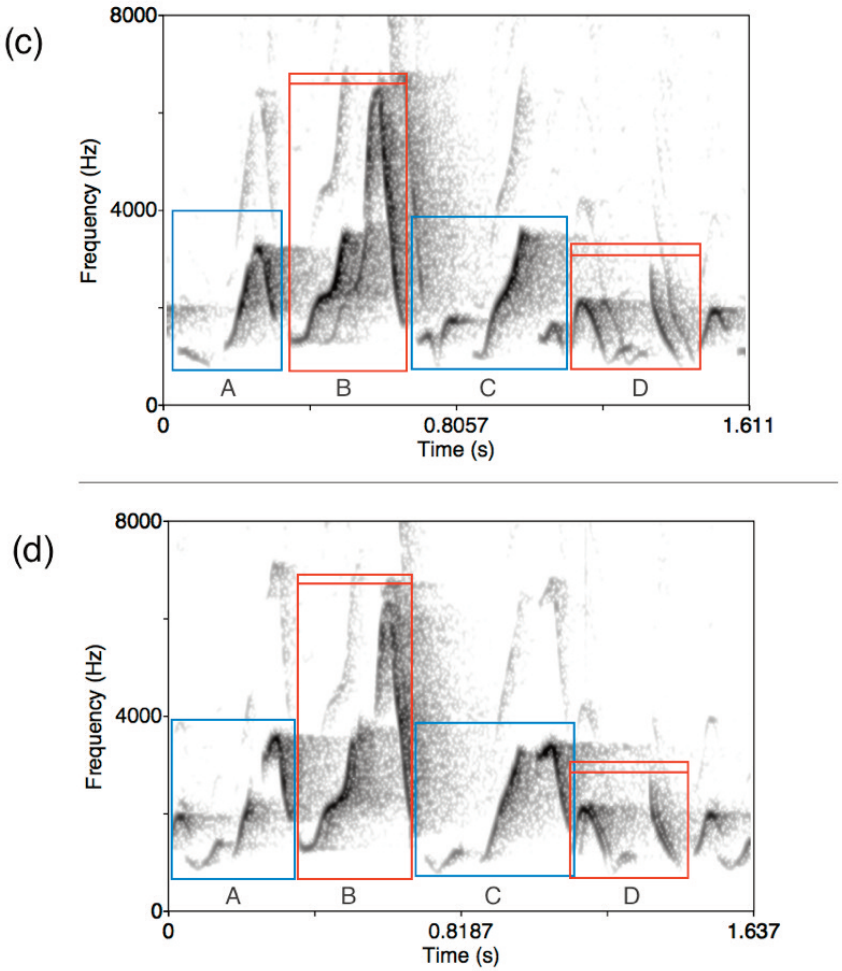

Figure 6. Spectrograms of one song type (c) and a variation of it (d), each is showing one cycle of ABCD.

Table 4. Number of song types (+ variants) given as playback response by our focal groups (n = number of birds within a group).

Subject group	n (male/female)	Number of song types
G1	2 (1/1)	9
M1	2 (1/1)	7
Y3	3 (2/1)	4
S1	4 (1/3)	18 (+2)
6	4 (1/3)	9
S2	4 (2/2)	7
S5	4 (2/2)	4
5	4 (2/2)	12 (+2)
M2	4 (1/3)	18
S4	4 (2/2)	11
16A	5 (3/2)	8
1A	5 (2/3)	18 (+1)
B2	5 (3/2)	7
9	7 (4/3)	10

3.2. Intensity of response

3.2.1. Effect of number of intruders (effect of playback treatment)

A comparison between duet and chorus playback treatment among all focal plain-tailed wren groups showed no significant difference in the 'intensity of response' depending on the number of simulated intruders (Wilcoxon signed-rank test for matched pairs, Z = - 0.534, p = 0.594, 2-tailed, n = 14).

3.2.2. Effect of number of territory holders

The relationship between the 2 covariates group and treatment and the dependent variable PC1 scores did not differ significantly as a function of the number of territory holders. The 'intensity of response' was not affected by the number of territory holders (ANCOVA: $F_{4, 21} = 0.767$, p = 0.558). Only 12.8 % of the total variance in the 'intensity

of response' was accounted for by the number of territory holders controlling for the effect of group and treatment.

3.2.3. Effect of numeric odds

3.2.3.1. Odds ratio

The relationship between the covariates group and treatment and the dependent variable PC1 scores did not differ significantly as a function of the numeric odds. The 'odds ratio' did not influence the 'intensity of response' (ANCOVA: $F_{8, 17} = 0.387$, $p = 0.913$). 15.4 % of the total variance in PC1 scores could be accounted for by the odds ratio controlling for the effect of group and treatment (Figure 7).
The patterns of results remained the same, if PC1 scores were replaced with any single playback response variable.

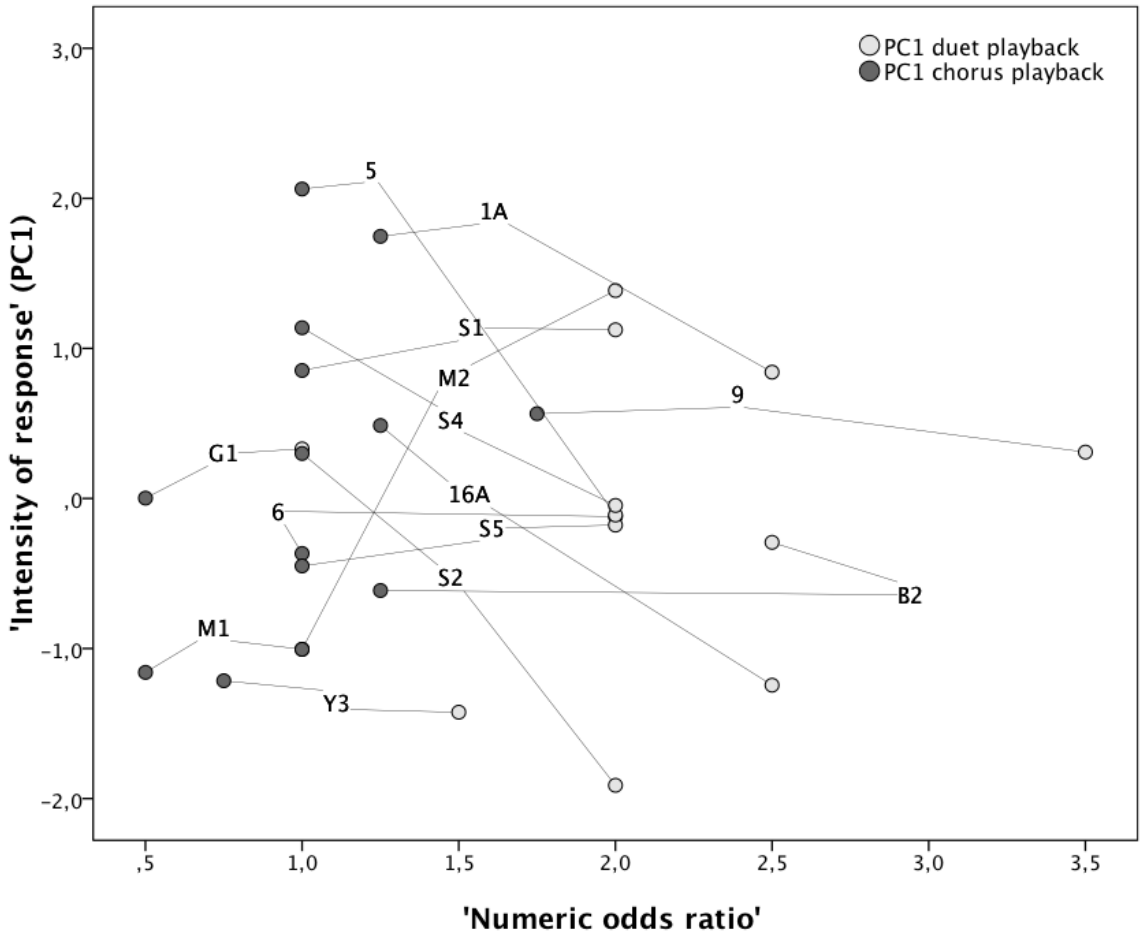

Figure 7. Numeric odds ratio versus PC1 scores ('intensity of response') of the 14 subject groups.

Analysis of the 3 response variables that were excluded from PCA showed a significant difference between the duration of the first song as a function of the numeric odds (ANCOVA: $F_{8, 17} = 9.229$, $p < 0.001$). The 'odds ratio' accounted for 81.3 % of the total variance in the duration of the first song controlling for the effect of group and treatment. There was no significant difference between the maximum number of group members simultaneously contributing to a song as a function of the numeric odds (ANCOVA: $F_{8, 17} = 0.760$, $p = 0.641$). 26.3 % of the total variance in the maximum number of group members could be explained by the 'odds ratio' controlling for the effect of group and treatment.
The mean values of max pitch of the most frequent female B phrase were not affected by the numeric odds (ANCOVA: $F_{8, 17} = 1.064$, $p = 0.431$). 33.4 % of the total variance was accounted for by the 'odds ratio' controlling for the effect of group and treatment.

3.2.3.2. Categories of numeric odds

When numeric odds where categorized into 3 groups ('In favour', 'Even', 'Outnumbered'), no significant difference in PC1 scores could be found in 7 focal groups of 4 birds between 'In favour' (duet playback treatment) and 'Even' (chorus playback treatment) (Wilcoxon signed-rank test, 2-tailed, $Z = -0.169$, $p = 0.938$).

Table 5. Numeric odds classified in 3 categories. In favour = more territory holders than intruders, Even = same size of defending and opposing group, Outnumbered = more intruders than territory holders.

		PC1 scores			
Subject group	**Group size**	In favour		Even	Outnumbered
G1	**2**			0,33	0,00
M1	**2**			-1,00	-1,16
Y3	**3**	-1,42			-1,22
S1	**4**	1,12		0,85	
6	**4**	-0,12		-0,37	
S2	**4**	-1,91		0,30	
S5	**4**	-0,18		-0,45	
5	**4**	-0,11		2,06	
M2	**4**	1,39		-1,01	
S4	**4**	-0,05		1,14	
16A	**5**	-1,24	0,49		
1A	**5**	0,84	1,75		
B2	**5**	-0,29	-0,61		
9	**7**	0,31	0,56		

Playback treatment: Duet | Chorus

As only two of the subject groups were pairs, 3 consisted of 5 birds and one was a large group of 7, it was not possible to compare statistically their PC1 scores between numeric odds categories (Table 5 and Figure 8).

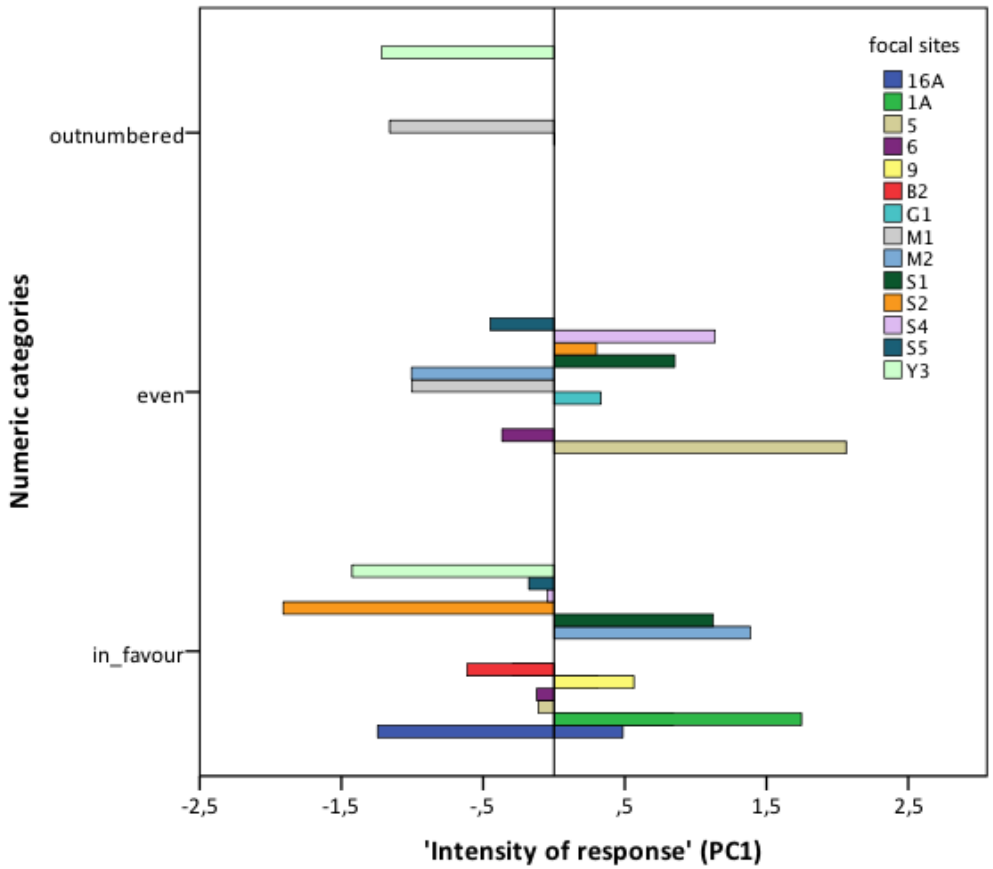

Figure 8. 'Intensity of response' (PC1 scores) versus numeric categories. Different colours indicate different subject groups.

Analysis of the 3 response variables, that were excluded from PCA, showed no significant difference between the two levels of threat (Wilcoxon signed-rank test, 2-tailed, n = 7; 'duration of the first song': Z = -0.338, p = 0.813; 'proportion of max number of group members simultaneously contributing to a song': Z = -0.970, p = 0.344; 'mean values of max pitch of the most frequent B phrase': Z = -1.153, p = 0.313).

3.2.4. Effect of number of males and number of females

There was no significant difference between the mean ranks of PC1 depending on the number of males within a group in both playback treatments (Kruskal-Wallis test, df = 2, n = 14; duet: H = 2.364, p = 0.307; chorus: H = 1.440, p = 0.487) (Figure 9).

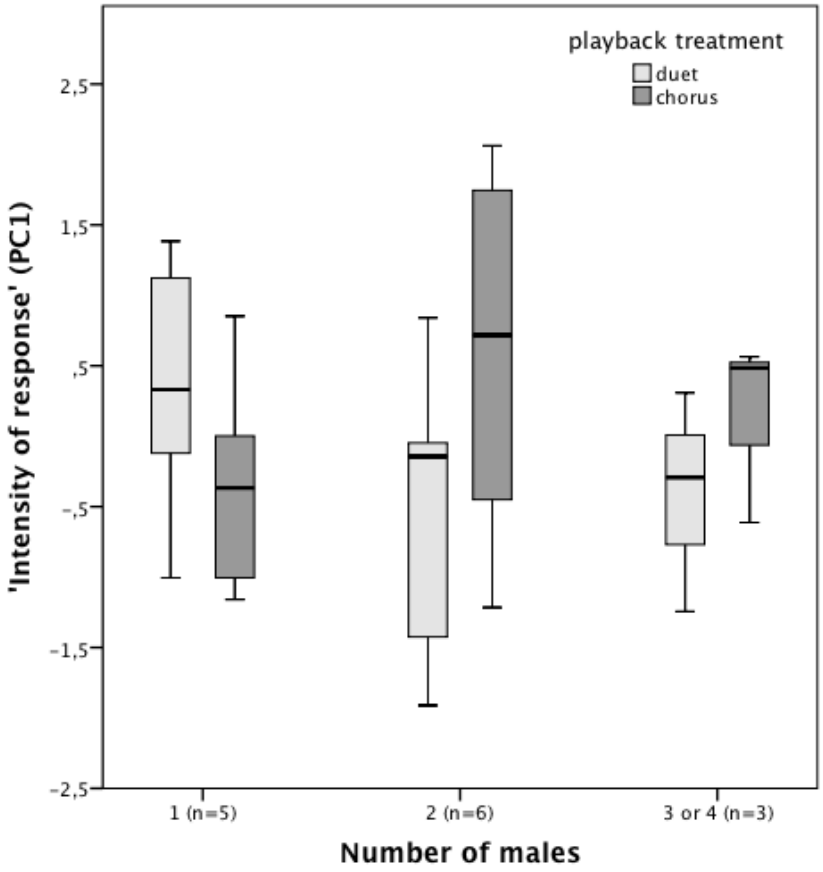

Figure 9. 'Intensity of response' (PC1) depending on the number of males within the focal groups – classified in 3 categories, n = number of groups. The top and bottom of the box are the 25th and 75th percentiles, the horizontal line within the box denotes the median value.

There was a statistically significant difference between the mean ranks of the 'intensity of response' depending on the number of females within a group in duet playback treatment (Kruskal-Wallis test, df = 2, n = 14, H = 6.097, p = 0.047), but there was no significant difference in chorus playback (Figure 10). Post-hoc analysis was conducted with a Bonferroni correction (significance level at $p < 0.017$) and showed a significant difference in the 'intensity of response' between groups with 2 and groups with 3 females (Mann-Whitney test, U = 2, Z = -2.373, p = 0.017).

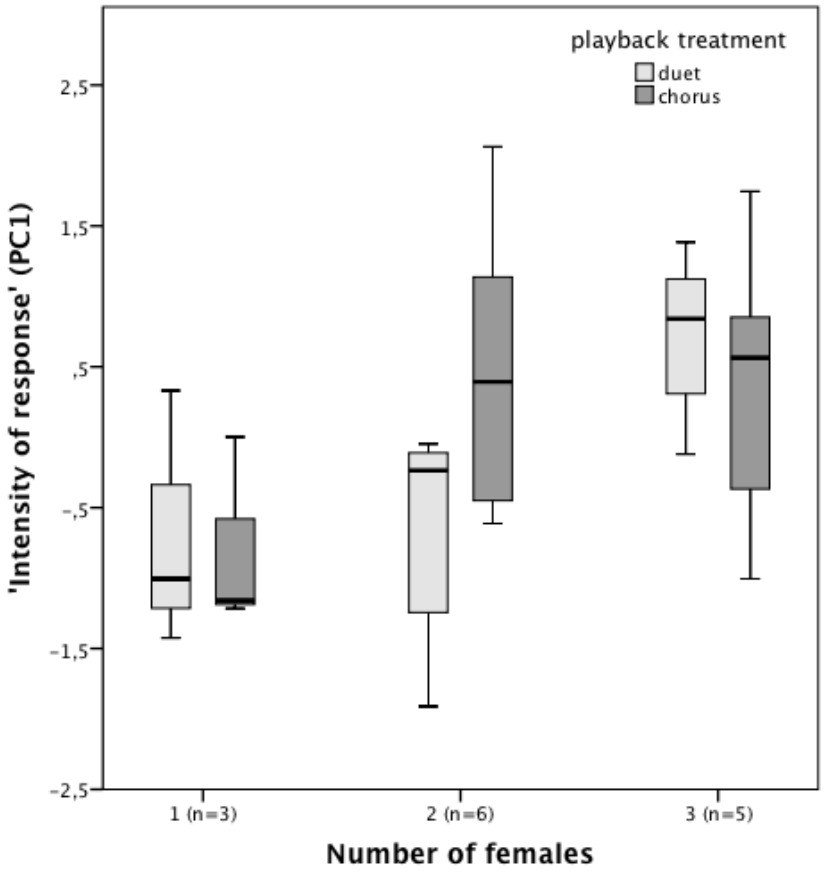

Figure 10. 'Intensity of response' (PC1) depending on the number of females within the focal groups – classified in 3 categories, n = number of groups. The top and bottom of the box are the 25th and 75th percentiles, the horizontal line within the box denotes the median value.

3.2.5. Effect of number of males to number of females

3.2.5.1. Effect of male-to-female sex ratio

The relationship between the 2 covariates group and treatment and the dependent variable PC1 scores did not differ significantly as a function of the male-to-female sex ratio. The 'intensity of response' was not affected by the 'sex ratio' (ANCOVA: $F_{5, 20} = 1.708$, $p = 0.179$). 29.9 % of the total variance in PC1 scores could be accounted for by the 'sex ratio' controlling for the effect of group and treatment (Figure 11).

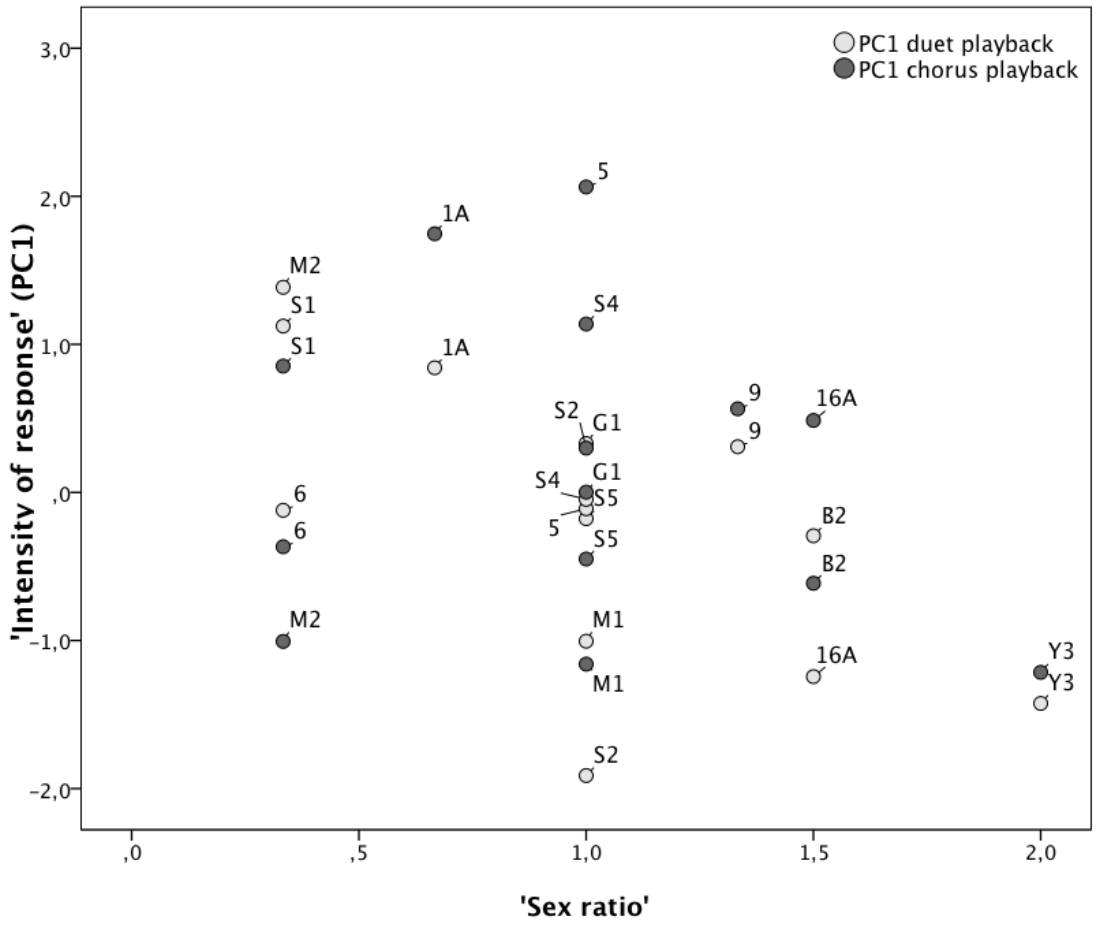

Figure 11. 'Sex ratio' (number of males to number of females in the subject group) versus PC1 scores ('intensity of response') of the 14 subject groups.

3.2.5.2. Categories of group composition

When subject groups were classified into 3 categories, depending on the number of males and females ('even', 'more females', 'more males'), we found no significant difference in PC1 scores between the 3 categories in both playback treatments (Kruskal-Wallis test, df = 2, n = 14; duet: H = 5.429, p = 0.066; chorus: H = 0.729, p = 0.695) (Figure 12).

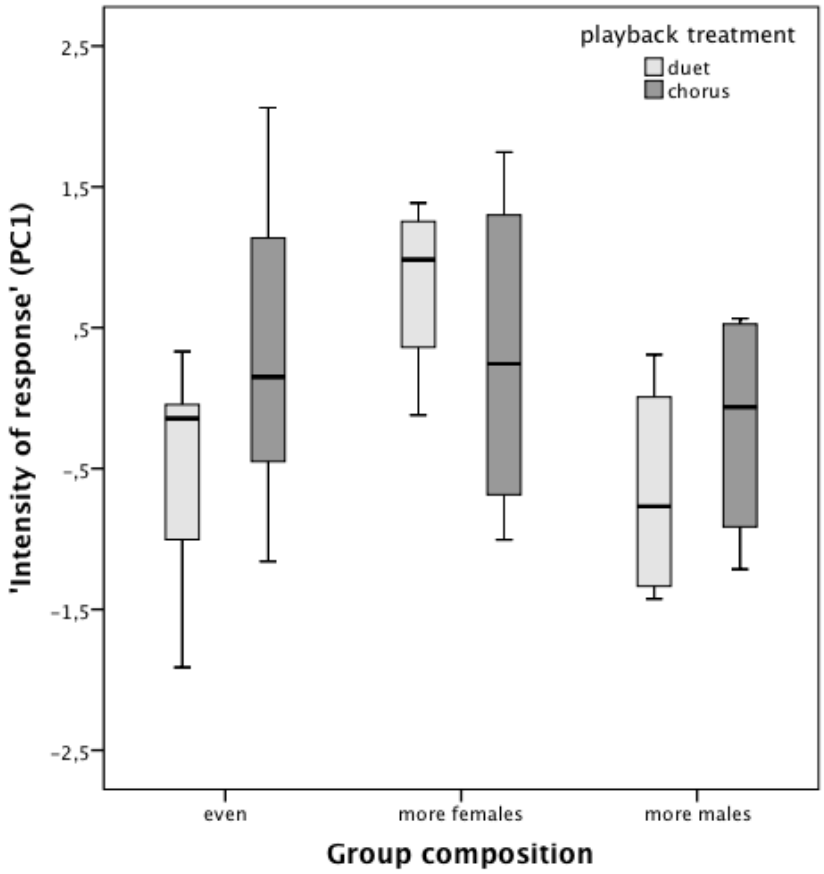

Figure 12. 'Intensity of response' (PC1) depending on the number of males and females in a group, n (even) = 6, n (more females) = 4, n (more males) = 4. The top and bottom of the box are the 25th and 75th percentiles, the horizontal line within the box denotes the median value.

3.3. Song initiation and termination

Although statistically not significant, there was a difference in the proportion of male and female song initiation phrases depending on group composition (Wilcoxon signed-rank tests, 2-tailed) (Figure 13). In groups with more females than males, females initiated songs more often than males (n = 4, median = 0.89, Z = -1.826, p = 0.068). Groups with more males than females initiated songs more often with male phrases (n = 4, median = 0.70, Z = -1.826, p = 0.068). Groups with an even number of males and females showed an equal distribution of males and females song initiations (n = 6, Z = -0.943, p = 0.345).

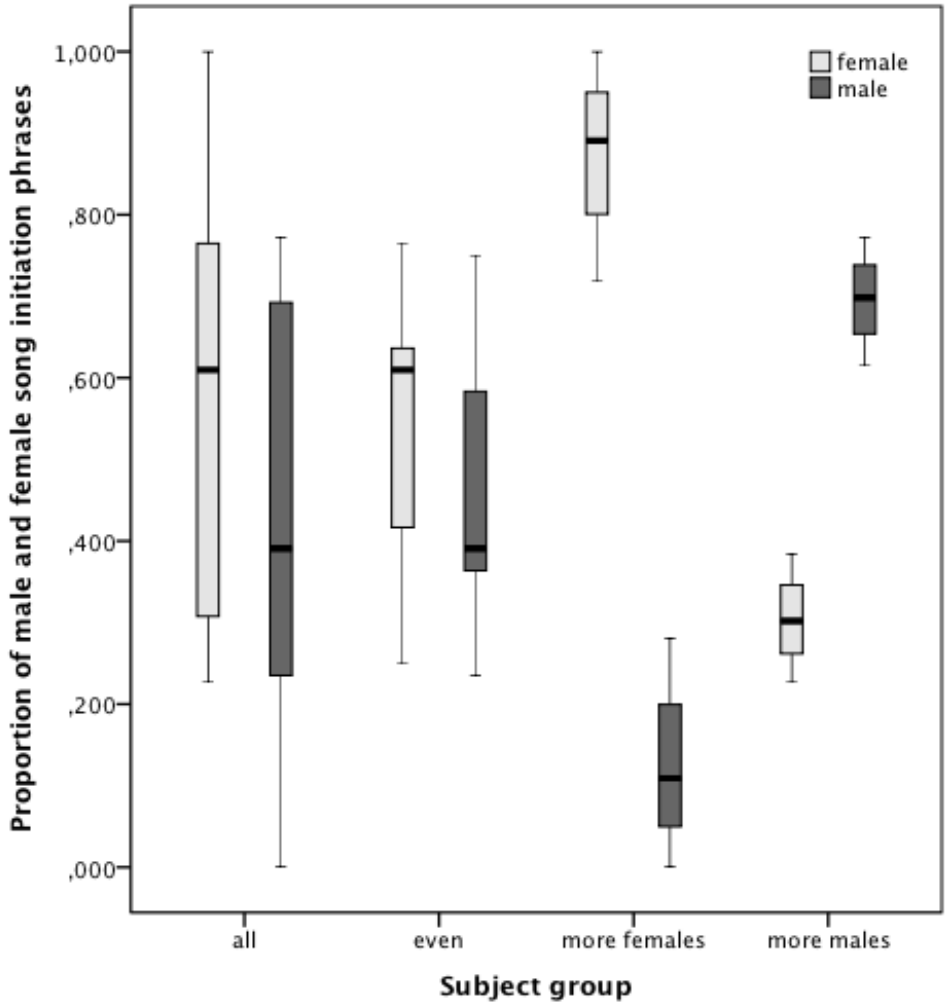

Figure 13. Proportion of male and female song initiation phrases depending on group composition. all = all focal groups (n = 14), even = groups that consist of the same number of males and females (n = 6), more females than males (n = 4), more males than females (n = 4). The top and bottom of the box are the 25th and 75th percentiles, the horizontal line within the box denotes the median value.

There was no statistically significant variation in the mean ranks of A, B, C, D as initial phrases in all subject groups, in groups with an even number of males and females and in groups with more males than females (Friedman test, df = 3, $p > 0.05$ in all 3 cases). In groups with more females than males the mean ranks of A, B, C and D as initial phrases showed significant differences, $\chi^2 = 7.816$, $p = 0.05$. Post-hoc analysis with Wilcoxon signed-rank tests was conducted with a Bonferroni adjustment on the results (significance level at $p < 0.008$). But however, there were no significant differences between each of the four phrases.

When looking at the termination phrases of the playback responses of all subject groups, songs usually ended with female phrases (Wilcoxon signed-rank test, n = 14, median = 0.75, Z = -3.109, p = 0.002).

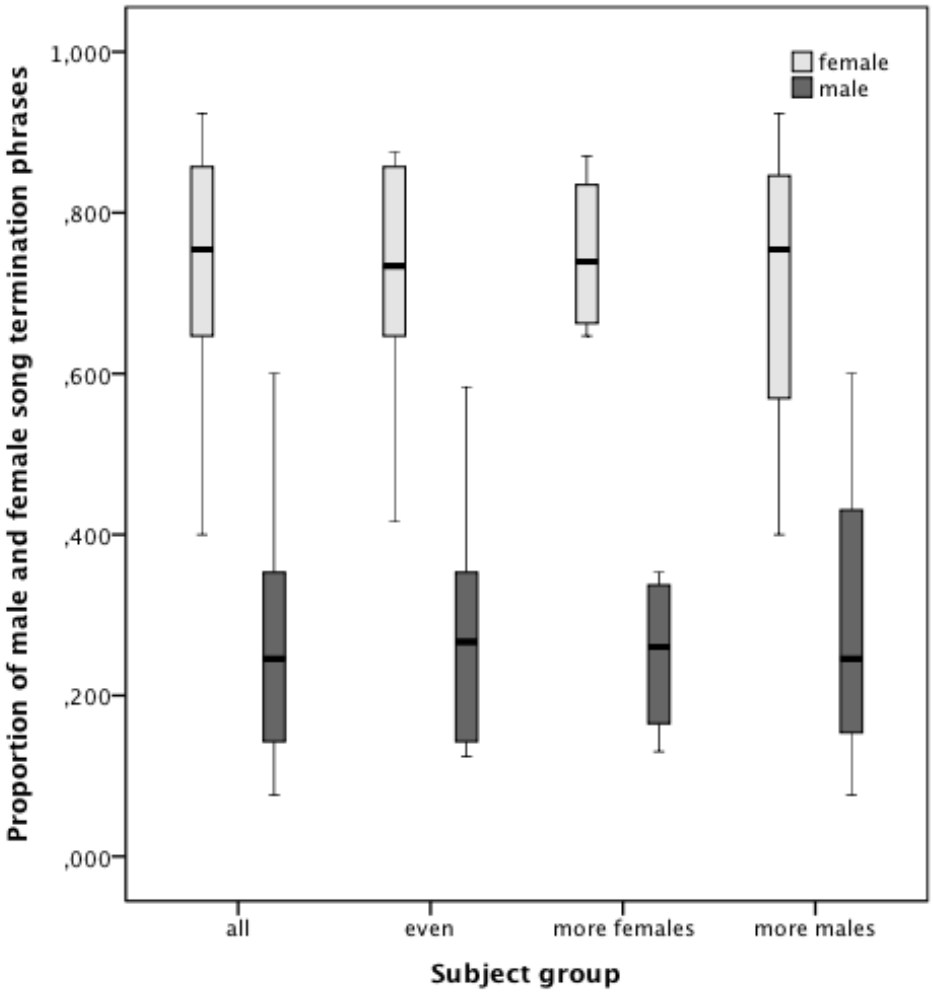

Figure 14. Proportion of male and female song termination phrases depending on group composition. all = all focal groups (n = 14), even = groups that consist of the same number of males and females (n = 6), more females than males (n = 4), more males than females (n = 4). The top and bottom of the box are the 25th and 75th percentiles, the horizontal line within the box denotes the median value.

The proportion of female termination phrases was also statistically significantly higher in groups with an equal number of both sexes (n = 6, median = 0.73, Z = -1.992, p = 0.046). As Figure 14 shows, groups with more females than males and vice versa also showed more female song terminations, although statistically not significant in both cases ('more females': n = 4, median = 0.74, Z = -1.826, p = 0.068 / 'more males': n = 4, median = 0.75, Z = -1.461, p = 0.144).

There was a statistically significant difference between the mean ranks of A, B, C and D as termination phrases in the subject groups 'all' (χ^2 = 19.993, p < 0.001), 'even' (χ^2 = 8.018, p = 0.046) and 'more females' (χ^2 = 7.923, p = 0.048). The test was marginally not significant for groups with more males than females (χ^2 = 7.5, p = 0.058). Post-hoc

tests (Bonferroni correction: significance level at $p < 0.008$) showed significant differences in the proportion of termination phrases in all subject groups: Most of the songs were terminated by the female D (median = 0.45), which differed significantly from female B (median = 0.22, Z = -2.845, p = 0.004), male C (median = 0.15, Z = -3.140, p = 0.002) and male A (median = 0.10, Z = -3.296, p = 0.001).

3.4. Proportion of solo, duet and chorus songs as response to playback

All of the songs the two plain-tailed wren pairs (G1 and M1) produced were duets; there were no solo songs as response to duet or chorus playback.
Groups with more than two birds (n = 12), showed very similar levels of response to both playback treatments for the proportions of solos, duets or choruses (Wilcoxon signed-rank tests, $p > 0.05$ in all 3 cases) (Figure 15).

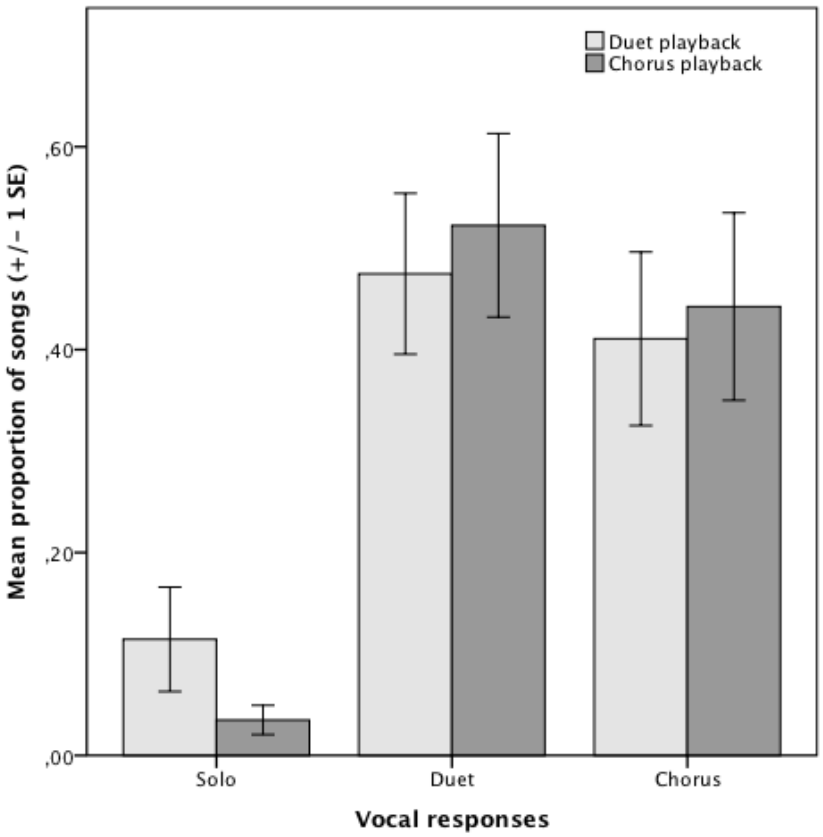

Figure 15. The three types of vocal responses of plain-tailed wrens to duet and chorus song playback. Bars show the mean proportion +/- standard error of solos, duets and choruses produced by the focal groups as response to the stimuli; n (groups with more than 2 birds) = 12.

There was significant variation in the mean ranks of the production of solo songs, duets and choruses in both playback treatments (duet: $\chi^2 = 7.277$, $p = 0.026$, chorus: $\chi^2 = 17.149$, $p < 0.001$). Post-hoc analyses (Bonferroni correction: significance level at $p < 0.017$) showed that the proportions of duet songs differed significantly from solo songs (duet: $Z = -2.667$, $p = 0.008$, chorus: $Z = -3.059$, $p = 0.002$). The production of chorus songs was also significantly higher than solo songs during chorus playback ($Z = -2.934$, $p = 0.003$), but however, the difference between the proportion of solo and chorus songs during duet playback was not significant after Bonferroni correction ($Z = -2.045$, $p = 0.041$) (Figure 15).

4. Discussion

All study groups responded to playback by duetting or chorusing. The proportions of duet and chorus songs differed significantly from solo songs in both treatments, indicating that coordinated songs play an important role in territory defence.
We observed that duetting and chorusing mostly occurred during morning hours and would often trigger a chain reaction, with neighbouring groups starting to sing as well. These communal songs could work for intergroup spacing and territorial advertisement (Catchpole & Slater 2008). There are some characteristics of plain-tailed wren duets and choruses that provide indirect evidence that these coordinated vocalisations function to communicate with other groups. Coordinated songs can be of high amplitude (Fortune et al. 2011) and carry much greater distances than would be necessary for a signal that only functions in intragroup communication.

The playback stimuli we used during the experiment varied in the number of birds contributing, but all started with the female B phrase, because it was suggested that females usually initiate songs in plain-tailed wrens (Mann et al. 2006). Female leadership in duetting has been discussed in only a few other bird species (e.g. bay wren: Levin 1996b, cocos flycatcher: Kroodsma et al. 1987). But our present results show that in groups with a sex ratio biased towards males, males initiated songs at a higher level. Whereas groups consisting of more females were more likely to start their songs with a female phrase and groups with an even number of both sexes showed an equal distribution of male and female song initiations. Mann et al. (2006) found that females generally initiated coordinated songs at a higher level. The plain-tailed wren groups that were used for analysis differed in size and also in composition from each other. But, however, song output of only four different groups was analysed (2 groups with an even number of both sexes: 1m1f, 2m2f, one slightly male biased: 3m2f and one strongly female biased: 2m5f). All of them showed a high level of female initiation rates. On the one hand this might have been influenced by the strong female bias in one group. On the other hand, as demonstrated in the present study, 50 % of the groups with an even number of males and females mostly started their songs with female phrases and the other 50 % initiated songs with male phrases (see *Appendix V*). Therefore it is likely that the small sample size (n = 4) compared to n = 14 could have been another explanation for achieving different results in the present study.
If we assume that the bird that initiates the song is at the same time the vocal leader (Rogers et al. 2004), our results may not support the theory that female leadership in

duetting / chorusing occur in this species. Further, we found that most of the time songs were terminated by female phrases (either B or D), as previously shown by Mann et al. (2006), who suggested that songs come to an end when males fail to contribute.

Given the intergroup nature of these vocal signals, we were particularly interested in understanding whether plain-tailed wrens were capable of numerical assessment, adjusting their response to territorial intruders on the basis of the number of individuals of their own and the opposing group. Many studies (e.g. McComb et al. 1994, Wilson et al. 2001, Seddon & Tobias 2003, Kitchen 2004) show that it is likely that animals are able to assess the number of opponents based on the length and the intensity of vocal signals. Groups respond more collectively and are less likely to approach playback as the relative number of intruders increases. Further, we investigated if group composition (sex ratio, number of males to females in defending group) had an influence on the intensity of response, as it was demonstrated in subdesert mesites (Seddon & Tobias 2003).
We found that the intensity of response did not depend on group size or sex ratio. Subject groups did not produce graded responses to different playback treatments, suggesting that both, duet and chorus playbacks were perceived as similar levels of threat. Our predictions of a variation of intensity of response to a relative number of opponents were not met. Therefore we suggest that in plain-tailed wrens group vocalisation is not used to assess rival group size.
Our result stands in contrast to earlier mentioned playback studies that investigated the ability of numerical assessment during territory encounters. But there are a few studies that show similar results. Fedy & Stutchbury (2005) investigated the effect of number of intruders in duetting white-bellied antbirds (*Myrmeciza longipennis*) and found that they respond equally to female solo, male solo and duet playback. They suggested that duetting does not function in territory defence and mate-guarding. Mennill & Vehrencamp (2008) found that rufous-and-white wrens created duets at similar levels in response to solo and duet playback, suggesting that duetting is a multifunctional signal that varies with context. Bradley & Mennill (2009b) presented three different playback stimuli (solo, duet, chorus) to territorial groups of rufous-naped wrens (*Campylorhynchus rufinucha*), but the strength of response did not vary across treatments, leading to the conclusion that all stimuli represented equally strong threats to the subjects. However, as the playback stimuli used in these studies were solo songs from males and females and in the case of Bradley & Mennill (2009b) a chorus of 3 birds, the sex relationship of simulated intruders was not the same as in our experiment. Therefore these findings might not be directly comparable to our study.

Even though we generated chorus playback by combining two duets such that the second one started with an offset of 0.2 s to simulate the naturally occurring asynchrony of contribution, a chorus of 4 birds might have been received as a duet. Choruses of the plain-tailed wren are so extremely synchronized and precise, with birds of the same sex matching each other's timing and selection of phrase types (Mann et al. 2006 & 2008), that we could argue that birds might not be able to assess the exact number of participants. Hence, chorus songs might not function to provide a reliable indication of the number of individuals contributing. But, however, this argument might be questionable since individuals are able to locate different sound sources (Douglas & Mennill 2010).

We observed that some groups of plain-tailed wrens produce highly synchronized choruses while others show temporal overlap between phrases or no synchronization at all. Those birds that fail to synchronize may be still learning this complex song behaviour. This might be the case for juveniles with a late dispersal that stay in their parent's territory and join in their parent's duets, with a young female/male matching her/his mother's/father's song. It is important to measure patterns of relatedness to clarify whether groups consist of a primary breeding pair and their non-dispersal offspring or of unrelated birds. The results, in combination with morphometric and acoustic data, would definitely help to understand hierarchical social structures, which could be the reason why some birds continue to sing throughout the song and therefore might choose different song types, while others drop out and/or join in later during the song.

It is very likely that duetting and chorusing function in territory defence, but coordinated songs may also have other functions in plain-tailed wrens as it has been suggested for other group-territorial species (Seddon 2002, Hale 2006). Given the fact that plain-tailed wrens live in thick bamboo forest, and Thorpe (1972) suggested that duetting/chorusing is important for group members to keep in contact with each other in a densely foliated habitat, this is likely to be a function of communal singing in plain-tailed wrens.

We found that all subject groups had their unique vocal signature, which means that they produced distinct duet types as they responded to the playbacks. The combination of A and C and B and D phrases differed between territories and thus could be assigned to a certain group. This group specificity could therefore serve as mutual recognition and coordination during territory encounters, as well as help discriminate neighbours from intruding strangers (reviewed by Nottebohm 1972, e.g., stripe-backed wren: Wiley & Wiley 1976).

To get a better insight into the biological significance and the mechanisms for chorusing in plain-tailed wrens, there is also a need to understand the roles of the sexes in territory defence and the function of song initiation and song answering during territorial encounters.

It would be good to investigate if divergent seasonal patterns of vocalisation in both sexes exist. It is expected that song contributions vary seasonally and therefore the function of chorusing might also change over the year. Territorial aggression and resource demand are usually higher during breeding season, because access to resources is vital in order to provide food for the offspring (Bradley & Mennill 2009b). During our fieldwork we observed one individual carrying nesting material, one group, which was not part of the playback experiment had a young, and three females of different groups had brood patches. Variation in resource demand could have been a reason for not finding differences in response to varying levels of threat in plain-tailed wrens. This has been suggested by Bradley & Mennill (2009b), who reported a lack of graded response levels in rufous-naped wrens (*Campylorhynchus rufinucha*). Their study was performed in a period while groups either had recently fledged offspring or were unsuccessful in the first breeding attempt and perhaps preparing to nest again. As a result of this higher resource demand during breeding season, aggression levels may have been so high, that the responses to all playback treatments were similarly high, regardless of the number of simulated intruders. Bradley & Mennill (2009b) further suggest that playback studies that would be conducted during the pre-breeding period could provide an interesting comparison.

To have better insight into the functions and to understand the ecology and evolution of this highly coordinated form of vocal communication it is important to analyse the social environment in which communal songs are produced.
Duets and choruses of the plain-tailed wren may serve multiple independent functions that vary with context. Why members of plain-tailed wren groups join to produce these highly coordinated songs still remains uncertain. Social groups could use chorus songs to identify and localize other group members and to coordinate their foraging or other within-group activities. Another important point is that chorusing signals a group member's readiness to participate in a coordinated song with others and also in cooperative territorial defence, maybe indicating: "we are a unit". It could be that group vocalisation is a mechanism to enhance within-group cohesion and reduction of conflict within a group, as previously discussed in the Australian magpie (Brown & Farabaugh 1991, Baker 2009) and the laughing kookaburra (Reyer & Schmidl 1988).

5. References

Audacity Team 2011. Audacity, Version 1.3.13–beta [Computer program]. Retrieved November 13, 2011, from http://audacity.sourceforge.net/

Baker MC 2004. The chorus song of cooperatively breeding laughing kookaburras (Coraciiformes, Halcyonidae: *Dacelo novaeguineae*): Characterization and comparison among groups. *Ethology,* 110, 21–35.

Baker MC 2009. Information content in chorus songs of the group-living Australian magpie (*Cracticus tibicen dorsalis*) in Western Australia. *Ethology,* 115, 227–238.

Boersma P & Weenink D 2011. Praat: doing phonetics by computer, Version 5.3.03 [Computer program]. Retrieved January 16, 2012, from http://www.praat.org/

Burt J 2006. Syrinx, Version 2.6h [Computer program]. Retrieved November 13, 2011, from http://syrinxpc.com/syrinxinstall/syrinxinst.exe/

Bradley DW & Mennill DJ 2009a. Solos, duets and choruses: vocal behaviour of the rufous-naped wren (*Campylorhynchus rufinucha*), a cooperatively breeding neotropical songbird. *Journal of Ornithology,* DOI 10.1007/s10336-009-0393-3.

Bradley DW & Mennill DJ 2009b. Strong ungraded responses to playback of solos, duets and choruses in a cooperatively breeding neotropical songbird. *Animal Behaviour,* DOI 10.1016/j.anbehav.2009.01.037.

Brown ED, Farabaugh SM & Veltman CJ 1988. Song sharing in a group-living songbird, the Australian magpie, *Gymnorhina tibicen.* Part I. Vocal sharing within and among social groups. *Behaviour,* 104 (1/2), 1–28.

Brown ED & Farabaugh SM 1991. Song sharing in a group-living songbird, the Australian magpie, *Gymnorhina tibicen.* Part III. Sex specificity and individual specificity of vocal parts in communal chorus and duet songs. *Behaviour,* 118(3/4), 244–274.

Catchpole CK & Slater PJB 2008. *Bird song. Biological themes and variations.* Cambridge, 2nd ed. UK: Cambridge University Press.

Curry RL 1988. Group structure, within-group conflict and reproductive tactics in cooperatively breeding Galápagos mockingbirds, *Nesomimus parvulus. Animal Behaviour,* 36, 1708–1728.

Dawson DA, Burke T, Hansson B, Pandhal J, Hale MC, Hinten GN & Slate J 2006. A predicted microsatellite map of the passerine genome based on chicken-passerine sequence similarity. *Molecular Ecology,* 15, 1299–1320.

Dawson DA, Chittock J, Jehle R, Whitlock A, Nogueira D, Pellatt J, Birkhead T & Burke T 2005. Identification of 13 polymorphic microsatellite loci in the zebra finch, *Taeniopygia guttata* (Passeridae, Aves). *Molecular Ecology Notes,* 5, 298–301.

Dawson DA, Horsburgh GJ, Küpper C, Stewart IRK, Ball AD, Durrant KL, Hansson B, Bacon I, Bird S, Klein Á, Krupa AP, Lee JW, Martín-Gálvez D, Simeoni M, Smith G, Spurgin LG & Burke T 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility – as demonstrated for birds. *Molecular Ecology Resources,* 10, 475–494.

DuVal EH 2007. Social organization and variation in cooperative alliances among male lance-tailed manakins. *Animal Behaviour,* 73, 391–401.

Douglas SB & Mennill DJ 2010. A review of acoustic playback techniques for studying avian vocal duets. *Journal of Field Ornithology,* 81(2), 115–129.

Fedy BC & Stutchbury BJM 2005. Territory defence in tropical birds: are females as aggressive as males? *Behavioural Ecology and Sociobiology,* 58, 414–422.

Fortune ES, Rodríguez C, Ball GF & Coleman MJ 2011. Neural mechanisms for the coordination of duet singing in wrens. *Science,* 334, 666–670.

Franklin SB, Gibson DJ, Robertson PA, Pohlmann JT & Fralish JS 1995. Parallel Analysis: A Method for Determining Significant Principal Components. *Journal of Vegetation Science,* 6(1), 99–106.

Gill SA, Vonhof MJ, Stutchbury BJM, Morton ES & Quinn JS 2005. No evidence for acoustic mate-guarding in duetting buff-breasted wrens (*Thryothorus leucotis*). *Behavioural Ecology and Sociobiology,* 57, 557–565.

Griffiths R, Daan S & Dijkstra C 1996. Sex identification in birds using two CHD genes. *Proceedings of the Royal Society of London B*, 263, 1249–1254.

Griffiths R, Double MC, Orr K & Dawson RJG 1998. A DNA test to sex most birds. *Molecular Ecology,* 7, 1071–1075.

Hale AH 2006. The structure, context and functions of group singing in black-breasted wood-quail (*Odontophorus leucolaemus*). *Behaviour,* 143(4), 511–533.

Hall ML 2000. The function of duetting in magpie-larks: conflict, cooperation, or commitment? *Animal Behaviour,* 60, 667–677.

Hall ML 2004. A review of hypotheses for the functions of avian duetting. *Behavioural Ecology and Sociobiology,* 55, 415–430.

Kitchen DM 2004. Alpha male black howler monkey responses to loud calls: effect of numeric odds, male companion behaviour and reproductive investment. *Animal Behaviour,* 67, 125–139.

Kroodsma DE, Ingalls VA, Sherry TW & Werner TK 1987. Songs of the cocos flycatcher: vocal behavior of a suboscine on an isolated oceanic island. *The Condor,* 89(1), 75–84.

Langmore NE 1998. Functions of duet and solo songs of female birds. *Trends in Ecology and Evolution.* 13(4), 136–140.

Langmore NE 2002. Vocal duetting: definitions, discoveries and directions. *Trends in Ecology and Evolution,* 17, 451–452.

Levin RN 1996a. Song behaviour and reproductive strategies in a duetting wren, *Thryothorus nigricapillus:* I. Removal experiments. *Animal Behaviour,* 52, 1093–1106.

Levin RN 1996b. Song behaviour and reproductive strategies in a duetting wren, *Thryothorus nigricapillus:* II. Playback experiments. *Animal Behaviour,* 52, 1107–1117.

Logue DM & Gammon DE 2004. Duet song and sex roles during territory defence in a tropical bird, the black-bellied wren, *Thryothorus fasciatoventris*. *Animal Behaviour,* 68, 721–731.

Logue DM 2005. Cooperative defence in duet singing birds. *Cognitie, Creier, Comportament / Cognition, Brain, Behaviour,* 9(3), 497–510.

Mann NI, Marshall-Ball L & Slater PJB 2003. The complex song duet of the plain wren. *The Condor,* 105, 672–682.

Mann NI, Dingess KA & Slater PJB 2006. Antiphonal four-part synchronized chorusing in a neotropical wren. *Biology Letters*, 2, 1–4.

Mann NI, Dingess KA, Barker FK, Graves JA & Slater PJB 2008. A comparative study of song form and duetting in neotropical *Thryothorus* wrens. *Behaviour,* 146, 1–43.

Marshall-Ball L, Mann NI & Slater PJB 2006. Multiple functions to duet singing: hidden conflicts and apparent cooperation. *Animal Behaviour,* 71(4), 823–831.

McComb K, Packer C & Pusey A 1994. Roaring and numerical assessment in contests between groups of female lions, *Panthera leo. Animal Behaviour,* 47, 379–387.

McGregor PK 2000. Playback experiments: design and analysis. *Acta Ethologica,* 3, 3–8.

Mennill DJ & Rogers AC 2006. Whip it good! Geographic consistency in male songs and variability in female songs of the duetting eastern whipbird *Psophodes olivaceus. Journal of Avian Biology,* 37, 93–100.

Mennill DJ & Vehrencamp SL 2008. Context-dependent functions of avian duets revealed by microphone-array recordings and multispeaker playback. *Current Biology,* 18, 1314–1319.

Molles LE & Waas JR 2006. Are two heads better than one? Responses of the duetting kokako to one- and two-speaker playback. *Animal Behaviour,* 72(1), 131–138.

Nottebohm F 1972. The origins of vocal learning. *The American Naturalist,* 106(947), 116–140.

O'Connor BP 2000. SPSS and SAS programs for determining the number of components using parallel analysis and Velicer's MAP test. *Behaviour Research Methods, Instruments, & Computers,* 32(3), 396–402.

Radford AN 2003. Territorial vocal rallying in the green woodhoopoe: influence of rival group size and composition. *Animal Behaviour,* 66, 1035–1044.

Reyer HU & Schmidl D 1988. Helpers have little to laugh about: group structure and vocalization in the laughing kookaburra *Dacelo novaeguineae. Emu,* 88(3), 150–160.

Rogers AC 2005. Male and female song structure and singing behaviour in the duetting Eastern whipbird, *Psophodes olivaceus. Australian Journal of Zoology,* 53(3), 157–166.

Rogers AC, Ferguson JE, Harrington HM, McDowell S, Miller A & Panagos J 2004. Use of stereo duet playback to investigate traditional duet playback methods and mechanisms of cooperative territorial defence in magpie-larks. *Behaviour,* 141(6), 741–753.

Rogers AC, Langmore NE & Mulder R 2007. Function of pair duets in the eastern whipbird: cooperative defense or sexual conflict? *Behavioural Ecology,* 18(1), 182–188.

Seddon N 2002. The structure, context and possible functions of solos, duets and choruses in the subdesert mesite (*Monia benschi*). *Behaviour,* 139, 645–676.

Seddon N, Butchart SHM & Odling-Smee L 2002. Duetting in the subdesert mesite *Monia benschi*: evidence for acoustic mate defence? *Behavioural Ecology and Sociobiology,* 52, 7–16.

Seddon N & Tobias JA 2003. Communal singing in the cooperatively breeding subdesert mesite *Monia benschi*: evidence of numerical assessment? *Journal of Avian Biology,* 34, 72–80.

Slater PJB & Mann NI 2004. Why do females of many bird species sing in the tropics? *Journal of Avian Biology,* 35, 289–294.

Sonnenschein E & Reyer HU 1983. Mate-guarding and other functions of antiphonal duets in the slate-coloured boubou (*Laniarius funebris*). *Zeitschrift für Tierpsychologie,* 63, 112–140.

SPSS 2011. SPSS Version 20.0 [Computer Program]. Chicago, Illinois, SPSS Inc.

Stern MJ 1995. An inter-Andean forest relict: vegetation change on Pasochoa volcano, Ecuador. *Mountain Research and Development,* 15, 339–348.

Templeton CN, Rivera-Cáceres KD, Mann NI, Slater PJB 2011. Song duets function primarily as cooperative displays in pairs of happy wrens. *Animal Behaviour,* 82, 1399–1407.

Thorpe WH 1972. Duetting and antiphonal song in birds: Its extent and significance. *Brill Archive, 18, Google Books.* Web. February 22, 2013, from http://books.google.at/

Topp SM & Mennill DJ 2008. Seasonal variation in the duetting behaviour of rufous-and-white wrens (*Thryothorus rufalbus*). *Behavioural Ecology and Sociobiology,* 62(7), 1107–1117.

Wiley RH & Wiley MS 1976. Recognition of neighbors' duets by stripe-backed wrens *Campylorhynchus nuchalis. Behaviour,* 62(1/2), 10–34.

Wilson ML, Hauser MD & Wrangham RW 2001. Does participation in intergroup conflict depend on numerical assessment, range location, or rank for wild chimpanzees? *Animal Behaviour,* 61, 1203–1216.

Wingfield JC & Lewis DM 1993. Hormonal and behavioural responses to simulated territorial intrusion in the cooperatively breeding white-crowned sparrow-weaver *Plocepasser mahali. Animal Behaviour,* 45, 1–11.

6. Appendices

Appendix I: Ammonium Acetate (NH_4Ac) DNA extraction protocol

Appendix II: Molecular Sexing: P2/P8 technique

Appendix III: Microsatellite analysis

Appendix IV: 'Intensity of response': response variables

Appendix V: Song initiation and termination

Appendix VI: Proportion of solo, duet and chorus songs

Appendix I: DNA Extraction

Ammonium Acetate (NH_4Ac) DNA extraction protocol (Recipes for Digsol buffer and Low TE on www.parisveltsos.com/research, February 22, 2012)

1. Place in a 1.5 ml eppendorf tube 125 µl Digsol buffer and 5 µl Proteinase K (20 mg/ml).
2. Cut a small piece of FTA card, transfer to the eppendorf and mix.
3. Add 125 µl Digsol buffer.
4. Wrap the eppendorf rack in tissue paper and elastic bands and place in rotating oven at 55° C (3 hours) or 40° C (overnight).
5. Add 3 µl RNase A (10 µg/µl) and incubate at 37° C for 45 minutes.
6. Add 260 µl 4M Ammonium Acetate to each sample.
7. Vortex several times over a period of at least 15 minutes at room temperature to precipitate the proteins.
8. Centrifuge for 10 minutes at 13.000 rpm.
9. Aspirate 500 µl supernatant into clean, labelled 1.5 ml eppendorfs.
10. Add 1 ml 100% ethanol and mix by inverting (about 20 times). The DNA should precipitate out of solution, but may not be visible.
11. Leave samples in freezer at -80° C for 10 minutes.
12. Centrifuge for 10 minutes at 13.000 rpm.
13. Pour off ethanol in a smooth movement so as not to lose DNA pellet.
14. Add 500 µl 70% ethanol and invert several times to rinse pellet.
15. Centrifuge for 5 minutes at 13.000 rpm.
16. Pour off ethanol in a smooth movement and stand tubes upside down on clean tissue until they are completely dry (1 hour or overnight).
17. When dry, add Low TE (amount dependent on pellet size) (20 µl).
18. Place tubes in incubator for 30 minutes (50° C) to dissolve pellet (flicking every 10 minutes) or leave overnight at room temperature. Or leave for longer periods at 4° C flicking occasionally (slightly viscous solution).
19. Store at -20° C (long term).

Appendix II: Molecular Sexing

Protocol for molecular sexing using P2/P8 method (Griffiths et al. 1996 & 1998)

1. Primer sequence information:

Forward primer P2 = 5'-TCT GCA TCG CTA AAT CCT TT-3'
Reverse primer P8 = 5'-CTC CCA AGG ATG AGR AAY TG-3'

2. PCR reaction mixture:

The P2/P8 test was carried out in a 10 µl reaction containing the following chemical constituents: 7.1 µl H_2O, 1.0 µl NH_4 buffer, 0.1 µl of P2 and P8 primers (1µM), 0.4 µl $MgCl_2$ (2.0 mM), 0.2 µl of each dNTP (0.2 mM), 0.1 µl *Taq* polymerase and 1 µl DNA solution (about 20 ng/µl) per sample.

3. Reaction profile used with P2/P8 primers:

DNA was amplified in a thermal cycler (PTC-100 Programmable Thermal Controller, MJ Research. Inc.) using the following program: initial denaturation step at 95° C for 4 minutes, followed by 35 cycles of 95° C for 20 seconds, 53° C for 30 seconds, and 72° C for 45 seconds, followed by a final extension cycle of 5 minutes at 72° C. After completion the PCR products were kept at 4° C.

4. Gelelectrophoresis

PCR products were visualized on a 2.5 % agarose gel (300 ml), which was run at 120 volts. This method resulted in one PCR product of around 350 bp corresponding to the amplified fragment of the CHD1-Z gene in both sexes, and an additional female specific PCR product of around 380 bp, which corresponds to the longer fragment of CHD1-W.

Appendix III: Microsatellite Analysis

Introduction

Group living is a fundamental aspect of the complex chorus of the plain-tailed wren (*Pheugopedius euophrys*). It could be that juveniles remained in their natal territory throughout the following breeding season and perhaps beyond. Delayed or non-dispersal is associated with helping behaviour in many tropical bird species. In such a cooperative breeding system young birds (non-breeding) contribute to territorial defence and brood care (of their own siblings) (e.g. Cockburn 1998 & 2003, Komdeur 1994).
There have been no studies so far, whether groups consist of a primary breeding pair and their offspring from previous breeding attempts or of unrelated individuals.
Microsatellites have proven to be powerful tools for kinship analysis. In this study we have chosen to test a large set of avian microsatellite primers which are known to be of high cross-species utility (Dawson et al. 2010, 2006, 2005).
Understanding the relatedness patterns within a group is important for testing questions about the species' complex vocal behaviour. If we assume that plain-tailed wrens do indeed reproduce cooperatively, the chorus song could have a synchronizing effect on reproductive efforts by members of a group. The patterns of participation in the chorus might help to coordinate the timing of breeding (Slater & Mann 2004).

Materials and Methods

All molecular analyses were done at the Molecular Ecology Lab of the University of St Andrews, UK. 54 microsatellite loci that appeared suitable for analysis, because of a high polymorphism rate across other species, were tested for variability on a panel of 12 different plain tailed wren individuals of the same population. I used microsatellite loci that were isolated previously from the zebra finch (*Taeniopygia guttata;* Dawson et al. 2010), the house sparrow (*Passer domesticus;* Griffith et al. 1999), the Seychelles warbler (Richardson et al. 2000), the Scottish crossbill (*Loxia scotica;* Piertney et al. 1998), the white-bearded manakin (*Manacus manacus;* Piertney et al. 2002), the banded wren (*Thryothorus pleurostictus*; Brar et al. 2007), the song wren (*Cyphorhinus phaeocephalus*, Hughes & Robinson 2001) and the house wren (*Troglodytes aedon*, Cabe & Marshall 2001) (see Table 1). For each locus, optimal priming conditions were determined by attempting amplification at different annealing temperatures (T_a) and $MgCl_2$ concentrations. Reactions (10µl) contained 10–20 ng of template DNA, 1.0 µl NH_4 buffer, 1.0 µl primer mix (forward and reverse primer, 0.25 µM each), $MgCl_2$ spe-

cific to each locus (Table 1), 0.2 mM of each dNTP, 0.5 unit *Taq* polymerase per sample. Polymerase chain reactions were performed in eight-strip 0.2 ml tubes using a thermal cycler (PTC-100 Programmable Thermal Controller, MJ Research, Inc. or G-STORM 3.3.0.0, Gene Technologies). I used three different PCR profiles, depending on the microsatellite primer (see Table 1). The cycling profile 'A' started with 3 minutes at 94° C, followed by 35 cycles of 30 seconds at 94° C, 30 seconds at the locus-specific annealing temperature (T_a), and 30 seconds at 72° C, followed by a final extension at 72° C for 10 minutes. The cycling profile 'B' was 1 cycle at 95° C for 4 minutes, 40 cycles of 20 seconds at 95° C, 45 seconds at T_a, and 45 seconds at 72° C, followed by a final extension cycle of 5 minutes at 72° C. The third cycling profile, labelled as 'C' (Table 1) started with 95° C for 3 minutes, 35 cycles of 1 minute at 95° C, 1 minute at T_a, and 1 minute at 72° C, followed by a final extension at 72° C for 5 minutes.

PCR products were separated by electrophoresis (60 volts) in either 2.5 % agarose gels (stained with ethidium bromide) or high-resolution 3.5 % MetaPhor® agarose (FMC Bioproducts, USA) gels, which were post-stained with ethidium bromide, and visualized under UV light. Four loci, that have shown low allelic variation in some individuals, were chosen again for test run on a few individuals using fluorescently labelled forward primers WELLRED™ Oligos Sigma-Aldrich® (see Table 1). Genotypes of 7 plain-tailed wren individuals were scored using Beckman Coulter CEQ™ capillary sequencer with a 400-bp size standard and deionized formamide. CEQ 8000 Genetic Analysis System software was used to analyse sequencing results.

Results

All except five primer pairs amplified robustly in the plain-tailed wren. There was no sufficient allelic variation present at any of the tested microsatellite loci that would have made paternity and kinship analysis possible.

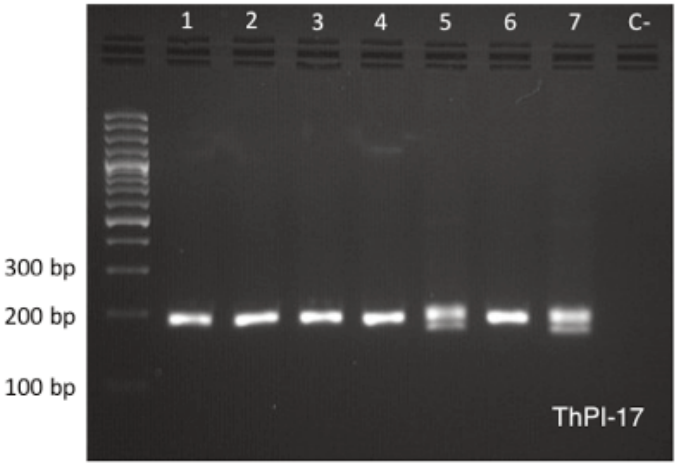

Figure 1. Example of PCR products amplified from plain-tailed wren DNA using microsatellite primer ThPI-17. Lanes 1 to 7 corresponds to products of 7 different individuals. Bands 5 and 7 show possible signs of polymorphism. The left lane shows the 100 bp ladder (GeneRuler™).

Table 1. Details of all microsatellite primer sets that were tested on individuals of plain-tailed wrens. See references for characteristics of each locus.

Locus	T_a (°C)	$MgCl_2$ (mM)	n	PCR profiles		References
TG01-000	50–60	2.0	7	A	na	Dawson et al. 2010
TG01-040	59.0	2.0	20	A		Dawson et al. 2010
TG01-077	51.9	2.0	7	A		Dawson et al. 2010
TG01-092	53.0	2.0	7	A		Dawson et al. 2010
TG01-114	50.9	2.0	7	A		Dawson et al. 2010
TG01-124	51.9	2.0	7	A		Dawson et al. 2010
TG01-147	53.0	2.0	9	A		Dawson et al. 2010
TG01-148	50–60	2.0	8	B	na	Dawson et al. 2010
TG01-058	51.9	2.0	7	A		Dawson et al. 2010
TG02-078	50.9	2.0	10	A		Dawson et al. 2010
TG02-088	56.9	2.0	8	B		Dawson et al. 2010
TG02-120	51.9	2.0	7	A		Dawson et al. 2010
TG03-002	51.9	2.0	7	A		Dawson et al. 2010
TG03-031	55.6	2.0	7	A		Dawson et al. 2010
TG03-034	53.0	2.0	7	A		Dawson et al. 2010
TG03-035	53.0	2.0	7	A		Dawson et al. 2010
TG03-098	50–60	2.0	8	B	na	Dawson et al. 2010
TG04-004	51.9	2.0	7	A		Dawson et al. 2010
TG04-012	59.0	2.0	8	A		Dawson et al. 2010
TG04-012A	58.4	2.0	10	A		Dawson et al. 2010
TG04-041	55.6	2.0	8	A		Dawson et al. 2010
TG04-061	58.4	2.0	8	A		Dawson et al. 2010
TG05-030	56.9	2.0	9	A		Dawson et al. 2010
TG05-046	55.6	2.0	7	A		Dawson et al. 2010
TG05-053	53.0	2.0	7	A		Dawson et al. 2010
TG06-009	51.9	2.0	8	A		Dawson et al. 2010
TG07-022	59.0	1.5	11	A	f	Dawson et al. 2010
TG08-024	53.0	2.0	7	A		Dawson et al. 2010
TG09-014	56.9	2.0	10	A		Dawson et al. 2010
TG11-011	53.0	2.0	7	A		Dawson et al. 2010
TG12-015	59.0	1.5	11	B	f	Dawson et al. 2010
TG13-009	58.4	2.0	7	A		Dawson et al. 2010
TG13-016	50–60	2.0	7	A	na	Dawson et al. 2010
TG13-017	55.6	2.0	7	A		Dawson et al. 2010

Locus	T_a (°C)	$MgCl_2$ (mM)	n	PCR profiles		References
TG22-001	53.0	2.0	9	A		Dawson et al. 2010
TGZ-040	54.3	2.0	7	A		Dawson et al. 2010
TGZ-037	53.0	2.0	9	A		Dawson et al. 2010
Pdoμ5	59.0	2.0	8	A		Griffith et al. 1999
Ase18	60.0	2.0	12	A		Richardson et al. 2000
Lox1	55.0	2.0	12	A		Piertney et al. 1998
Man13	62.0	1.0	12	A	na	Piertney et al. 2002
ThPl-01	62.0	1.5	8	A		Brar et al. 2007
ThPl-14	60.0	2.0	9	A		Brar et al. 2007
ThPl-17	60.0	1.0	9	A	f	Brar et al. 2007
ThPl-27	59.0	1.5	8	A		Brar et al. 2007
ThPl-30	60.0	3.0	8	A		Brar et al. 2007
CpAAT-18	59.0	1.0	9	C	f	Hughes & Robinson 2001
CpAAT-34	60.0	1.5	4	C		Hughes & Robinson 2001
CpAAT-39	60.0	2.0	4	C		Hughes & Robinson 2001
TA-B4-2	57.0	2.0	8	A		Cabe & Marshall 2001
TA-A5-2	57.0	2.0	8	A		Cabe & Marshall 2001
TA-A5-15	55.0	1.5	8	A		Cabe & Marshall 2001
TA-C3(B)-2	57.0	1.5	8	A		Cabe & Marshall 2001
TA-C6-7	57.0	1.5	8	A		Cabe & Marshall 2001

T_a = annealing temperature, n = number of DNA samples, na = no amplification, f = fluorescent marker

Discussion

Due to the fact that just two of all the tested microsatellite primer pairs were polymorphic in some individuals, I could not genotype families. This unusually low microsatellite allele variation has also been discussed for the banded wren (*Thryothorus pleurostictus*: Brar et al. 2007). Brar et al. suspect that the low allelic diversity is a consequence from past demographic bottlenecks (glaciation events), despite the relative abundance of banded wrens at present time. Plain-tailed wrens also seem to have particularly low levels of genetic variation. They are habitat specialists, like e.g. banded wrens or rufous-and-white wrens, and live in *Chusquea* bamboo forests in the Andes. The habitat of our study population extends over the steep slopes and the valley of the collapsed crater of the extinct Pasochoa Volcano. Pasochoa has high conservation value as it protects this patch of relict cloud forest that continues to be threatened by human disturbance. Today it is surrounded by agricultural fields and dairy farms (Stern 1995). As a consequence, this population fragmentation and probably isolation could have led to barriers in dispersal, a reduction in gene flow and therefore a greater chance for genetic drift (e.g. greater prairie-chicken: Johnson et al. 2003). This could be an explanation for the paucity of polymorphic microsatellite loci in our study population of plain-tailed wrens.

As a new approach, potentially useful microsatellites and single nucleotide polymorphisms (SNPs) will be identified by genome sequencing (454 flex+ technology). This part of the study will be carried out in collaboration with Dr ED Jarvis and J Howard from Duke University.

References

Brar RK, Schoenle LA, Stenzler LM, Hall ML, Vehrencamp SL & Lovette IJ 2007. Eleven microsatellite loci isolated from the banded wren (*Thryothorus pleurostictus*). *Molecular Ecology Notes,* 7, 69–71.

Cabe PR & Marshall KE 2001. Microsatellite loci from the house wren (*Troglodytes aedon*). *Molecular Ecology Notes,* 1(3), 155–156.

Cockburn A 1998. Evolution of helping behaviour in cooperatively breeding birds. *Annual Review of Ecology and Systematics,* 29, 141–177.

Cockburn A 2003. Cooperative breeding in oscine passerines: does sociality inhibit speciation? *Proceedings of the Royal Society of London B,* 270, 2207–2214.

Dawson DA, Burke T, Hansson B, Pandhal J, Hale MC, Hinten GN & Slate J 2006. A predicted microsatellite map of the passerine genome based on chicken-passerine sequence similarity. *Molecular Ecology,* 15, 1299–1320.

Dawson DA, Chittock J, Jehle R, Whitlock A, Nogueira D, Pellatt J, Birkhead T & Burke T 2005. Identification of 13 polymorphic microsatellite loci in the zebra finch, *Taeniopygia guttata* (Passeridae, Aves). *Molecular Ecology Notes,* 5, 298–301.

Dawson DA, Horsburgh GJ, Küpper C, Stewart IRK, Ball AD, Durrant KL, Hansson B, Bacon I, Bird S, Klein Á, Krupa AP, Lee JW, Martín-Gálvez D, Simeoni M, Smith G, Spurgin LG & Burke T 2010. New methods to identify conserved microsatellite loci and develop primer sets of high cross-species utility – as demonstrated for birds. *Molecular Ecology Resources,* 10, 475–494.

Griffith SC, Stewart IRK, Dawson DA, Owens IPF & Burke T 1999. Contrasting levels of extra-pair paternity in mainland and island populations of the house sparrow (*Passer domesticus*): is there an 'island effect'? *Biological Journal of the Linnean Society,* 68, 303–316.

Hughes CR & Robinson TR 2001. Characterization of microsatellite loci developed for song wrens *Cyphorhinus phaeocephalus. Molecular Ecology Notes,* 1, 165–167.

Komdeur J 1994. The effect of kinship on helping in the cooperative breeding Seychelles warbler (*Acrocephalus sechellensis*). *Proceedings of the Royal Society of London B,* 256, 47–52.

Johnson JA, Toepfer JE & Dunn PO 2003. Contrasting patterns of mitochondrial and microsatellite population structure in fragmented populations of greater prairie-chickens. *Molecular Ecology,* 12, 3335–3347.

Piertney SB, Marquiss M & Summers R 1998. Characterization of tetranucleotide microsatellite markers in the Scottish crossbill (*Loxia scotica*). *Molecular Ecology,* 7(9), 1261–1263.

Piertney SB, Shorey L & Höglund J 2002. Characterization of microsatellite DNA markers in the white-bearded manakin (*Manacus manacus*). *Molecular Ecology Notes,* 2, 504–505.

Richardson DS, Jury FL, Dawson DA, Salgueiro P, Komdeur J & Burke T 2000. Fifty Seychelles warbler (*Acrocephalus sechellensis*) microsatellite loci polymorphic in Sylviidae species and their cross-species amplification in other passerine birds. *Molecular Ecology,* 9(12), 2226–2231.

Slater PJB & Mann NI 2004. Why do females of many bird species sing in the tropics? *Journal of Avian Biology,* 35, 289–294.

Stern MJ 1995. An inter-Andean forest relict: vegetation change on Pasochoa volcano, Ecuador. *Mountain Research and Development,* 15, 339–348.

Appendix IV: ‘Intensity of response’

The following 7 response variables were used for the principal component analysis (PCA):

				n songtypes			total duration of vocal response (s)				number of changes in songtypes					
		odds ratio		duet	chorus	duet			chorus		duet			chorus		
subject group	sex ratio	duet	chorus	all	all	pb1	pb2	all	pb1	pb2	all	pb1	pb2	all	pb1	pb2	all
G1	1,00	1	0,5	5	4	78,38	127,39	205,76	61,59	122,19	183,77	3	3	7	1	2	3
M1	1,00	1	0,5	4	3	60,74	69,21	129,94	47,38	96,77	144,16	2	1	3	1	1	2
Y3	2,00	1,5	0,75	2	2	70,72	39,59	110,30	90,04	146,43	236,46	1	0	1	1	0	1
S1	0,33	2	1	12	7	100,71	129,89	230,60	117,21	142,57	259,79	5	9	15	4	8	13
6	0,33	2	1	4	6	1,28	127,87	129,14	79,32	42,30	121,62	0	3	4	3	1	5
S2	1,00	2	1	2	5	20,32	14,13	34,45	149,50	143,20	292,70	1	0	1	4	5	10
S5	1,00	2	1	3	2	109,85	137,96	247,81	21,13	179,78	200,90	1	2	4	0	0	1
5	1,00	2	1	9	14	100,69	132,35	233,04	51,80	245,56	297,36	7	4	12	17	25	43
M2	0,33	2	1	17	3	31,63	158,24	189,86	2,44	39,24	41,68	2	22	25	0	3	3
S4	1,00	2	1	4	6	57,47	107,28	164,74	122,00	169,80	291,80	4	1	6	8	2	10
16A	1,50	2,5	1,25	2	8	50,69	26,75	77,44	133,10	127,76	260,86	1	1	3	2	7	9
1A	0,67	2,5	1,25	7	10	38,12	164,41	202,53	77,06	307,77	384,83	4	11	16	2	9	11
B2	1,50	2,5	1,25	3	4	56,15	113,12	169,26	104,67	42,33	147,01	0	2	2	2	6	9
9	1,33	3,5	1,75	4	8	100,67	210,21	310,88	0,00	237,40	237,40	2	1	3	-	8	8

				max pitch of B (Hz)		proportion of n birds 3m			min distance to one of the speakers (m)						duration of longest song (s)					
		odds ratio		duet	chorus	duet	chorus		duet			chorus			duet			chorus		
subject group	sex ratio	duet	chorus	all	all	all	all		pb1	pb2	all	pb1	pb2	all	pb1	pb2	all	pb1	pb2	all
G1	1,00	1	0,5	6221,54	6035,89	1	1		12	3	3	6	3	3	78,38	66,89	78,38	58,07	71,99	71,99
M1	1,00	1	0,5	6221,17	6178,19	0	0		12	12	12	12	12	12	60,74	69,21	69,21	47,38	51,32	51,32
Y3	2,00	1,5	0,75	6136,33	5424,33	0	0		12	12	12	12	12	12	38,59	24,66	38,59	29,57	57,76	57,76
S1	0,33	2	1	6784,00	6545,56	0,75	0,75		3	1	1	3	3	3	58,76	33,82	58,76	57,85	98,04	98,04
6	0,33	2	1	6132,33	5776,83	0,5	0,5		12	0	0	3	1	1	1,28	92,60	92,60	48,62	22,64	48,62
S2	1,00	2	1	5721,67	5561,67	0	1		12	12	12	3	1	1	6,79	12,46	12,46	34,29	30,75	34,29
S5	1,00	2	1	5331,38	5475,59	0,5	0,5		3	3	3	12	3	3	57,84	107,76	107,76	23,52	98,82	98,82
5	1,00	2	1	6280,27	6280,27	0	0,5		12	12	12	12	3	3	72,48	36,30	72,48	30,40	112,50	112,50
M2	0,33	2	1	7622,83	5770,27	0,25	0,5		3	12	3	12	1	1	8,50	26,98	26,98	2,44	16,19	16,19
S4	1,00	2	1	6816,00	6860,17	0,75	1		3	3	3	1	1	1	29,15	24,66	29,15	101,09	48,85	101,09
16A	1,50	2,5	1,25	5153,85	6220,13	0,4	1		3	3	3	3	3	3	31,18	14,55	31,18	27,71	27,77	27,77
1A	0,67	2,5	1,25	7171,88	7576,82	0,60	0,8		1	1	1	3	3	3	23,51	67,49	67,49	42,00	101,24	101,24
B2	1,50	2,5	1,25	6095,00	5908,33	0,6	0,2		3	3	3	3	3	3	11,44	76,93	76,93	28,87	25,07	28,87
9	1,33	3,5	1,75	6594,31	6401,77	0,29	0,43		12	3	3	12	3	3	22,79	96,41	96,41	0,00	109,42	109,42

Appendix V: Song initiation and termination

Proportions of males' and females' song initiations during playback response of each focal group, depending on their group composition:

initial even

	no. of songs	A	B	C	D	total	male	female
5 chorus	5	0,00	0,20	0,40	0,40	1,00	0,40	0,60
5 duet	6	0,33	0,17	0,00	0,50	1,00	0,33	0,67
G1 chorus	5	0,60	0,20	0,20	0,00	1,00	0,80	0,20
G1 duet	3	0,67	0,00	0,00	0,33	1,00	0,67	0,33
M1 chorus	3	0,00	0,33	0,33	0,33	1,00	0,33	0,67
M1 duet	2	0,00	0,00	0,50	0,50	1,00	0,50	0,50
S2 chorus	15	0,07	0,20	0,47	0,27	1,00	0,53	0,47
S2 duet	6	0,00	0,33	0,00	0,67	1,00	0,00	1,00
S4 chorus	11	0,05	0,45	0,18	0,32	1,00	0,23	0,77
S4 duet	6	0,25	0,42	0,00	0,33	1,00	0,25	0,75
S5 chorus	3	0,33	0,33	0,33	0,00	1,00	0,67	0,33
S5 duet	9	0,22	0,00	0,33	0,44	1,00	0,56	0,44

initial male

	no. of songs	A	B	C	D	total	male	female
9 chorus	6	0,00	0,17	0,67	0,17	1,00	0,67	0,33
9 duet	16	0,19	0,06	0,63	0,13	1,00	0,81	0,19
16A chorus	21	0,76	0,00	0,00	0,24	1,00	0,76	0,24
16A duet	6	0,50	0,17	0,00	0,33	1,00	0,50	0,50
B2 chorus	7	0,14	0,29	0,57	0,00	1,00	0,71	0,29
B2 duet	6	0,00	0,33	0,67	0,00	1,00	0,67	0,33
Y3 chorus	8	0,50	0,00	0,00	0,50	1,00	0,50	0,50
Y3 duet	5	0,20	0,00	0,60	0,20	1,00	0,80	0,20

initial female

	no. of songs	A	B	C	D	total	male	female
1A chorus	13	0,00	0,54	0,00	0,46	1,00	0,00	1,00
1A duet	10	0,00	0,60	0,00	0,40	1,00	0,00	1,00
6 chorus	6	0,17	0,17	0,00	0,67	1,00	0,17	0,83
6 duet	4	0,00	0,00	0,00	1,00	1,00	0,00	1,00
M2 chorus	6	0,00	0,17	0,50	0,33	1,00	0,50	0,50
M2 duet	26	0,00	0,08	0,23	0,69	1,00	0,23	0,77
S1 chorus	10	0,00	0,20	0,20	0,60	1,00	0,20	0,80
S1 duet	7	0,00	0,43	0,00	0,57	1,00	0,00	1,00

termination even

	no. of songs	A	B	C	D	total	male	female
5 chorus	5	0,00	0,20	0,40	0,40	1,00	0,40	0,60
5 duet	7	0,00	0,14	0,29	0,57	1,00	0,29	0,71
G1 chorus	5	0,00	0,00	0,00	1,00	1,00	0,00	1,00
G1 duet	3	0,33	0,00	0,00	0,67	1,00	0,33	0,67
M1 chorus	3	0,33	0,33	0,00	0,33	1,00	0,33	0,67
M1 duet	2	0,00	0,50	0,00	0,50	1,00	0,00	1,00
S2 chorus	8	0,25	0,25	0,00	0,50	1,00	0,25	0,75
S2 duet	6	0,00	0,67	0,00	0,33	1,00	0,00	1,00
S4 chorus	11	0,09	0,36	0,18	0,36	1,00	0,27	0,73
S4 duet	6	0,17	0,00	0,33	0,50	1,00	0,50	0,50
S5 chorus	3	0,00	0,00	1,00	0,00	1,00	1,00	0,00
S5 duet	9	0,11	0,11	0,33	0,44	1,00	0,44	0,56

termination male

	no. of songs	A	B	C	D	total	male	female
9 chorus	6	0,00	0,00	0,33	0,67	1,00	0,33	0,67
9 duet	14	0,50	0,00	0,21	0,29	1,00	0,71	0,29
16A chorus	17	0,00	0,24	0,35	0,41	1,00	0,35	0,65
16A duet	6	0,00	0,17	0,00	0,83	1,00	0,00	1,00
B2 chorus	7	0,00	0,71	0,00	0,29	1,00	0,00	1,00
B2 duet	6	0,17	0,00	0,00	0,83	1,00	0,17	0,83
Y3 chorus	8	0,13	0,00	0,00	0,88	1,00	0,13	0,88
Y3 duet	5	0,00	0,00	0,40	0,60	1,00	0,40	0,60

termination female

	no. of songs	A	B	C	D	total	male	female
1A chorus	13	0,00	0,38	0,23	0,38	1,00	0,23	0,77
1A duet	10	0,00	0,00	0,00	1,00	1,00	0,00	1,00
6 chorus	6	0,00	0,17	0,33	0,50	1,00	0,33	0,67
6 duet	4	0,00	0,75	0,00	0,25	1,00	0,00	1,00
M2 chorus	5	0,20	0,40	0,00	0,40	1,00	0,20	0,80
M2 duet	23	0,17	0,35	0,17	0,30	1,00	0,35	0,65
S1 chorus	10	0,20	0,10	0,10	0,60	1,00	0,30	0,70
S1 duet	7	0,43	0,29	0,00	0,29	1,00	0,43	0,57

Appendix VI: Proportion of solo, duet and chorus songs

Proportion of each type of song (solo, duet, chorus) as response to duet and chorus playback by all focal groups

focal groups	n songs		n cycles		solo songs		duet songs		chorus songs		duet/chorus songs	
	duet	chorus	duet	chorus	duet	chorus	duet	chorus	duet	chorus	duet	chorus
G1	3	5	140	126	0	0	1	1 x	x	x	1	1
M1	2	3	92	100	0	0	1	1 x	x	x	1	1
Y3	5	8	68	167	0,01	0,01	0,22	0,50	0,76	0,49	0,99	0,99
S1	7	10	159	173	0,05	0,02	0,16	0,15	0,79	0,83	0,95	0,98
6	4	6	82	79	0,04	0,06	0,34	0,57	0,62	0,37	0,96	0,94
S2	6	15	24	201	0,54	0,07	0,46	0,70	0,00	0,23	0,46	0,93
S5	9	3	171	141	0,00	0,00	0,93	1,00	0,07	0,00	1,00	1,00
5	7	5	157	198	0,00	0,00	0,85	0,88	0,15	0,12	1,00	1,00
M2	26	5	113	29	0,35	0,17	0,49	0,55	0,17	0,28	0,66	0,83
S4	6	11	115	193	0,01	0,05	0,48	0,46	0,51	0,49	0,99	0,95
16A	8	22	59	144	0,07	0,01	0,86	0,90	0,07	0,08	0,93	0,99
1A	10	12	137	258	0,01	0,02	0,31	0,16	0,69	0,82	0,99	0,98
B2	6	7	109	99	0,01	0,01	0,48	0,37	0,51	0,62	0,99	0,99
9	16	6	130	149	0,29	0,00	0,12	0,02	0,58	0,98	0,71	1,00

Acknowledgements

I am very grateful to Dr Nigel I. Mann (SUNY Oneonta) who gave me the opportunity to join him, Prof Dr Peter J. Slater and Dr Chris N. Templeton (University of St Andrews) on a field trip to Ecuador in November 2011 to study the song behaviour of the plain-tailed wren *(Pheugopedius euophrys),* which formed the basis of my diploma-thesis. I would like to thank him for his support and encouragement throughout the whole time.
I am also very grateful to Prof Peter Slater for his support, advice and valuable comments on the write-up. I want to thank him and his wife Elisabeth Slater for their kindness and generous hospitality and for making me feel so welcome in their home during the time in St Andrews.
I would like to thank Dr Chris Templeton for his help and understanding, for his advice and his valuable comments and suggestions on the write-up.
I am also especially grateful to Prof Dr Jeff Graves who gave me the opportunity to work in the Molecular Ecology Lab at the University of St Andrews. I am very thankful for his great support and guidance.
Additionally I would like to thank Dr Lilian Manica, Dr Sonia Pascoal, Dr Paris Veltsos, Tanya Sneddon and Harry Hodge for their help and advices in the lab.
Finally I wish to thank Prof Dr W. Tecumseh Fitch for his support and for being my supervisor. A research scholarship supporting this thesis was kindly granted by the Department of Cognitive Biology and the University of Vienna.

Abstract

In a few group-living bird species, members join together to perform chorus songs, of which the function is not completely understood. These communal songs often play an important role in territory defence and can play a role in assessing relative group size, with birds adjusting their behaviour according to the 'numeric odds' (the number of individuals in their own compared to the number of individuals in the rival group) to avoid costs of fighting when outnumbered.

This study examined the complex song behaviour of a population of group-living plain-tailed wrens (*Pheugopedius euophrys*) that inhabit the dense bamboo forest in Pasochoa, Ecuador. To find out whether chorusing functions as a tool to advertise or assess rival's group size in this species, we tested the 'numeric odds hypothesis' on 16 different groups of plain-tailed wrens. Duet and chorus playback were presented to groups of different numbers of birds ranging from 2 to 7. All study groups responded to playback by duetting or chorusing. The proportions of duet and chorus songs were significantly higher than solo songs, indicating that coordinated songs play an important role in territory defence. The intensity of response did not depend on group size or sex ratio. Further, plain-tailed wrens did not produce graded responses to different types of simulated territory intrusions (duet and chorus playback) as a function of numeric odds. This result stands in contrast to many playback studies that investigated the effect of number of intruders. Therefore we suggest that duet and chorus songs do not function in numerical assessment of rival group size in this species. Instead, they might just reveal, in addition to group-cohesion, the presence of a group in its territory and its motivation to participate in communal defence.

Zusammenfassung

In einigen gruppenlebenden Vogelarten finden sich Mitglieder zu Chorgesängen zusammen, deren Funktion nicht vollständig verstanden wird. Diese Gruppengesänge spielen häufig eine wichtige Rolle in der Revierverteidigung, können jedoch auch beim Ermessen relativer Gruppengröße wichtig sein, in dem Vögel ihr Verhalten nach den 'numeric odds' (Anzahl der Individuen in ihrer eigenen im Vergleich zu der Anzahl der Individuen in der rivalisierenden Gruppe) adjustieren, um Kampfkosten zu vermeiden, wenn in der Unterzahl.

Diese Studie untersucht das komplexe Gesangsverhalten einer Population von in Gruppen lebenden Fraser-Zaunkönigen (Pheugopedius euophrys), die von einem dichten Bambuswald in Pasochoa, Ecuador, beherbergt wird. Um herauszufinden, ob in dieser Vogelart der Chorgesang dazu dient Gruppengröße zu signalisieren bzw. zu ermessen, wird die 'numeric odds' -Hypothese auf 16 verschiedene Gruppen getestet, welche aus 2 bis 7 Individuen bestanden. Alle untersuchten Gruppen antworteten auf Duett- und Chor-Play-back. Der Anteil an Duett- und Chorgesang war signifikant höher als der Anteil an Sologesang. Dies zeigt, dass koordinierte Gesänge eine wichtige Rolle in der Verteidigung des Territoriums spielen. Dabei hängt die Intensität der Reizantwort nicht von der Gruppengröße oder dem Verhältnis von Männchen und Weibchen innerhalb einer Gruppe ab. Weiters zeigen Fraser-Zaunkönige auch keine abgestuften Reaktionen auf verschiedene Arten von simuliertem Eindringen in ein Territorium (Duett- und Chor-Play-back) als Funktion der 'numeric odds'. Dieses Ergebnis steht im Gegensatz zu vielen Play-back-Studien, die die Reaktion der untersuchten Gruppe auf die Anzahl der Eindringlinge untersuchten. Daher schlagen wir vor, dass Duett- und Chorgesang nicht in numerischer Beurteilung der rivalisierenden Gruppengröße dieser Art funktionieren können. Stattdessen könnten sie, zusätzlich zu Gruppenzusammenhalt, die Anwesenheit einer Gruppe in ihrem Territorium und ihre Motivation sich in gemeinsamer Verteidigung zu beteiligen, signalisieren.

Curriculum Vitae

Persönliche Daten

Name	Christine Wu
Geburtsdatum	6. Juli 1987
Geburtsort	Wien
Email	a0501205@univie.ac.at
	christinewu@gmx.net

Bildungsweg

2005–2013	Diplomstudium Biologie Studienzweig: Zoologie
1997–2005	Bundesrealgymnasium BRG 6 Marchettigasse, 1060 Wien
1993–1997	Öffentliche Volkschule VS Corneliusgasse, 1060 Wien

Sprachen

Deutsch
Englisch
Französisch