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Elodie F. Briefer

Queen Mary University of London

Thierry Aubin

French National Center for Scientific Research

Fanny Rybak

French National Center for Scientific Research

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Response to Displaced Neighbours in a Territorial Songbird with a Large Repertoire

Elodie Briefer, Thierry Aubin, and Fanny Rybak
French National Center for Scientific Research

KEYWORDS

neighbour recognition, song analysis, playback experiment, oscine, *Alauda arvensis*

ABSTRACT

*Neighbour recognition allows territory owners to modulate their territorial response according to the threat posed by each neighbour and thus to reduce the costs associated with territorial defence. Individual acoustic recognition of neighbours has been shown in numerous bird species, but few of them had a large repertoire. Here, we tested individual vocal recognition in a songbird with a large repertoire, the skylark *Alauda arvensis*. We first examined the physical basis for recognition in the song, and we then experimentally tested recognition by playing back songs of adjacent neighbours and strangers. Males showed a lower territorial response to adjacent neighbours than to strangers when we broadcast songs from the shared boundary. However, when we broadcast songs from the opposite boundary, males showed a similar response to neighbours and strangers, indicating a spatial categorization of adjacent neighbours' songs. Acoustic analyses revealed that males could potentially use the syntactical arrangement of syllables in sequences to identify the songs of their neighbours. Neighbour interactions in skylarks are thus subtle relationships that can be modulated according to the spatial position of each neighbour.*

Introduction

Few studies have investigated the perceptual bases used by songbirds to recognise individually their territorial neighbours. Discriminating conspecific potential rivals may be a useful ability in the context of territory defence. For example, distinguishing neighbours from strangers may result in the 'dear-enemy effect' (Fisher 1954), i.e. a reduced aggression from territorial animals towards familiar individuals. This effect, shown in numerous species (e.g. Falls and McNicholl 1979; Leiser and Itzkowitz 1999; Bee 2003), minimises the energy expended on aggressive behaviour between adjoining neighbours.

According to Falls (1982), individual recognition is expected between animals that are in continuous or repeated contact. Immediate neighbours are conspecifics that it might be indeed advantageous to keep track of, as they may intend to appropriate the owner's territory or mate (Catchpole and Slater 1995). According to the relative threat hypothesis (Temeles 1994), the strength of a resident's territorial response against an intruder depends on the threat presented by this intruder. Neighbours may differ by

their level of aggression (Hyman and Hughes 2006) or by the threat they pose (Olendorf et al. 2003; Poesel et al. 2007). Discriminating between threatening neighbours and less threatening ones could allow a resident to save time and energy by modulating its territorial response according to the threat posed by each neighbour.

To summarise, neighbour recognition provides a precise, reliable method of assessing individual agonistic qualities. Thus, individual recognition is expected to be particularly important within small groups of neighbours, where repeated interactions among individuals with differing intentions occur. In such communication networks, recognition of neighbours must be an important aspect of territoriality, by enabling individuals to establish and maintain a particular spacing pattern among their territories and by regulating social relationships (Naguib 2005; Tibbetts and Dale 2007).

To our knowledge, individual vocal recognition of neighbours has been shown in three bird species with large repertoires (European robin *Erithacus rubecula*, 100–250 phrases per individual: Brindley 1991; European starlings *Sturnus vulgaris*, 11–90 song types: Gentner and Hulse 1998; thrush nightingale *Luscinia luscinia*, 12–40 song types per individual: Naguib and Todt 1998), but the song parameters coding for individual recognition (the individual signatures) have never been examined. Songbirds with large repertoire raise the question of cognitive abilities required to decode information such as emitter identity. The ‘repertoire constraint’ hypothesis (Krebs and Kroodsma 1980; Falls 1982) suggests that large repertoires may interfere with birds’ ability to recognise their neighbour’s songs. This ability could be even more difficult for species with large repertoires that do not remain in or near their territory year round, the duration of neighbours’ association being short in this case (Godard 1993). Lambrechts and Dhondt (1995) hypothesised that species with very large repertoires learn only a part of their neighbours’ repertoires. Another phenomenon that might hinder individual recognition is song sharing among neighbours (Stoddard 1996). Shared songs of neighbours may indeed be particularly difficult to distinguish because of their high similarity. Song sharing may at least delay the song learning process required for neighbour recognition (Stoddard et al. 1992).

In the present study, we investigated individual variation in songs and neighbour vocal recognition in a territorial bird with a very large repertoire, the skylark, *Alauda arvensis*. During the breeding season, from February to the end of July, skylarks settle in pairs in territories that are concentrated in particular locations due to habitat heterogeneity. Both male and female have a strong tendency to return to the same breeding location from year to year (Delius 1963; Jenny 1990). Males are thus gathered in distinct small groups of ‘neighbours’ spaced from ‘strangers’ by a few kilometres. Within a group, males are established in adjacent and stable territories and show a strong territorial behaviour by flying towards intruders, exhibiting threat postures and even fighting. As part of this territorial behaviour, males produce a flight song to deter intruders (Delius 1963; Hedenström 1995), in which species identity is mainly encoded by temporal parameters (Aubin and Brémond 1983). Unlike songs of most songbirds which are relatively short and discontinuous and which can be categorised in discrete song types, the skylark flight song is very long and continuous. It consists of series of song units, syllables, produced between 2 and 6 kHz. With an estimated syllable repertoire of up to 300 different syllables per individual, this song is one of the most complex among oscines, giving rise to a huge potential for variation at the syntactic level (Aubin 1981, 1982). Some sequences of syllables are shared by all individuals of a given location (microdialect). These shared sequences constitute a group signature used by birds for neighbour-stranger discrimination (Briefer et al. 2008a). Such discrimination results in a less aggressive territorial response to neighbours than to strangers when territories are well established and stable (Briefer et al. 2008b).

We examined both neighbour recognition and the potential acoustic basis supporting such recognition. To assess the existence of inter-individual variation in songs, which could be potentially used by birds to

discriminate their neighbours, we first analysed both the repertoire composition and the acoustic structure of songs. We then tested adjacent neighbour recognition with playback experiments, using the method developed by Falls and Brooks (1975), in which songs of neighbours and strangers are broadcast at two boundaries: the boundary shared with the neighbour whose song is used as a stimulus and the opposite boundary. We hypothesised that, despite a very large repertoire, limited period of neighbour association during the year and considerable song sharing, skylark males would respond differently to the neighbour song broadcast at the shared boundary than to that broadcast at the opposite one, thus showing that they are able to associate their neighbours with a particular position. Further, we particularly focused on the conspicuous individual characteristics of songs that may constitute a basis for the process of identification.

Materials and methods

Study area, subjects and song recordings

Our study was carried out during the breeding seasons 2005 to 2007 in nine locations situated in the fields surrounding the University of Paris 11, France. During the breeding season, site fidelity is very strong (Jenny 1990; Delius 1963). Once territories are well established (boundaries between adjoining territories are stable and males are strongly confined inside (Aubin 1981)), subjects are easily identifiable by observing their position and movements, especially when they perform flight songs. Consequently, we estimated the boundaries of the studied territories after numerous and careful visual observations of the birds' movements at different times of day. Within a location, males, referred to as neighbours, held adjoining territories of circa 1 ha, measured using GPS coordinates (Garmin GPSMAP 76S). The mean \pm SE number of neighbours (group size) within each of the nine studied locations was 9.17 \pm 1.30 (Min=five neighbours; Max=13 neighbours). Males from two different locations, situated at least 2 km apart, were referred to as strangers (Briefer et al. 2008a).

We recorded several songs per individual between 0900 and 1200 hours Eastern Daylight Time using a Marantz PMD 690 digital recorder (sampling rate, 48,000 Hz) connected to a Sennheiser ME 64 K6 omnidirectional microphone (frequency response, 30 Hz to 20 kHz \pm 1 dB) mounted on a Telinga Universal parabola (diameter, 50 cm). We then transferred song files to a computer and high-pass filtered them (cut-off frequency, 1,600 Hz) to remove the background noise. We used Avisoft SASLab pro v4.31. software (Specht 2004) for subsequent analysis and the preparation of songs played back.

Song analysis

We carried out the song analysis on a total of 23 songs produced by nine males established in three different locations (three males per location), corresponding to two or three songs and a mean \pm SE duration of song analysis per individual of 370.7 \pm 42.4 s. In a previous study, we examined the sequential organisation of syllables using a custom Matlab programme (The MathWorks, Natick, MA, USA; see Lehongre et al. 2008) and showed that syllables belong to three kinds of sequences: sequences that are not repeated ('individual non-repeated sequences'), sequences repeated several times by the same individual ('individual phrases') and sequences shared by different individuals ('non-individual phrases' or 'shared phrases'; Briefer et al. 2008a). We focused the present analysis on the individual phrases and sequences, not analysed previously. We analysed these individual parts of the song at both the syntactical level (repertoire composition) and the featural level (acoustic characteristics).

Repertoire composition

For each individual, the number of new syllables produced against their time of occurrence in the songs reached an asymptote, the value of which corresponded to the repertoire size. For each individual and for

each song, we established the syllable repertoire and the phrase repertoire (respectively the number of different syllables and the number of different phrases produced). A coefficient of syllable repertoire similarity (RS) allowed us to compare, within each location, either the syllable repertoire of songs produced by the same individual or the syllable repertoire of songs produced by different individuals. Thus, for each pair of songs, the number of syllables present in the two songs was related to the number of syllables present in only one song using the following equation: $RS = Z / ((X + Y) - Z)$, with X and Y being the total number of different syllables produced in the songs x and y , respectively, and Z being the number of syllables present in both songs x and y (Hultsch and Todt 1989). We used the same procedure to calculate coefficients of phrase repertoire similarity. As conventional parametric and non-parametric tests are not suitable for analyses in which each individual or song is included several times in the different pair-wise comparisons (Sokal and Rohlf 1995; Mundry 1999), we used two-tailed exact permutation tests to compare the RS.

Within each of the three locations, we calculated the proportion of individual syllables in the repertoire of each individual. We then measured the proportion of these individual syllables and non-individual syllables that were repeated in at least two songs of a given individual. The same procedure was applied for the phrases. As the data were not normally distributed, we compared proportions using two-tailed Wilcoxon matched pair tests.

Acoustic characteristics

Based on previous studies on individual recognition (e.g. Aubin et al. 2004; Lehongre et al. 2008), we chose to analyse the following temporal and frequency parameters of the individual parts of the song, i.e. the individual nonrepeated sequences and the individual repeated sequences (individual phrases), in order to assess intra- and interindividual variability.

Temporal parameters. For each sequence, we measured the duration of syllables, the duration of silences between two successive syllables and the total duration of the sequence on oscillograms. We used these parameters to calculate the rhythm (sound to silence duration ratio) and the tempo (number of syllables per unit of time). We retained the following four temporal parameters for subsequent analyses: the duration of sequences (DurSe), the mean duration of syllables in the sequences (DurSyl), the tempo (Tem) and the rhythm (Rhy).

Frequency parameters. For each sequence, we measured the following four frequency parameters on an averaged linear amplitude spectrum applied to the whole sequence: the frequency of maximum amplitude (MaxFre) and the frequency values at the upper limit of the first (Fre25%), second (Fre50%) and third (Fre75%) quartiles of energy.

For each individual, we measured temporal and frequency parameters on ten sequences of each kind (individual nonrepeated sequences and individual phrases), and we log-transformed the measures to normalise distributions. We used discriminant function analyses (DFA) to identify potential differences between individuals. We treated frequency and temporal parameters separately. We used each DFA to calculate a success rate of individual discrimination (correct classification rate). For external validation, we used a 'jack-knifed' or 'leave-one-out' cross validation, in which the syllable sequence to be classified is not used in the creation of the classification function (Lachenbruch and Mickey 1968). We compared the success rates of individual discrimination obtained with the cross validation with those expected by chance using χ^2 tests.

Additionally, we made comparisons between individuals for each parameter measured using one-way ANOVA and calculated coefficients of variation. We computed between- and within-individual coefficients

of variation (respectively CV_b and CV_w) as follows: $CV = 100 \times (1 + 1/(4 \times n)) \times (SD/X_{\text{mean}})$, where SD is the standard deviation, X_{mean} the mean of the sample and n the sample size (Scherrer 1984; Sokal and Rohlf 1995). We then calculated a potential of individual coding (PIC) for each parameter with the ratio CV_b/mean CV_w, where mean CV_w is the mean value of the CV_w of all individuals. A PIC value greater than 1 indicates that this parameter is likely to be used for individual recognition as its intra-individual variability is smaller than is inter-individual variability (Scherrer 1984; Robisson et al. 1993).

Last, in order to assess the distribution of individual phrases in the song, we used a Friedman test to compare the number of individual phrases between three song parts: beginning, middle and end.

We used R (R development core team 2007) and Statistica v6.0. (StatSoft 2001) for these statistical analyses. All results are stated as mean ± SE. The significance level was taken at $\alpha \leq 0.05$ to assess differences.

Playback experiment

We made the playback experiments in two locations in 2006 and four locations in 2007. We tested a total of 15 males in May when territories were well established and stable.

Signals tested

We broadcast two songs from either category to each subject: a familiar neighbour song ('N', i.e. the song of an adjacent neighbour sharing a territory boundary with the tested subject) and a stranger song ('S', i.e. the song of an unfamiliar individual established in another location). We recorded these songs during the same breeding season as the playback experiment, and we adjusted all of them to the same duration by taking the first 90 s. Using Goldwave v5.11. (Craig 2000), we rescaled each song to match the root mean square amplitude of the different songs at the same output level. To avoid pseudo-replication (McGregor et al. 1992), we prepared a different N song for each individual and two or three different S songs for each location using songs recorded in the most distant locations from the tested one.

Playback procedure

For each subject, we broadcast the same N and S songs from two positions in its territory (two songs, four playbacks per subject): at the boundary shared with the neighbour whose song was used to prepare the N song (in the following named 'shared boundary') and at the opposite boundary (in the following named 'opposite boundary'). The experimenter positioned the loudspeaker on the ground at about 5 m inside the territory of the tested bird and stood 20 m away from the loudspeaker. We played back songs with a Marantz PMD 690 numeric recorder connected via a 20 m cable to a 10-W Megavox Pro mega-6000 loudspeaker (frequency response, 400 Hz–10 kHz, ±3 dB), at the intensity estimated to be normal for the birds (mean ± SE, 90.8 ± 0.8 dB measured at 1 m from the loudspeaker with a Brüel & Kjaer 2235, linear setting). We carried out trials between 0900 and 1200 hours Eastern Daylight Time. We did not carry out experiments during rainy or windy weather. To avoid habituation (Aubin 1982), we tested each subject only once with each playback. We carried out the four playbacks in the same day for each subject in a random order spaced by at least a 5-min delay to avoid a confounding effect of habituation or increasing aggression due to an order effect. We initiated the playback when the subject was standing on the ground inside its territory at more than 10 m from the loudspeaker and when adjacent neighbours were quiet. The song played back never elicited any song production by the neighbours of the tested subject. Thus, the responses observed and scored could not be attributed to an external stimulus.

Measures of responses and statistical analyses

For each trial, the experimenter scored the response of the bird during 180 s, corresponding to the broadcast of 90 s of continuous song and 90-s period of post-playback silence. We recorded the following 11 measures of response to assess the effects of the different categories of songs played back: total duration of movements; duration of movements between 10 and 5 m and at less than 5 m from the loudspeaker; time spent between 10 and 5 m and at less than 5 m from the loudspeaker; total number of calls, duration of songs and latency before the first song produced in response; latency before the first movement and latency to approach at less than 10 m and at less than 5 m from the loudspeaker. When the tested subject did not produce any song or did not approach at less than 10 m or at less than 5 m from the loudspeaker, a value of 180 s (corresponding to the total trial duration) was attributed for these latter measures of latency. We used a principal components analysis (PCA) based on the correlation matrix in order to create a composite score with the measures of response scored during the 180 s of trial, which are partially correlated (McGregor 1992).

We compared the scores of the first principal component (PC1) using a two-factors repeated-measures ANOVA, the first factor being the position of the loudspeaker (shared or opposite boundary) and the second factor being the category of song (N or S). We made further post hoc comparisons using two-tailed Tukey HSD tests. We made all analyses using Statistica v6.0. (StatSoft 2001).

Table 1 Results of the multivariate analyses of variance made on four frequency parameters and four temporal parameters showing the effects and interactions (×) of the factors ‘kind of sequence’ (N=2), ‘location (N=3) and ‘individual’ (N=9; ten measures per kind of sequence and per individual). Values indicate MANOVA F, df and P values.

Factors	Parameters					
	Frequency			Temporal		
	F	df	P	F	df	P
Sequence	1.94	4,159	0.11	3.39	4,159	0.01
Location	1.68	8,320	0.10	2.60	8,320	0.009
Individual	2.26	24,648	0.0006	1.93	24,648	0.005
Sequence × location	1.77	8,320	0.08	0.96	8,320	0.47
Sequence × individual	1.93	24,648	0.005	0.61	24,648	0.93

Results

Song analysis

Repertoire composition

Within each location, songs produced by the same individual were significantly more similar than songs produced by different individuals. Thus, two songs produced by the same individual had significantly more syllables in common (coefficient of $RS=0.39\pm 0.02$) than two songs produced by different individuals of a given location ($RS=0.33\pm 0.04$; exact permutation test: $N=77$, $P<0.0001$). Two songs produced by the same individual also had significantly more phrases in common ($RS=0.24\pm 0.03$) than two songs produced by different individuals of a given location ($RS=0.15\pm 0.02$; exact permutation test, $N=77$; $P<0.0001$).

Syllables produced by only one bird of a given location represented $16.71\pm 1.89\%$ of the syllable repertoires of this bird, making a total of 56.67 ± 9.35 different individual syllables per individual ($N=9$).

individuals). Of these individual syllables, $28.28 \pm 4.95\%$ were repeated in more than one song of the same individual. Comparatively, $56.77 \pm 5.23\%$ of nonindividual syllables were repeated in at least two songs of the same individual. The difference between these proportions of repeated individual and non-individual syllables was significant (Wilcoxon matched pairs test calculated on the proportions: $Z=2.67$, $N=9$, $P=0.008$).

Phrases produced only by one bird of a given location represented $29.27 \pm 2.87\%$ of the phrase repertoire of this bird, making a total of 37.89 ± 4.54 different individual phrases per individual ($N=9$ individuals). Of these individual phrases, $43.44 \pm 6.81\%$ were repeated in at least two songs of the same individual. Comparatively, $32.51 \pm 4.65\%$ of non-individual phrases were repeated in more than one song of the same individual. The difference between these proportions of repeated individual and non-individual phrases was significant (Wilcoxon matched pairs test on the proportions: $Z=2.19$, $N=9$, $P=0.028$).

Acoustic characteristics

Before plotting the different kinds of sequences (individual non-repeated sequences and individual phrases), the different locations (three locations) and the individuals (nine individuals) in DFAs, we carried out MANOVA on the temporal and frequency parameters measured to assess the effects of these three different factors on the parameters and the potential interactions between them. Results of these MANOVAs are shown in Table 1. Due to a significant interaction between 'kind of sequence' and 'individual' on the frequency parameters, we carried out separate DFAs for individual non-repeated sequences and individual phrases ($N =$ nine individuals, ten measures per individual).

DFAs correctly assigned individual non-repeated sequences and individual phrases to individuals with weak percentages, and similar correct classification rates were obtained for the two kinds of sequences. The correct classification rates (CR) obtained with the cross validation were not different than expected by chance alone (11.1%; χ^2 test, frequency parameters of non-repeated sequences (CR=17.8%), $\chi^2=2.30$, $P=0.97$; frequency parameters of individual phrases (CR=17.8%), $\chi^2=2.48$, $P=0.96$; temporal parameters of non-repeated sequences (CR=12.2%), $\chi^2=1.77$, $P=0.99$; temporal parameters of individual phrases (CR=13.3%), $\chi^2=1.12$, $P=0.10$). Thus, as shown by Fig. 1, neither the frequency parameters nor the temporal parameters were consistently different between individuals.

Results of the ANOVA (Table 2) indicated that the rhythm, the duration of the sequences and all frequency parameters measured except the frequency values at the third quartile of energy differed significantly between individuals. However, all coefficients of within-individual variation (CVw) were greater than coefficients of between-individual variation (CVb). Resulting PICs had values lower than 1, indicating that none of the frequency or temporal parameters had a potential for individual coding.

When we divided the songs in three parts (beginning, middle and end), individual phrases were equally distributed in these three parts (Friedman test, $F=0.63$; $N=23$ songs; $P=0.73$).

Playback experiment

Although the four playbacks (N and S played back from the shared and opposite boundary) were conducted for each subject in a random order, we tested the possibility of a confounding effect of habituation or increasing aggression due to an order effect. A repeated-measures ANOVA with the order of presentation of playback treatments as repeated measures and the responses of subjects as the dependant variables did not reveal such effect ($F_{3,56}=0.017$, $P=0.99$).

The PC1 explained 30.1% of the variance in the responses measured. The examination of the component loadings, shown in Table 3, revealed that the latencies to approach at less than 10 and 5 m from the

loudspeaker, the duration of movements at different distances from the loudspeaker and the time spent at less than 5 m from the loudspeaker loaded highly on PC1 compared to the other responses. Higher positive values of PC1 corresponded to a stronger response, i.e. the subjects spent more time in movements, approached closer to the loudspeaker after a shorter latency and produced more calls. A comparison of PC1 scores revealed a significant interaction between the position of the loudspeaker (shared or opposite boundary) and the category of song played back (N or S; repeated-measures ANOVA: $F_{1,56}=4.97$, $P=0.04$). As shown by Fig. 2, subjects responded significantly more to S songs than to N songs when songs were played back from the shared boundary (Tukey HSD test: $N=15$, $P=0.004$). However, when songs were played back from the opposite boundary, responses of subjects to S and N songs did not differ (Tukey HSD test: $N=15$, $P=0.71$). Neither the responses to the S songs (Tukey HSD test: $N=15$, $P=0.89$) nor those to the N songs (Tukey HSD test: $N=15$, $P=0.12$) were different according to the position of the loudspeaker in the subject's territory.

Fig. 1. Discriminant function analysis based on four frequency parameters (A) and four temporal parameters (B) measured in individual phrases ($N =$ nine individuals, ten measures per individual). Individuals are identified by a *number* corresponding to their location and a *letter* that differentiates them from their neighbours inside the location

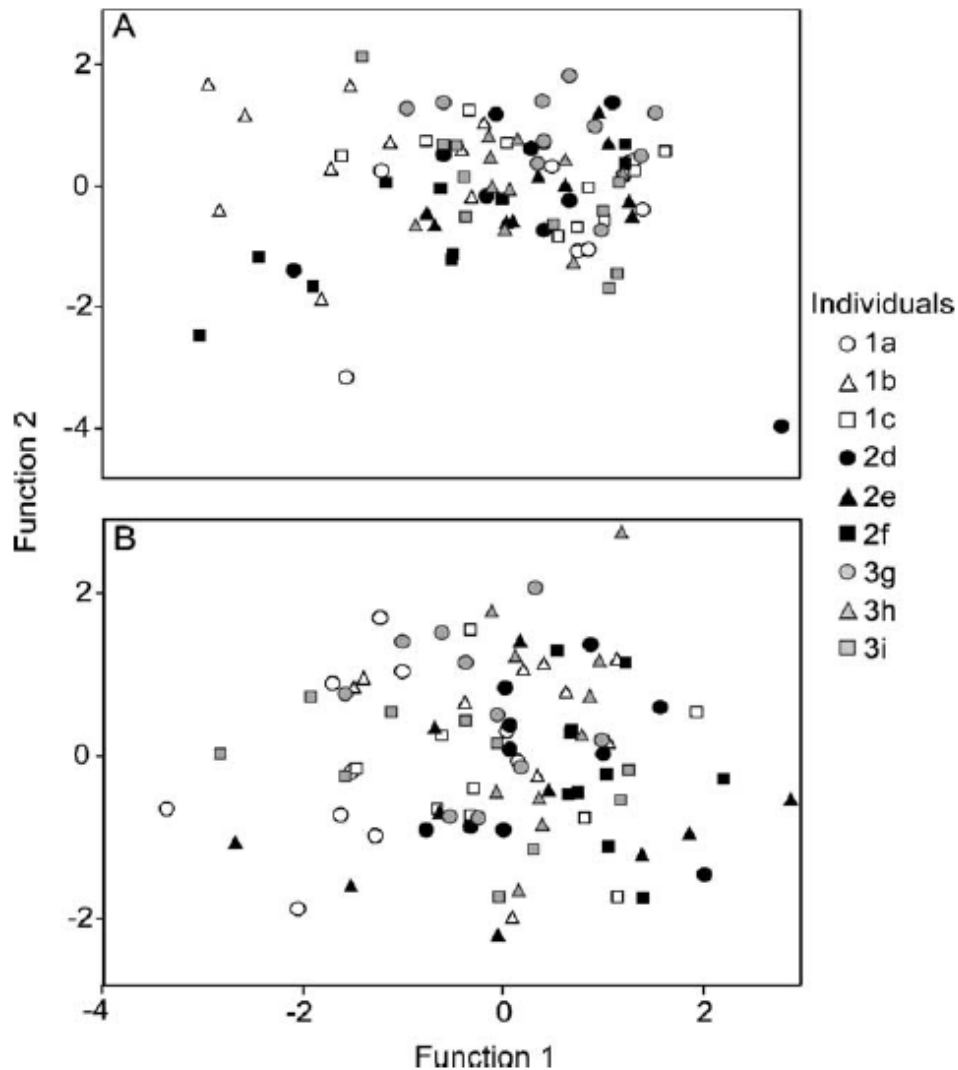


Table 2. Analysis of variance and comparison between individuals of four frequency parameters and four temporal parameters ($N=9$ individuals, 20 measures per individual)

Parameters	Mean CVw	CVb	PIC	F	P
MaxFre	16.29	6.94	0.43	3.18	0.002
Fre25%	9.37	3.15	0.34	2.12	0.04
Fre50%	7.85	2.99	0.38	2.73	0.007
Fre75%	7.14	2.34	0.31	1.68	0.11
DurSe	80.96	26.79	0.33	2.20	0.03
DurSyl	34.47	7.39	0.21	1.12	0.35
Tem	29.06	8.56	0.30	1.42	0.19
Rhy	33.83	11.09	0.33	2.68	0.008

One-way ANOVA results are given as $F_{8,71}$ and P values. See text for the description of measured parameters
 Abbreviations for the statistical parameters: CVw and CVb within and between-individual coefficients of variation, PIC potential of individual coding

Fig. 2 First principal component scores (mean \pm SE, $N=15$ subjects) for playbacks of neighbour (N) and stranger (S) songs broadcast from the shared boundary and from the opposite boundary. (Tukey HSD test, ** $P<0.01$)

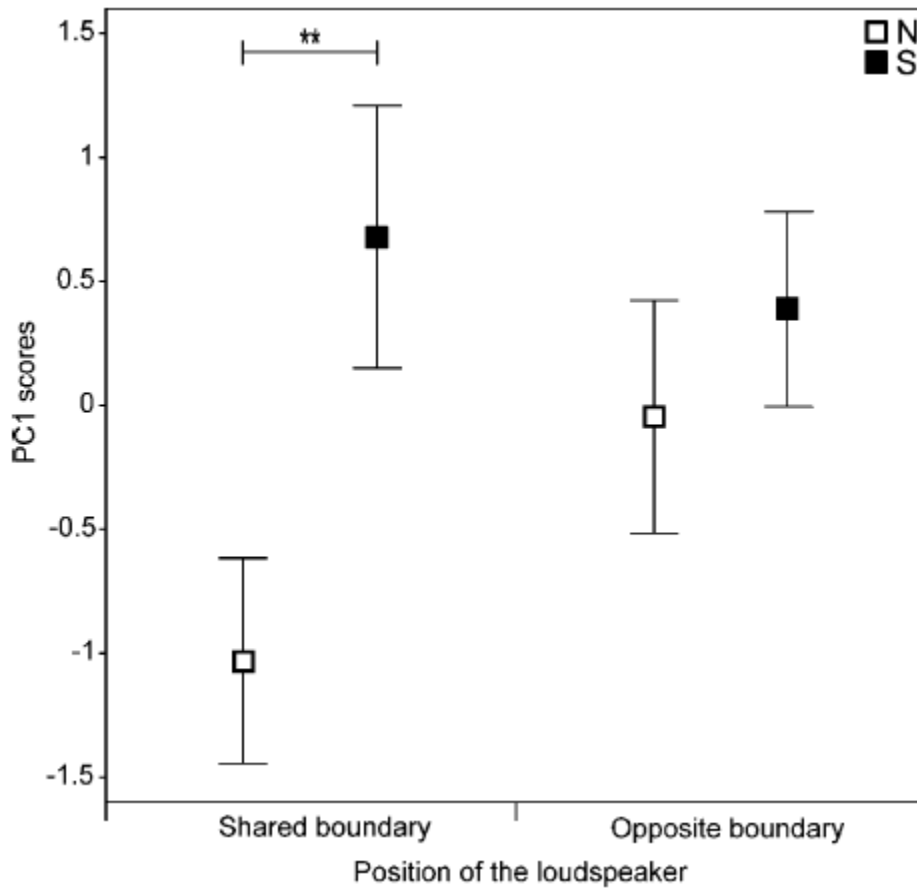


Table 3 Loadings of the response measures on the first (PC1) and second (PC2) principal component for playbacks of neighbour and stranger songs played from the shared and opposite boundaries

Response measures and statistics	PC1	PC2
Latency to approach at less than 10 m	-0.904	-0.106
Latency to approach at less than 5 m	-0.810	0.153
Duration of movements between 5 and 0 m	0.726	-0.297
Time spent between 5 and 0 m	0.623	-0.140
Duration of movements between 10 and 5 m	0.616	0.390
Total duration of movements	0.471	-0.031
Time spent between 10 and 5 m	0.368	0.464
Latency before the first movement	-0.330	-0.040
Total number of calls	0.273	0.062
Latency before the first song	-0.068	0.816
Duration of songs	0.036	-0.833
Percent of variance	30.122	17.032
Eigenvalue	3.313	1.874

Variance explained and eigenvalues of PC1 and PC2 are indicated. The total variance explained by PC1 and PC2 was 47.15%. Measures that contributed most to the particular compound variables (loadings >0.45) are in bold

The second principal component (PC2) explained 17.0% of the variance in the response measured. As shown in Table 3, the duration of songs, the latency before first song emission and the time spent between 10 and 5 m from the loudspeaker loaded highly on PC2. There was no interaction between the position of the loudspeaker (shared or opposite boundary) and of the category of song played back (N or S) on PC2 scores (repeated-measures ANOVA: $F_{1,56}=0.08$, $P=0.78$).

Discussion

Neighbour–neighbour discrimination and spatial categorisation

Our playback experiments showed that males respond differently to neighbour (N) and stranger (S) songs when these signals are played back at the boundary shared with the neighbour, but not when they are played back at the opposite boundary. When a territorial intrusion was simulated by playback at the shared boundary, males showed indeed a stronger territorial response to S song than to N song, spending more time moving, approaching closer to the loudspeaker and after a shorter latency and producing more calls. However, at the opposite boundary, males reacted in a similar way to N and S songs. Males thus show a dear-enemy effect, i.e. reduced aggression towards familiar compared to unfamiliar individuals, only at the shared boundary. Hence, skylarks discriminate not only songs (N and S) but also positions of sound sources (shared and opposite boundary), indicating that they are able to spatially categorise songs. This association of particular songs with particular positions is a more subtle task than simply distinguishing between familiar and unfamiliar conspecifics (Lambrechts and Dhondt 1995). Indeed, if N songs were only recognised as a class of familiar sounds, then it should not matter at what side of the boundary the song is played since neighbours occur on both sides of the territory (Falls 1982). Such differences in responses according to the position of playbacks has been found in similar studies on individual recognition of neighbours (e.g. Naguib and Todt 1998; Lovell and Lein 2005).

Temeles (1994) proposed that the dear-enemy effect could be explained by the relative threat presented by neighbours compared to strangers. Residents may lose both their territory and their mate because of

interactions with strangers, whereas they may lose only their mate from interactions with neighbours already in possession of a suitable territory. As a consequence, residents respond more aggressively to strangers that constitute a more serious threat than neighbours. In the same way, a displaced neighbour could represent a more threatening situation than a non-displaced neighbour. In our study, despite a less aggressive response to the neighbour than to the stranger song at the shared boundary and a similar response to the two categories of songs at the opposite boundary, the response to the neighbour song did not differ significantly between the two territory sides. This could be explained by similar levels of threat presented by these two situations. It also may be possible that a neighbour, even displaced, is first seen as a member of the group that may represent a weaker menace than a stranger. This could indicate that males rely more on the group identity contained in the neighbour song than on the individual identity to assess the strength of their territorial response. This hypothesis is in accordance with our previous study showing that the insertion of the group signature in a stranger song was sufficient to obtain a reduced territorial response, even if this group signature had been extracted from the song of more than one neighbour (Briefer et al. 2008a). In other terms, group identity, revealed by our previous study, has a prevalent effect over individual identity.

In our experiment, the spatial categorisation of songs of neighbours could be the result of a habituation process (Temeles 1994). Habituation is defined as a learning process that produces a response decrement to repeated stimulation (Thomson and Spencer 1966). Male skylarks could be habituated to individually distinct characteristics present in their adjacent neighbours' songs and to the position from which these songs are produced. This habituation should lead to a decrement in the aggressive response to neighbour songs. When neighbour songs are produced from an unusual position, like at the opposite boundary, they will then be perceived as a novel signal and will induce a recovery of the aggressive response. Bee and Gerhardt (2001) demonstrated that territorial animals could indeed be habituated to a distinct property of an acoustic signal and to the position from which it was played. Nevertheless, in a previous study, we found that the response to the N song was modified during the breeding season according to social and ecological circumstances, with a recovery of the aggressive response to neighbour songs at the end of the season (Briefer et al. 2008b). This indicates that the process underlying reduced aggression between neighbouring male skylarks cannot be habituation alone. Thus, spatial categorisation of songs observed in this study is more likely the result of 'true' individual recognition. True individual recognition occurs when the resident learns its neighbour's individually distinct characteristics and links them with individualspecific information about the neighbour (Tibbetts and Dale 2007). Male skylarks may individually recognize their adjacent neighbours using individually distinct characteristics of their songs and could respond differentially to N and S songs in contexts where the adjacent neighbour is less threatening than a stranger. However, field playback experiments do not allow us to differentiate between a habituation process and a true individual recognition. Further experiments in laboratory, like operant conditioning, are needed to distinguish between these hypotheses (e.g. Gentner and Hulse 1998).

Individual and group signatures

We attempted to highlight individually distinct characteristics of songs that male skylarks could rely on to show the observed spatial categorisation of neighbour songs. Our analysis showed that syllable and phrase compositions were individually distinct, as two songs of the same individual were more similar than two songs produced by different individuals of a given location. Furthermore, on average, 17% of the syllable repertoire and 30% of the phrase repertoire of each individual was not produced by any other studied birds of its location.

To allow an efficient recognition by conspecifics, the individual signature should be encoded in characteristics that remain constant between the different renditions of an individual. Our results showed

that on average 43% of individual phrases were repeated in more than one song of a given male, which is a greater percentage than the one of repeated non-individual phrases. On the other hand, only about 28% of individual syllables were repeated in at least two different songs of the same individual, compared to 56% of non-individual syllables. Thus, phrases are more stereotyped within individuals and more different between individuals than syllables. Analyses of variance showed some differences between individuals for the acoustic parameters measured in individual phrases and individual non-repeated sequences, especially for frequency parameters. However, coefficients of variation and discriminant analyses demonstrated that these parameters do not seem to show enough within-individual constancy to enable individual recognition. Thus, the spatial categorisation observed during the playback experiment more likely relies on individually distinct characteristics of the phrase repertoire. This requires further investigations by playing back modified stranger songs with individual phrases artificially inserted or modified neighbour songs with individual phrases artificially removed.

Our results show that male skylarks show a categorization of their neighbours' songs despite a very large repertoire (more than 300 syllables per male: Aubin 1982; Aubin and Brémond 1983; Briefer et al. 2008a), despite considerable song sharing (half of the song duration is composed of shared sequences: Briefer et al. 2008a) and, as a half-migratory species, despite a limited neighbour association period during the year. According to our analyses, the process of neighbour recognition in skylarks cannot be based on simple particular acoustic temporal or frequency features of syllables of a bird's repertoire ('individual voice characteristics'), such as found in great tits for example (Weary and Krebs 1992). Males more likely use the ordering of syllables within-individual phrases. Each male produces almost 40 different individual phrases. Thus, learning all individual phrases of each adjacent neighbour may require considerable cognitive abilities. As skylarks show strong phylopatriy and could thus potentially have the same neighbours each year, they may learn their neighbours' whole phrase repertoire and retain the memory of this repertoire from a given year to the next, as shown in the hooded warblers *Wilsonia citrina* (Godard 1991).

In a previous study, we found that non-individual phrases, i.e. phrases shared by neighbouring birds, were used by birds to discriminate a neighbour from a stranger song, thus supporting group identity (Briefer et al. 2008a). The continuous song of the skylark thus includes some phrases that are potentially distinguishable from the rest of the song and that carry a particular meaning for the birds, e.g. the group and the individual identities. Both individual and shared phrases have been found to be equally distributed in the song, indicating that the skylark song differs from songs of other species that are markedly stereotyped at the beginning but more plastic with individual characteristics at the end (e.g. tree pipit *Anthus trivialis*: Bjerke 1971; indigo bunting *Passerina cyanea*: Emlen 1972; goldcrest *Regulus regulus*: Becker 1974). Such homogeneous repartition of the group and individual signatures in the skylark song may be an efficient way to transmit the bird's identity during the entire song production.

Group and individual identity are part of the different messages conveyed by bird song, inducing several conflicting selection pressures to act upon song structure (Emlen 1972). To encode group identity, the songs of all birds established in the same location must contain similar features. In contrast, to encode individual identity, the songs of all these birds must contain features that differ among individuals. Thus, a conflict exists between group recognition that favours convergence and individual recognition that favours divergence. This conflict can be partially resolved by using different features or different ranges of the same feature to convey different information (Marler 1960). Skylarks seem to use such segregation of information by conveying group and individual identities, both at level of the syntax, but in different components of their song, i.e. in phrases shared by the neighbourhood and in individual phrases respectively, like found in other species (Schubert 1969; Bjerke 1971; Becker 1974; Bergmann et al. 1975; Thomson and Baker 1993; Nelson and Poesel 2007).

The segregation of information enables birds to produce songs with distinguishable components, allowing both group and individual recognition. However, in skylarks, the percent of shared phrases in the phrase repertoire (70.7%) seems great compared to the percent of individual phrases (29.3%), and the percent of shared syllables in the syllable repertoire (83.3%) seems also great compared to the percent of individual syllables (16.7%). This difference between the amount of shared components and individual components in the song could indicate that the conflict existing between group and individual identities has evolved in favour of the group in skylarks, inducing a convergence more than a divergence of neighbour songs. In our playback experiments, responses to the neighbour song played from the shared and opposite boundary were not different. Thus, results of both song analysis and playback experiment tie up with the hypothesis that group information is more important than individual information.

To conclude, we showed that male skylarks show a spatial categorisation of their neighbours' songs, potentially using individual variation in the syntactical arrangement of syllables in sequences. Dear-enemy relationships in skylarks are thus subtle relationships that can be modulated according to the spatial position of each neighbour.

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