



Differences in Frequency of Shared Song Types Enables Neighbour-Stranger Discrimination in a Songbird Species with Small Song Repertoire

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Received: January 2, 2014
Initial acceptance: March 12, 2014
Final acceptance: April 27, 2014
(M. Manser)

doi: 10.1111/eth.12260

Keywords: Neighbour-Stranger discrimination, acoustic cues, identity coding, territorial defence

Abstract

Acoustic Neighbour-Stranger (N-S) discrimination is widespread in birds and has evolved to settle territorial disputes with low costs. N-S discrimination was found both in song-learning oscines and non-song-learning bird taxa, irrespective of the repertoire sizes they have. Therefore, it seems that more than just a single mechanism enable N-S discrimination. Species with larger repertoires, where males have unique phrases or syllables may rely on such interindividual differences. The majority of birds have rather small repertoires, which often are shared among neighbours. In this case, males are facing the problem of individual recognition when rivals produce songs, at least superficially, identical. To better understand the acoustic basis of N-S discrimination in species with small and shared repertoires, I studied the ortolan bunting (*Emberiza hortulana*). Males of this small oscine species are able to N-S discrimination based on a single song rendition when presented in a playback experiment, regardless of song-type diversity and song-sharing level within a particular population. It was also found that songs of the same type sung by different males differ in the frequency of the initial song phrases and these differences persist over years. Here, I tested whether males are able to discriminate among the natural songs and the artificially modified songs of their neighbours in which the frequency was experimentally changed by relatively small value in comparison with the variation range found in this population. Subjects responded significantly more aggressively to the songs with an artificially modified frequency, suggesting that males treat such songs as having come from the repertoire of a non-neighbour. These results confirm an earlier prediction that differences in the frequency of shared song types enable N-S discrimination. The study presents one of the possible mechanisms enabling N-S discrimination in songbirds with small repertoires and stress the role of within-song-type variation, which is still understudied song characteristic.

Introduction

Individual recognition is widespread among different animal taxa and has been demonstrated in different behavioural contexts and in different communication modalities (Tibbets & Dale 2007). For example, king penguins (*Aptenodytes patagonicus*) use calls in parent/offspring discrimination (Aubin & Jouventin 1998), and African elephants (*Loxodonta africana*) can

recognize different females and family group members from the cues present in a urine-earth mix (Bates et al. 2008). Individual recognition is not limited to vertebrates. For example, paper wasps (*Polistes fusca-tus*) use individually distinct facial markings to recognize nest mates (Tibbets 2002). In general, individual recognition seems to be common whenever individuals meet repeatedly and when it is economically important to differentiate behaviour directed towards

different known individuals or known and unknown individuals (Sherman et al. 1997).

Neighbour-stranger discrimination is typically used to describe the behaviour of territorial animals, which defend a territory and differentiate their responses towards known neighbours and unknown strangers. Territory owners usually respond more strongly to an intruding stranger than to known neighbours. This is known as the 'dear enemy phenomenon'. The term was coined by Fisher (1954) and explains the reduction of aggression towards neighbours that minimizes energy expenditure and unnecessary conflicts. An obvious prerequisite for the occurrence of the 'dear enemy phenomenon' is the ability to recognize individuals or, at least, to discriminate between neighbours and strangers. Neighbour-stranger (N-S) discrimination has been demonstrated most commonly for songbirds defending their territories (Brooks & Falls 1975a; review in Stoddard 1996), but it has also been described in fish, amphibians, and mammals (e.g. Bee & Gerhardt 2001; Leiser 2003; Palphramand & White 2007). Most studies of N-S discriminations in territorial bird song have been based on the discriminative responses of males to playbacks of the songs of neighbours and strangers. Little is known about the song features that are used in such discrimination. The problem of identifying these song features is complicated because bird song is extremely diverse. There are species showing N-S discrimination that have huge repertoires of hundreds (or more) of different syllables (Briefer et al. 2008). N-S discrimination seems also to be common in species that have repertoires of just a single song type or only a few song types that are shared by neighbours (review in Stoddard 1996). This finding suggests that the acoustic cues responsible for N-S discrimination most likely vary among bird species (Stoddard 1996).

Many acoustic signals are complex and often carry different sets of information at one time. One signal can carry such information as the identity of the species and individual, the singer's fitness and current motivation (Gil & Gahr 2002). Identifying the characteristics of a signal that are responsible for its individually unique character may therefore be difficult and should be confirmed by testing animal responses using experimentally manipulated signals.

The ortolan bunting (*Emberiza hortulana*) is a small passerine species that breeds in Europe in a wide spectrum of habitats, including farmland, peat bogs and clear-cut or burned forest areas. Males usually have a small repertoire of 2–3 song types with a local dialect in which the final part of a song is shared by all or a vast majority of individuals (Cramp & Perrins 1994).

However, some exceptional populations have also been described that lack a local dialect and exhibit larger average male repertoires (Osiejuk et al. 2003).

Males establish territories in Europe after wintering in sub-Saharan Africa and advertise their territories by singing from a limited number of song posts within the defended space. Males often interact with each other with chasing and even physical fights during the first few days after arrival. As the situation becomes more stable and new males cease to arrive, most of the potential conflicts are resolved by signal exchange (Cramp & Perrins 1994; Skierczyński 2009). However, intrusion by a stranger is always met with a quick response by the territory owner, who signals his aggressive motivation by switching from singing to using calls and approaching the rival (Osiejuk et al. 2007a,b; Skierczyński et al. 2007; Skierczyński & Osiejuk 2010).

Ortolan bunting males have been shown to discriminate between neighbours and strangers in populations that differ in average repertoire sizes and levels of song-type sharing with neighbours. These results suggest that the individual acoustic cue that is used for N-S discrimination is encoded in some song characteristics that are not directly (or solely) related to song-type repertoire (Skierczyński et al. 2007; Skierczyński & Osiejuk 2010). Such individual non-repertoire cues seem to be especially necessary in populations in which individual male repertoires are small and song types are strongly shared by neighbours. Small repertoires and shared song types would cause the song type itself to be non-informative in N-S discrimination. For example, in the population in western Poland where this study was conducted, we found an average of only 11 different song types per 100 males recorded, and we found that neighbouring males often shared whole repertoires (Łosak 2007).

How can neighbours recognize each other and detect a stranger if all of them are commonly singing the exact same song types? One of the suggested mechanisms is the existence of subtle differences between renditions of the same song types sung by different males (Draganoiu et al. 2014). Osiejuk et al. (2005) revealed that the song types shared by males in the ortolan bunting population in Norway are not identical. The initial phrases of the same song types of different males, as classified by the visual inspection of spectrograms, showed frequency differences. These differences were difficult for humans to detect under field conditions, but the frequency of the songs of different males differed by as much as 2.5 kHz, and each male had its own specific frequency band that did not change over subsequent years. These results suggest

that the specific frequency of the initial phrase of ortolan bunting song is most likely an individual vocal signature that allows N-S discrimination even when the birds share complete song repertoires.

The main aim of this study was to test experimentally whether subtle differences in shared song types occurring among males enable N-S discrimination. As a model species, I used the ortolan bunting and the song feature on which I have focused was the frequency of initial song phrase (varied among males and stable within a male). I also present some basic data that show the quantitative characteristics of the frequency characteristics of songs in the study population. I did this for two reasons. First, I planned to conduct experiments in a population from western Poland that has a high level of song-type sharing and small repertoires per male. This population differs markedly from the isolated Norwegian population in which the frequency variation had previously been found (Osiejuk et al. 2005). The existence of the frequency differences in the Polish population should be confirmed before further experiments are conducted. Second, knowledge about variation of song frequency was necessary to plan the specific manipulations of the frequency properly.

Methods

Study Area and Subjects

This study was conducted in farmland habitats that are located south of the city of Poznań in Wielkopolska National Park and the surrounding area in western Poland (coordinates of the centre of the study area: 52°17'N and 16°56'E). Ortolan buntings are common in this area and breed preferentially along forest edges and in tree lines surrounded by cultivated fields. The estimated size of the ortolan bunting population in Poland is 150 000 breeding pairs, and in the Wielkopolska region, their distribution is more or less continuous within farmland landscapes (Kuczyński & Chylarecki 2012). Singing males in the study area have been counted and recorded since 1998. More than 50 territorial males were found each year on six well-defined farmland habitat patches that were separated by forest (Łosak 2007; T. S. Osiejuk, pers. obs).

Measurements of natural level of frequency variation

I recorded and analysed song repertoires of 40 males from the study area before I conducted any playback experiments. Each male was recorded for at least 10 min to capture his full repertoire. These males

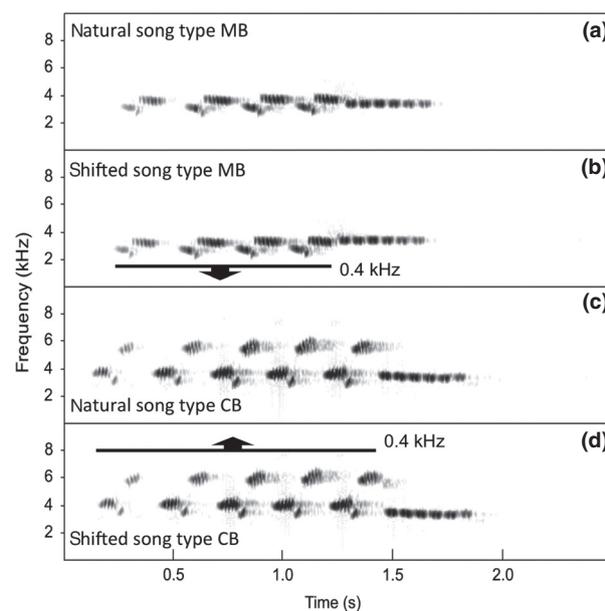


Fig. 1: Spectrograms of exemplary songs of the ortolan bunting used for the playback experiments. Illustrated are two different song types from the repertoire of a single male: MB and CB. Different letters indicate different syllable types. All song types shared the same dialect B final phrase. Presented are natural songs (a, c) and songs with shifted frequency of the initial song phrase (b, d).

included the group of birds with which subsequent playback experiments were conducted and their neighbours, whose songs were later used as experimental stimuli. The males sang a total of 12 different song types (Fig. 1). A song type is defined as song phrases that consist of the same syllable types arranged in the same order (details in Osiejuk et al. 2003). Approximately, 10 good quality songs of each type were measured for each male (on average 21.7 songs per male). They included separate frequency measurements for the initial and the final song phrase (for details of methods see Osiejuk et al. 2005).

Playback Equipment and Song Stimuli

For the playback experiments, I used an Apple iPod Classic 4th generation player (Shenzhen, China) with a wireless Sekaku WA-320 (Taichung, Taiwan) loudspeaker that had a 20 W amplifier (frequency range 50–15 000 Hz and a linear frequency response within the species-specific frequency range, that is, 1.8–6.6 kHz).

The songs used in the experiments were recorded in the field using a Marantz PMD661 (D&M Professional, Kanagawa, Japan) solid state recorder coupled with a Sennheiser MKH70 microphone equipped with an MZW 60-1 basket windshield and an MZH 60-1 long

hairy cover Sennheiser Electronic GmbH & Co.KG, Wademark, Germany). All of the songs used for playback were of good quality, recorded at short distances during windless mornings and free of background noise. The songs selected for playback had a minimum frequency of 2.7 kHz. I first applied a 2 kHz high-pass filter (Avisoft SASLab Pro 5.1.x; Raimund Specht, Berlin, Germany) and then adjusted the amplitude to match 86 dB SPL (A-weighting) at 1 m from the loudspeaker. The SPL value was set according to the amplitude level of typical ortolan bunting song, which had previously been measured in the field with a CHY 650 (Ningbo, China) sound level meter. The amplitude manipulations were small and did not affect the song structure. All of the sounds recorded and used for playback were PCM WAVE files with a 48 kHz sampling rate and 16-bit resolution.

I used 32 different song renditions from 16 different males during the experiment. Each song rendition belonged to one of two types of stimuli: (1) songs from an adjacent neighbour of a focal male (N-treatment) or (2) songs from the same adjacent neighbour that were shifted in frequency (S-treatment). Because most of the males had only two different song types in their repertoires (some had three), for the playback I always selected exactly two of the most frequently sung song types from each male's repertoire. In cases of N-treatment, the songs that were used were only manipulated to remove low-frequency noise and adjust amplitude to the same level, as described above. In cases of S-treatment, the additional manipulation was done, that is, the frequency of one of the two chosen song types was shifted up by 0.40 kHz and that of the second was shifted down by 0.40 kHz. The frequency changes were applied only to the initial song phrase. The final phrase remained at the original level. The value of the shift was only approximately half of the smallest frequency difference found for song types shared among tested birds (compare above). The frequency shift was performed using the Avisoft SASLab Pro 5.1.x function: Frequency Domain Transformation | Frequency Shift.

Playback Experiment Protocol

The experimental protocol was generally based on earlier experience with the study species (e.g. Osiejuk et al. 2007a). It was modified only slightly to address the specific question of this study. The experiments were conducted between 2 and 11 May 2011, between 05:00 and 10:00 local time. Each male was subjected to two identical treatments, except different sounds were played back in one treatment, that is,

unaltered songs of a neighbour (N-treatment) or shifted neighbour songs (S-treatment). The experimental period for each male was chosen on the basis of behavioural observations to correspond to the time when unpaired males defended their territories intensively and after the territorial borders with neighbours had been well established for at least a few days. All subject males and their neighbours had been recorded and their territories mapped (especially song posts locations) prior to the experiments. Each territory was visited at least every second day to record a target male and his neighbours and to check the current social situation (e.g. pairing status, the presence of neighbours, spatial segregation of birds). The experiments were conducted only when the arrangement of territories of male subjects and neighbours remained unchanged between the initial field inspection and the following experiments (2–4 days later). This ensured that the songs used in N-treatments were derived from the repertoire of a well-known neighbour. The loudspeaker was fixed in a tree approximately 2 m above the ground before each experiment, and its location was changed slightly (5–10 m) between subsequent treatments with the same male to avoid habituation. In both treatments, songs were played back within the focal male territory from a place near the border of the neighbour male whose song was used in the N-treatment. The loudspeaker was always within the subject male's territory and placed at a distance of 20–40 m from the song-post used by the focal male during equipment set-up. I tried to place the loudspeaker in a way that allowed the focal male to fly towards it and to land in a tree or another elevated place at distances less than 1 m from the speaker. Each male could therefore approach the loudspeaker without getting closer to the observer, who was located perpendicular to and 20–30 m away from the loudspeaker–focal male line. The N- and S-treatments for each subject were conducted in a random order and were separated by a 1-day period. Experiments were only conducted if subject males were regularly singing and their neighbours were silent and not present at typical song posts.

Each treatment consisted of three phases: a 2-min recording of a male just before the playback started (PRE), a 2-min playback (PLAY) and a 2-min post-playback recording (POST). The behaviour of the focal male was observed, and his songs and calls were recorded during all phases. The vocalizations of the focal male were recorded using a Marantz PMD661 solid-state recorder coupled with a Sennheiser MKH70 microphone directed towards him, and observations of his behaviour were dictated into a second

microphone (Sennheiser ME62) attached to a second channel of the same recorder. Recordings and notes on behaviour were transcribed within few days using Raven Pro 1.4 software (Cornell Lab of Ornithology), which enabled the extraction of response variables with ≤ 1 s accuracy.

Two song types were played back with a species-typical rate of six songs per minute during each treatment. The first song type that was used was played 6 times during the first minute of PLAY, and the second song type was played back 6 times during the second minute of PLAY. Switching between song types and song rate imitated natural way of singing (Osiejuk et al. 2003). The order of use of particular song types for each male was random, but once chosen for a male, it remained the same in both the N- and S-treatments.

Nine original response measures were recorded: flight latency towards loudspeaker (s), latency of the closest approach to the loudspeaker, closest approach to the loudspeaker (m) and the numbers of flights, songs and calls during and after the playback. Distance measurements were made using a Bushnell Yardage Pro Sport 450 laser rangefinder (1 m accuracy).

Statistical Analysis

The data on frequency of initial and final song phrase variation among shared song types met parametric assumption and were analysed with general linear models (GLM). Birds' responses to playback often have a multidimensional character and were therefore measured using a set of response variables. Separate tests on the original variables would not be statistically independent and would not reveal the multivariate character of the response (Rice 1989; McGregor 1992). I therefore combined all original variables into orthogonal principal components using a principal components analysis. I obtained two principal components with eigenvalues over 1.0 that were logically related to the original variables that described the birds' responses (Table 1). I assessed the factorability of the data: according to the Bartlett test of sphericity (130.45, $p < 0.001$), the data set should be considered appropriate. The Kaiser–Meyer–Olkin measure of sampling adequacy had a value of 0.761, suggesting that the degree of common variance among the seven original variables was adequate (Pallant 2001; Tabachnick & Fidell 2001). I therefore tested the effect of treatments on obtained principal components with generalized estimating equations (GEEs), assuming a normal error distribution, an

Table 1: Eigenvalues, variance explained and weightings of the original variables in the first two principal components extracted from the ten original variables of the response to the playback. Measures that contributed most to the particular compound variable are in bold

Statistics and original response variables	Component	
	PC1	PC2
Eigenvalue	3.885	1.235
% of variance	55.50	17.65
Cumulative %	55.50	73.15
Flight latency	0.75	0.27
Approach 5 m latency	0.80	0.13
Time within 5 m distance	-0.71	-0.30
Closest distance	0.85	0.10
Flights after playback start	-0.89	0.05
Songs after playback start	-0.03	0.95
Calls after playback start	-0.54	-0.71

independent working correlation matrix structure, and an identity link function. GEEs are a modification of generalized linear models that account for a nested structure in an experimental design (Liang & Zeger 1986; Hardin & Hilbe 2003). I found no statistically significant differences between the N- and S-treatments when comparing a particular male's distance to the loudspeaker before the experiment (paired *t*-test, $t_{15} = 0.453$, $p = 0.657$) or to the number of songs sung before playback began (paired *t*-test, $t_{15} = -1.088$, $p = 0.294$). This finding suggests that each male was tested twice under conditions that were comparable in the sense that N-treatment should be a control for S-treatment, and the only noticeable difference between treatments is the shifted frequency of initial song phrases. However, because the initial distance of a male to the loudspeaker depends on the different spatial features of each territory (e.g. trees available, song post-distribution) and cannot be standardized perfectly under field conditions, I included this variable in the analysis. I also used the number of songs sung by focal males during PRE playback as another covariate, which should enable better model estimation if there is a relationship between song effort before simulated intrusion and the strength of a male's later response. I therefore built several models in which males that had been tested twice were the primary sampling units, PC1 or PC2 were the explained variables and the factors included in the models were treatment, treatment order, initial distance of a male to the loudspeaker, number of songs given by a male before the playback began and interactions among these variables. Using an information-theoretic approach (Burnham & Anderson 2002), I selected and present

here only the best model with the lowest quasilielihood information criterion (QIC) (Pan 2001). All statistics were calculated using IBM SPSS Statistics v.21.

Results

Characteristics of Frequency Variation Among Shared Song Types

The males sang a total of twelve different song types (Fig. 1). Each of the twelve song types that I recorded in this experiment consisted of two different syllable types and belonged to the same dialect, as characterized by a final phrase that consisted of repeated narrow band B-syllables (letters indicate different syllable types). With one exception, all of the song types were shared by some of the males (numbers of males sharing particular song type given in brackets) that were recorded in this experiment: song type HB (19 males), JB (18 males), AB (nine males), UB (eight males), KB (six males), CB and QB (five males), OB (four males), NB and RB (three males), MB (two males), and the unshared type LB (one male). I also found song types that consisted of three (eight cases) or even four different syllable types (one case), but such songs were given rarely. These nine cases, combined with cases of shortened song (i.e. in which strophes lacked a final phrase), constituted only approximately 1% of all songs recorded ($N = 2597$). I found that the frequency of maximal amplitude (FMA) of the initial phrase varied, showing a pattern similar to that found in the Norwegian population (Osiejuk et al. 2005). The initial phrases of a particular type and from a specific male were invariable but differed significantly among males (all GLM, $p < 0.001$). The frequency differences of the averaged FMAs of different males varied between 0.75 kHz (song type RB) and 2.63 kHz (song type HB). The magnitude of the frequency differences was positively correlated with the number of males that shared a song type ($r = 0.66$, $N = 11$, $p = 0.027$), which simply means that the more males shared particular song type the more likely was finding song type versions produced at different frequency levels. However, the final song phrase showed minimal variability regardless of the individual or the song type (average FMA varied between 3.37 and 3.46 kHz).

Males Response to Neighbour and Modified Neighbour Songs

All of the approach-related response measures were strongly correlated with PC1. Lower values corresponded to stronger responses, that is, males

approached faster, approached more closely, made more flights and stayed longer in close proximity to the speaker (Table 1). PC2 was negatively correlated with the number of calls given after the playback began and was positively related to the number of songs given (Table 1). PC2 is a good reflection of the typical vocal response of ortolan bunting males that are faced with territorial intrusion: they stop singing and start calling (e.g. Osiejuk et al. 2007a,b).

Males approached the loudspeaker significantly more quickly and more closely (Fig. 2) during S-treatment than during N-treatment (GEE: Wald $\chi^2 = 9.73$, $p = 0.002$; details in Table 2). In their vocal responses, males tend to switch to a stronger call response during S-treatment (Fig. 3, GEE: Wald $\chi^2 = 4.75$, $p = 0.029$; details in Table 3). However, the treatment effect was significant only when treatment order, distance to the speaker before the playback and the number of songs sung before the playback were included in the model. The second significant factor in this model was the number of songs sung by males before the playback began. (GEE: Wald $\chi^2 = 6.49$, $p = 0.011$). Males that had sung more before the playback tended to sing more afterwards ($r = 0.41$, $N = 32$, $p = 0.021$), but the overall relationship was an effect of males' responses during N-treatment ($r = 0.61$, $N = 16$, $p = 0.012$), not S-treatment ($r = 0.27$, $N = 16$, $p = 0.318$).

Discussion

The presented results here show that ortolan bunting males respond more strongly to the playback of

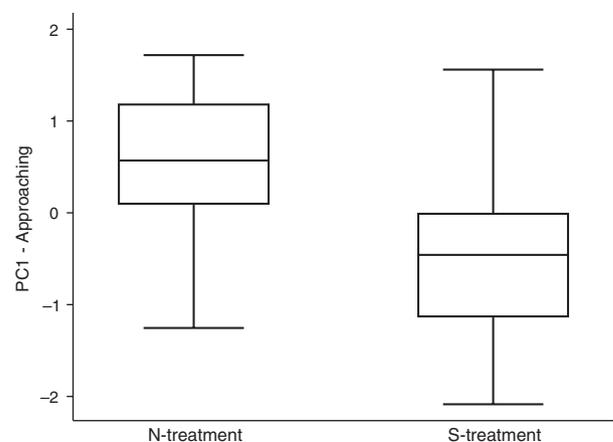


Fig. 2: Box plots showing PC1 – Approaching response measure to playback of natural neighbour songs (N-treatment) and shifted in frequency neighbour song (S-treatment). The lower and upper edges of the boxes represent the first and third quartiles; the median divides each box. The vertical lines ('whiskers') include the minimal and maximal values.

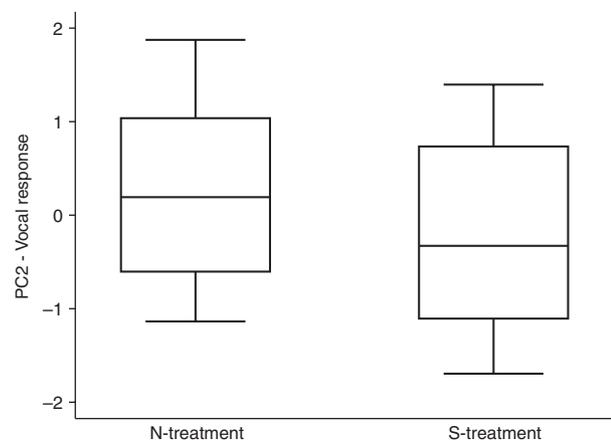
Table 2: GEE on factors affecting males' response measured by PC1 – Approaching and estimates of parameters associated with males' responses. The best model presented with QIC/QICC equal 27.136

Factors	Wald χ^2	df	p
Intercept	0.00	1	1
Treatment	9.73	1	0.002

	Coefficient estimate	Standard error
Intercept	−0.50	0.21
Treatment: N	0.99	0.32
Treatment: S	0 ^a	

Significant p values are indicated in bold.

^aBaseline categories of the categorical variable; p values pertain to the significance of estimates (slopes of covariates or differences between subsets of categorical variables).

**Fig. 3:** Box plots showing PC2 – Vocal response measure to playback of natural neighbour songs (N-treatment) and shifted in frequency neighbour song (S-treatment). The lower and upper edges of the boxes represent the first and third quartiles; the median divides each box. The vertical lines ('whiskers') include the minimal and maximal values.

neighbour songs that have a frequency-shifted initial phrase than they do to natural neighbour song. The differences involved both approach and vocal responses, and the males' behaviour was similar to the pattern found when natural neighbour and stranger songs were used for playback (Skierczyński et al. 2007; Skierczyński & Osiejuk 2010). These results therefore support the hypothesis that the variation of frequency of initial song phrases in shared song types is perceived by males and is used in N-S discrimination (Osiejuk et al. 2005). In a broader sense, it supports the idea that relatively subtle differences within shared song types are likely functional and may play an important role in N-S discrimination (Draganoiu et al. 2014).

Only a relatively small number of bird species have been shown experimentally to use acoustic cues to

Table 3: GEE on factors affecting males' response measured by PC2 – Vocal response and estimates of parameters associated with males' responses. The best model presented with QIC/QICC equal 32.142/32.85

Factors	Wald χ^2	df	p
Intercept	0.05	1	0.819
Treatment	4.75	1	0.029
Order	0.12	1	0.731
Distance to speaker before playback	2.56	1	0.109
Songs before playback	6.49	1	0.011

	Coefficient estimate	Standard error
Intercept	−0.60	0.75
Treatment: N	0.75	0.34
Treatment: S	0 ^a	
Order: N-S	0.11	0.32
Order: S-N	0 ^a	
Distance to speaker before playback	−0.03	0.02
Songs before playback	0.09	0.03

Significant p values are indicated in bold.

^aBaseline categories of the categorical variable; p values pertain to the significance of estimates (slopes of covariates or differences between subsets of categorical variables).

discriminate between neighbours and strangers (Stoddard 1996). Penguins are one of the best known models. Penguin species that differ in nesting habits and environmental conditions have been shown to use different acoustic coding systems to locate their partners, parents or chicks. The penguins use simple frequency analysis or a complex temporal analysis of modulation, depending on both what other cues were available (e.g., topographic cues of nest location) and on ambient noise level (Aubin et al. 2000; Aubin & Jouventin 2002; Jouventin & Aubin 2002). Penguins use individual recognition in parent/offspring context, which is functionally different from N-S discrimination during territory defence. They are not relatives of Oscines, thus the evolution of individual recognition in this group potentially might be based on different pre-adaptations. However, studies on penguins indicate that recognition processes are highly variable and flexible, even among closely related species, and this may also apply for N-S recognition mechanisms.

There are surprisingly few studies that directly test for the acoustic cues involved in N-S discrimination in songbirds. Males of the white-throated sparrow (*Zonotrichia albicollis*) rely on the three-first notes of their song for N-S discrimination. Small (5–10%) changes in the frequency of the first note and possibly in the frequency change between the first and second notes have been shown to interfere with recognition of a neighbour's song (Brooks & Falls 1975b). Nelson

(1989) demonstrated the importance of frequency differences for species and individual recognition in the field sparrow (*Spizella pusilla*). Nelson and Poesel (2007) experimentally manipulated songs of the white-crowned sparrow (*Zonotrichia leucophrys*) and found that acoustic cues for individual and dialect identity are segregated within a strophe. Males respond more strongly to neighbour song in which the note complex is replaced with a copy from a stranger. A similar segregation of information about local dialect and species recognition was suggested for the grey-cheeked fulvetta (*Alcippe morrisonia*) (Shieh et al. 2013). Briefer et al. (2008) showed experimentally that skylarks (*Alauda arvensis*), a species with continuous song and a huge repertoire of syllables, share some phrases as a group signature for N-S discrimination. The available data generally suggest that different bird species accomplish N-S discrimination regardless of the size of their repertoires and that the discrimination process most likely depends on song variation at a level that differs from repertoire variation (Lambrechts & Dhondt 1995). For example, Weary and Krebs (1992) have shown that males of the great tit (*Parus major*) can distinguish different individuals by their individual voice characteristics, although there are some cognitive constraints. McGregor and Avery (1986) showed that the ability of great tits to recognize new neighbours is inversely related to the number and similarity of neighbours' songs that have been experienced previously. European starlings (*Sturnus vulgaris*) are also capable of individual recognition, and this process is mediated by memorizing individual-specific song types, their sequential organization and, most likely, some individual-specific spectral characteristics across song types (Gentner & Hulse 1998).

The cited studies usually indicate only what part of a song or which syllable sequences are responsible for N-S discrimination. The current study goes a step further to prove experimentally that the songs of known neighbours are treated as songs of strangers by ortolan bunting males with only a slight change in frequency. This is important because an earlier analysis of this frequency differences in a Norwegian population of ortolan buntings (Osiejuk et al. 2005) and data from other populations (this study and others in preparation) suggest that the individually specific frequency of the initial phrases is typical for the ortolan bunting and may serve commonly as an identity cue. Although the manipulation of the frequency of neighbour song was relatively small (± 0.40 kHz) compared with the range of variation within the studied population it was clearly large enough to be perceived by the

birds. As indicated Dooling (1982) within the typical for the ortolan bunting song frequency range (2.5–5.7 kHz in studied population, Łosak 2007) birds should be able to discriminate auditory frequency differences as small as 25 to 120 Hz. Similar to the outcome of the cited study of white-throated sparrows (Brooks & Falls 1975b), Aubin et al. (2004) showed experimentally that the strength of the response to the modified neighbour song of the white-browed warbler (*Basileuterus leucoblepharus*) increases with only relatively small shifts in time or frequency of a very simple song (compare also Mathevon et al. 2008).

Bird song is well recognized as a multiple signal, and different song characteristics clearly may serve different functions (Gil & Gahr 2002). Frequency is only one characteristic and is relatively easy to measure and interpret. In general, larger birds produce lower sounds (Wallschläger 1980). However, within-species comparisons of individuals, including comparisons of individual ortolan buntings (Osiejuk et al. 2005), often do not conform to this pattern (e.g. Cardoso et al. 2008). Several species have been found to be very flexible in song frequency modification, and these changes are functional. For example, black-capped chickadee (*Poecile atricapillus*) males match a rival's song pitch in an agonistic context (Shackleton & Ratcliffe 1994; Otter et al. 2002). Relationships between the pitch of notes that constitute songs are a reliable indicator of relative male quality during dawn chorusing (Christie et al. 2004). Many species have recently been found to increase the pitch of their song in response to anthropogenic noise (e.g. Slabbekoorn & Peet 2003), and individual dynamic syllable pitch plasticity occurs in at least some species (Bermúdez-Cuamatzin et al. 2009, 2011). The information encoded in the frequency of simple whistles has also been shown to be well adapted to propagation through the harsh environment of a tropical forest (Mathevon et al. 2008).

The pattern that was found in the ortolan bunting nonetheless seems puzzling. Males have small repertoires of shared song types. The shared song types often vary in frequency among males, but these differences occur only in the initial part of the song. The final parts do not differ greatly among males. Males sing the same frequency version for a long time (most likely for their entire lives), but some males may sing more than one frequency version of a particular song type and use such versions like different song types, that is, with eventual variety. Finally, the frequency of their songs seems to be independent of body size, at least within the range in which the songs typically differ (Osiejuk et al. 2005; T. S. Osiejuk, pers. obs.).

Taken together, these data suggest that the frequency differences in shared song phrases results from specific learning mechanisms. A few scenarios are possible. Young males may acquire songs from many tutors and then 'choose' both preferred song types and the frequency versions that they will perform later. Alternatively, they may acquire only a template of song types and then individualize them by singing at a specific frequency. The possible mechanisms of individualization may result from morphological characteristics (e.g. the structure of the syrinx) and from social factors, especially by acquiring song templates that are available during memorization and songs sung by neighbours during territory establishment. These hypotheses would be difficult to test in the field because the studies would require tracking lifetime outcomes of individually marked males and descriptions of their entire social environments. It would be impossible to track all of the signals that are acquired by a young male in the wild, but such tracking could be accomplished in a lab setting, as done for other species (e.g. Burt et al. 2007). One of the most important unexplained phenomena is that the frequency of only the initial song phrases, but not of the final phrases, differ among males. It has recently been shown that despite its within-population homogeneity, the final song phrase is not the only signal of 'locality'. Both the initial and final local phrases of experimentally manipulated songs increase the responsiveness of males more than full foreign song (Osiejuk et al. 2012). One possible hint for explaining this phenomenon comes from syrinx morphology and biomechanics. The songbirds' syrinx comprises two sound generators, each of which may contribute differently to the song that is produced (Suthers 2004). In the majority of studied species, the left side of the syrinx contributes sound elements at a lower frequency range than the right side (Goller & Riede 2013). For example, in the European starling, the left side produces sounds at a frequency of 1–3.5 kHz and the right side generates sounds at a frequency of 3–10 kHz (Uchida et al. 2010). Such values fit the pattern observed in the ortolan bunting song well, but the details of the structure of the medial labia, which is responsible for the frequency of the generated sound, are not yet known for this species. However, if there is asymmetry in the ortolan bunting syrinx, it is likely that males use different sides of the syrinx to produce a higher and broader frequency range in the initial phrase and a lower and narrower range in the final phrase. Birds are also known to perceive relative pitch accurately (e.g. Cynx 1995; MacDoughall-Shackleton & Hulse 1996). Thus, a final phrase that is

stable in pitch in combination with individually varied initial phrases could be particularly useful in N-S discrimination (Christie et al. 2004).

In summary, I have shown that a relatively small change in frequency of the initial song phrase is perceived by ortolan bunting males, which respond to this manipulated neighbour song as if it were the song of strangers. The observed differences in frequencies of shared song types within a given population therefore most likely serve as cues for N-S discrimination in this species. There is also some indication that the specific system of song strophe structure of the two phrases, song sharing and individual differences are products of an interaction between genes (syrinx structure) and some still-unrecognized mechanisms of social learning. These results are representative for a single species, but likely apply to a broader range of other species. The majority of songbirds have small repertoires and often song variation among males within a particular area seems to be small. However, the N-S discrimination is likely advantageous also for them and it must have been based on some existing differences among males. Furthermore, our knowledge on how these subtle differences arise, the stability over time, and the recognition mechanisms is still insufficient (Stoddard 1996; Osiejuk et al. 2005; Draganoiu et al. 2014). All these questions could be easily addressed to other species, where the dear enemy effect is found.

Acknowledgements

I thank Kasia Łosak, Kamila Czarnecka and Piotr Chmielewski for their help with fieldwork, Marta Manser and two anonymous referees for helpful comments on the manuscript. This study was financially supported by a grant from the Polish National Science Centre (NN303807340). The study was conducted in accordance with Polish law and thanks to permission no. 2/2011 given by Director of Wielkopolski National Park.

Literature Cited

- Aubin, T. & Jouventin, P. 1998: Cocktail-party effect in king penguin colonies. *Proc. Biol. Sci.* **265**, 1665–1673.
- Aubin, T. & Jouventin, P. 2002: How to vocally identify kin in a crowd: the penguin model. *Adv. Study Behav.* **31**, 243–277.
- Aubin, T., Jouventin, P. & Hildebrand, C. 2000: Penguins use two-voice system to recognize each other. *Proc. Biol. Sci.* **267**, 1081–1087.
- Aubin, T., Mathevon, N., da Silva, M. L., Vielliard, J. M. E. & Sebe, F. 2004: How simple and stereotyped acoustic

- signal transmits individual information: the song of the White-browed Warbler *Basileuterus leucoblepharus*. *An. Acad. Bras. Cienc.* **76**, 335–344.
- Bates, L. A., Sayialel, K. N., Njiraini, N. W., Poole, J. H., Moss, C. J. & Byrne, R. W. 2008: African elephants have expectations about the locations of out-of-sight family members. *Biol. Lett.* **4**, 34–36.
- Bee, M. & Gerhardt, H. C. 2001: Neighbour-stranger discrimination by territorial male bullfrogs (*Rana catesbeiana*): I. Acoustic basis. *Anim. Behav.* **62**, 1129–1140.
- Bermúdez-Cuamatzin, E., Rios-Chelén, A. A., Gil, D. & Garcia, C. M. 2009: Strategies of song adaptation to urban noise in the house finch: syllable pitch plasticity or differential syllable use? *Behaviour* **146**, 1269–1286.
- Bermúdez-Cuamatzin, E., Rios-Chelén, A. A., Gil, D. & Garcia, C. M. 2011: Experimental evidence for real-time song frequency shift in response to urban noise in a passerine bird? *Biol. Lett.* **7**, 36–38.
- Briefer, E., Aubin, T., Lehongre, K. & Rybak, F. 2008: How to identify dear enemies: the group signature in the complex song of the skylark *Alauda arvensis*. *J. Exp. Biol.* **211**, 317–326.
- Brooks, R. J. & Falls, J. B. 1975a: Individual recognition by song in white-throated sparrows. I. Discrimination of songs of neighbours and strangers. *Can. J. Zool.* **53**, 879–888.
- Brooks, R. J. & Falls, J. B. 1975b: Individual recognition by song in White-throated Sparrows. III. Song features used in individual recognition. *Can. J. Zool.* **53**, 1749–1761.
- Burnham, K. P. & Anderson, D. R. 2002: Model Selection and Multimodel Inference: A Practical Information-Theoretic Approach. Springer, New York.
- Burt, J. M., O’Loughlen, A. L., Templeton, C. N., Campbell, S. E. & Beecher, M. D. 2007: Assessing the importance of social factors in bird song learning: a test using computer-simulated tutors. *Ethology* **113**, 917–925.
- Cardoso, G. C., Mamede, A. T., Atwell, J. W., Mota, P. G., Ketterson, E. D. & Price, T. D. 2008: Song frequency does not reflect differences in body size among males in two oscine species. *Ethology* **114**, 1084–1093.
- Christie, P. J., Mennill, D. J. & Ratcliffe, L. M. 2004: Pitch shifts and song structure indicate male quality in the dawn chorus of black-capped chickadees. *Behav. Ecol. Sociobiol.* **55**, 341–348.
- Cramp, S. & Perrins, C. M. 1994: Birds of the Western Palearctic: Handbook of the Birds of Europe, the Middle East and North Africa, Vol. **9**. Oxford Univ. Press, Oxford.
- Cynx, J. 1995: Similarities in absolute and relative pitch perception in songbirds (starling and zebra finch) and a nonsongbird (pigeon). *J. Comp. Psychol.* **109**, 261–267.
- Doolling, R. J. 1982: Auditory perception in birds. In: *Acoustic Communication in Birds*, Vol. **2**. (Kroodsma, D. E., Miller, E. H., eds). Academic Press, New York, pp. 95–130.
- Draganoiu, T. J., Moreau, A., Ravaux, L., Bonckaert, W. & Mathevon, N. 2014: Song stability and neighbour recognition in a migratory songbird, the black redstart. *Behaviour* **151**, 435–453.
- Fisher, J. 1954: Evolution and bird sociality. In: *Evolution as a Process*. (Huxley, J., Hardy, A. C., Ford, E. B., eds). Allen and Unwin, London, pp. 71–83.
- Gentner, T. Q. & Hulse, S. H. 1998: Perceptual mechanisms for individual vocal recognition in European starlings, *Sturnus vulgaris*. *Anim. Behav.* **56**, 579–594.
- Gil, D. & Gahr, M. 2002: The honesty of bird song: multiple constraints for multiple traits. *Trends Ecol. Evol.* **17**, 133–141.
- Goller, F. & Riede, T. 2013: Integrative physiology of fundamental frequency control in birds. *J. Physiol. Paris* **107**, 230–242.
- Hardin, J. W. & Hilbe, J. M. 2003: Generalized Estimating Equations. Chapman and Hall/CRC, Boca Raton, FL.
- Jouventin, P. & Aubin, T. 2002: Acoustic systems are adapted to breeding ecologies: individual recognition in nesting penguins. *Anim. Behav.* **64**, 747–757.
- Kuczyński, L. & Chylarecki, P. 2012: Atlas pospolitych ptaków lęgowych Polski. Rozmieszczenie, wybiórczość siedliskowa, trendy. GIOŚ, Warszawa.
- Lambrechts, M. E. & Dhondt, A. A. 1995: Individual voice discrimination in birds. *Curr. Ornithol.* **12**, 115–139.
- Leiser, J. K. 2003: When are neighbours ‘dear enemies’ and when are they not? The responses of territorial male variegated pupfish, *Cyprinodon variegatus*, to neighbours, strangers and heterospecifics. *Anim. Behav.* **65**, 453–462.
- Liang, K. Y. & Zeger, S. L. 1986: Longitudinal data analysis using generalized linear models. *Biometrika* **73**, 13–22.
- Łosak, K. 2007: Comparative analysis of ortolan bunting (*Emberiza hortulana*) song variation in two populations characterized by different conditions. Ph.D. Thesis, Adam Mickiewicz Univ., Poznań, Poland.
- MacDoughall-Shackleton, S. A. & Hulse, S. H. 1996: Concurrent absolute and relative pitch processing by European starling (*Sturnus vulgaris*). *J. Comp. Psychol.* **110**, 139–146.
- Mathevon, N., Aubin, T., Vielliard, J., da Silva, M.-L., Sebe, F. & Boscolo, D. 2008: Singing in the rain forest: how a tropical bird song transfers information. *PLoS ONE* **3**, e1580.
- McGregor, P. K. 1992: Quantifying responses to playback: one, many, or composite multivariate measures? In: *Playback and Studies of Animal Communication*. (McGregor, P. K., ed). Plenum Press, New York, pp. 79–96.
- McGregor, P. K. & Avery, M. I. 1986: The unsung songs of the great tits (*Parus major*): learning neighbours’ songs for discrimination. *Behav. Ecol. Sociobiol.* **18**, 311–316.

- Nelson, D. A. 1989: Song frequency as a cue for recognition of species and individuals in the field sparrow (*Spizella pusilla*). *J. Comp. Psychol.* **103**, 171—176.
- Nelson, D. A. & Poesel, A. 2007: Segregation of information in a complex acoustic signal: individual and dialect identity in white-crowned sparrow song. *Anim. Behav.* **74**, 1073—1084.
- Osiejuk, T. S., Ratyńska, K., Cygan, J. P. & Dale, S. 2003: Song structure and repertoire variation in Ortolan Bunting (*Emberiza hortulana* L.) from isolated Norwegian population. *Ann. Zool. Fenn.* **40**, 3—16.
- Osiejuk, T. S., Ratyńska, K., Cygan, J. P. & Dale, S. 2005: Frequency shift in homologue syllables of the Ortolan Bunting *Emberiza hortulana*. *Behav. Process.* **68**, 69—83.
- Osiejuk, T. S., Ratyńska, K. & Dale, S. 2007a: What makes a 'local song' in a population of ortolan buntings without common dialect? *Anim. Behav.* **74**, 121—130.
- Osiejuk, T. S., Łosak, K. & Dale, S. 2007b: Cautious response of inexperienced birds to conventional signal of stronger threat. *J. Avian Biol.* **38**, 644—649.
- Osiejuk, T. S., Bielecka, A. & Skierczyński, M. 2012: What exactly is 'local song' in a population of ortolan buntings with a common dialect? *J. Ethol.* **30**, 133—142.
- Otter, K., Ratcliffe, L., Njegovan, M. & Fotheringham, J. 2002: Importance of frequency and temporal song matching in black-capped chickadees: evidence from interactive playback. *Ethology* **108**, 181—191.
- Pallant, J. 2001: SPSS Survival Manual. Open Univ. Press, Buckingham.
- Palphramand, K. L. & White, P. C. L. 2007: Badgers, *Meles meles*, discriminate between neighbour, alien and self scent. *Anim. Behav.* **74**, 429—436.
- Pan, W. 2001: Akaike's information criterion in generalized estimating equations. *Biometrics* **57**, 120—125.
- Rice, W. R. 1989: Analyzing tables of statistical tests. *Evolution* **43**, 223—225.
- Shackleton, S. A. & Ratcliffe, L. 1994: Matched counter-singing signals escalation of aggression in black-capped chickadees (*Parus atricapillus*). *Ethology* **97**, 310—316.
- Sherman, P. K., Reeve, H. K. & Pfennig, D. W. 1997: Recognition systems. In: *Behavioural Ecology: An Evolutionary Approach*. (Krebs, J. R., Davies, N. B., eds). Blackwell Science, Oxford, pp. 69—120.
- Shieh, B. S., Liang, S. H., Youan, H. W. & Chen, C. C. 2013: Experimental evidence that distinct song phrases in the Great-cheeked Fulvetta *Alcippe morrisonia* permit species and local dialect recognition. *Ibis* **155**, 32—41.
- Skierczyński, M. 2009: The role of song in territorial defence in the ortolan bunting *Emberiza hortulana*. Ph.D. Thesis, Adam Mickiewicz Univ., Poznań, Poland.
- Skierczyński, M. & Osiejuk, T. S. 2010: Sharing song types within local dialect does not hinder neighbour-stranger recognition in ortolan bunting *Emberiza hortulana* males. *Behaviour* **147**, 333—351.
- Skierczyński, M., Czarnecka, K. M. & Osiejuk, T. S. 2007: Neighbour-stranger song discrimination in territorial ortolan bunting *Emberiza hortulana* males. *J. Avian Biol.* **38**, 415—420.
- Slabbekoorn, H. & Peet, M. 2003: Birds sing at higher pitch in urban noise. *Nature* **424**, 267.
- Stoddard, P. K. 1996: Vocal recognition of neighbors by territorial passerines. In: *Ecology and Evolution of Acoustic Communication in Birds*. (Kroodsma, D. E., Miller, E. H., eds). Comstock/Cornell Univ. Press, Ithaca, pp. 356—376.
- Suthers, R. A. 2004: How birds sing and why it matters. In: *Nature's Music: The Science of Birdsong*. (Marler, P., Slabbekoorn, H., eds). Academic Press, San Diego, CA, pp. 276—299.
- Tabachnick, B. G. & Fidell, L. S. 2001: *Using Multivariate Statistics*. Allyn and Bacon, Needham Heights, MA.
- Tibbets, E. A. 2002: Visual signals of individual identity in the wasp *Polistes fuscatus*. *Proc. Biol. Sci.* **269**, 1423—1428.
- Tibbets, E. A. & Dale, J. 2007: Individual recognition: it is good to be different. *Trends Ecol. Evol.* **22**, 529—537.
- Uchida, A. M., Meyers, R. A., Cooper, B. G. & Goller, F. 2010: Fibre architecture and song activation rates of syrinxal muscles are not lateralized in the European starling. *J. Exp. Biol.* **213**, 1069—1078.
- Wallschläger, D. 1980: Correlation of song frequency and body weight in passerine birds. *Experientia* **36**, 412.
- Weary, D. M. & Krebs, J. R. 1992: Great tits classify songs by individual voice characteristics. *Anim. Behav.* **43**, 283—287.