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Habitat fragmentation effects and variations in repertoire size and degree of song sharing among close Dupont's Lark *Chersophilus duponti* populations

Cristian Pérez-Granados[1] · Tomasz Osiejuk[2] · Germán Manuel López-Iborra[3]

Abstract We describe the song type repertoire variation and degree of song sharing in three isolated and declining Dupont's Lark (*Chersophilus duponti*) populations located close to each other in eastern Spain, studied over two years. We found no differences between sites either in song rate or individual repertoire size. We detected great differences in song type diversity at the population level, ranging from five to 18 song types. We used, for the first time, the SIMPROF test, a method that objectively discriminates significant groups resulting from agglomerative clustering methods, to study geographic variation in bird song. We found four microdialects in the study area with a low degree of song sharing among populations, which may be related to the influence of habitat fragmentation and the song learning process and ecology of the species. We detected different patterns of song sharing among sites. All males of two populations shared about 80–100 % of their song types throughout the habitat patch. At the other site, we found two microdialects within the same habitat patch, where song types were only shared among neighbouring males. The high degree of song sharing found can be explained by the reduced dispersal movements of the species. We also detected a high consistency in the song types between years. The creation of corridors and habitat management in potential habitat patches surrounding Dupont's Lark populations could act as stepping-stones, improving the connection between populations and thereby song transmission between patches.

Keywords Dupont's Lark · Habitat fragmentation · Repertoire size · SIMPROF test · Song sharing

Zusammenfassung

Habitatfragmentierungseffekte, Variation in der Größe des Gesangsrepertoires und das Ausmaß von gemeinsamen Gesangselementen zwischen nahe beieinanderliegenden Populationen der Dupontlerche (Chersophilus duponti)

Wir beschreiben Variation im Gesangstyprepertoire und das Ausmaß, in dem Männchen dieselben Gesänge benutzen („song sharing"), in drei isolierten und in ihrer Größe abnehmenden Populationen der Dupontlerche (*Chersophilus duponti*). Diese Populationen liegen nahe beieinander in Ostspanien und wurden über zwei Jahre hinweg untersucht. Wir fanden keine Unterschiede zwischen Standorten in der Gesangsrate oder der individuellen Größe des Gesangsrepertoires. Wir fanden jedoch große Unterschiede auf Populationsebene in der Diversität der Gesangstypen (5–18 verschiedene Gesangstypen). Wir haben zum ersten Mal den SIMPROF-Test angewendet, um geographische Variation in Vogelgesang zu untersuchen. Diese Methode unterscheidet auf objektive Weise signifikante Gruppen,

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die sich mittels agglomerativer Clustermethoden ergeben. Wir fanden im Untersuchungsgebiet vier Mikrodialekte und ein geringes Maß an „song sharing", was mit dem Einfluss der Habitatfragmentierung, dem Gesangslernen und der Ökologie der Art zusammenhängen könnte. Wir fanden jedoch unterschiedliche Muster des „song sharing" zwischen Standorten. Alle Männchen aus zwei Populationen teilten sich etwa 80–100 % ihrer Gesangstypen über die gesamte Habitatfläche hinweg. Am anderen Ende des Spektrums fanden wir zwei Mikrodialekte innerhalb derselben Habitatfläche, und Gesangstypen wurden lediglich von benachbarten Männchen gemeinsam genutzt. Dass wir „song sharing" in hohem Maße gefunden haben, kann mit dem schwach ausgeprägten Abwanderungsverhalten dieser Art erklärt werden. Wir fanden auch, dass sich die Gesangstypen in beiden Jahren sehr ähnelten. Das Schaffen von Korridoren und Habitatmanagement in potenziellen Habitatflächen, die Dupontlerchen-Populationen umgeben und als Trittsteine wirken könnten, sollten die Anbindung von Populationen und somit die Weitergabe von Gesang zwischen den Habitatflächen verbessern.

Introduction

Birdsong is a sexually-selected trait that evolves under intersexual selection through female choice and intrasexual selection through male–male competition (Catchpole and Slater 2008). Repertoire size, which is defined as the number of song units (e.g. song types, syllables) produced by a male, is a song parameter often considered important in sexual selection. In some species, it has been proposed that larger song repertoires are linked to male quality (Buchanan et al. 1999; Hesler et al. 2012), and, thus, repertoire size predicts male survival and reproduction (reviewed in Collins 2004). However, other song parameters such as song rate or song sharing are also related to sexual selection and male quality in other species (e.g. Wasserman and Cigliano 1991; Beecher et al. 2000; Nolan and Hill 2004; Poesel et al. 2012).

Natural or artificial barriers are a common cause of song differentiation. Therefore, population divergence in songs may be influenced by human activities that often lead to the fragmentation of natural habitats (Balmford et al. 2003). Habitat fragmentation produces a reduction of suitable patch size and an increase in distance between habitat patches (Saunders et al. 1991; Debinski and Holt 2000; Briefer et al. 2010). This may influence the original conditions under which male repertoire size was acquired (Sung and Park 2005), and the pattern of song sharing among neighbouring males (Briefer et al. 2010). Habitat fragmentation modifies the features of acoustic signals and

the evolution of vocal communication (see review in Laiolo 2010). Birdsong seems to be a good indicator of the effects of habitat fragmentation in the short term (Baker et al. 2003), owing to rapid cultural transmission and change (Laiolo 2010; Pavlova et al. 2012).

Habitat fragmentation effects on birdsong have been well studied in Dupont's Lark (*Chersophilus duponti*). The Dupont's Lark is a resident and endangered passerine distributed in southern Europe and North Africa, inhabiting flat scrublands (Suárez 2010). Its European population has been estimated to be around 2200 breeding pairs, and is patchily distributed and declining (Tella et al. 2005; Suárez 2010; Pérez-Granados and López-Iborra 2014). Prior studies have shown that the communication system of the species is altered by habitat fragmentation both at local and regional scales, which may lead populations to exhibit geographic song variations (dialects), and higher song dissimilarity between neighbouring males in fragmented habitats than in continuous ones (Laiolo and Tella 2005, 2006). Likewise, the number of song types uttered per male and the song type diversity within a population decline as patch size, population productivity and population size decrease. Thus, song diversity can be viewed as an indicator of the population viability of the species (Laiolo and Tella 2007; Laiolo et al. 2008).

However, there is a lack of detailed studies on the differences between and within populations in male repertoire size, number of distinct song types in a population and their patterns of song sharing degree. Differences within and among populations in song sharing degree can be used to infer the pattern of song learning and its adaptive significance in each population (Petrusková et al. 2010; Nowicki and Searcy 2014; Vargas-Castro 2015). Likewise, there are no studies testing the persistence of Dupont's Lark song types among years. In some species, male repertoire size may change so rapidly that most of the song types present in an area can disappear from one year to the next (Sorjonen 1987; Trainer 1989). The degree of male repertoire size variation among years provides evidence as to whether males are age-limited song learners (Nordby et al. 2002) or if they are able to modify their songs throughout their lifetime by adding new song types (Todt and Geberzahn 2003; Araya-Salas and Wright 2013).

The main goal of this paper is to describe the spatial pattern of song variation and degree of song sharing between individuals in three declining populations in eastern Spain. This may provide new information about song behaviour and the responses of the species to habitat fragmentation. We hypothesised that the communication system of the species is modified by habitat fragmentation. Thus, we expected that, in fragmented habitats compared to continuous ones, the degree of song sharing would be higher between neighbouring males and that there would be high levels of song dissimilarity among plots.

We also attempt to determine whether habitat fragmentation is reflected in the existence of song differentiation between the habitat patches. Geographic variations of birdsongs have often been described as dialects or microdialects, depending on the spatial scale considered (Mundinger 1982; Briefer et al. 2010; Camacho-Schlenker et al. 2011). Dialect definition has changed over time and between species and studies. In some species, dialects have sharp borders and may be easily defined on the basis of particular simple variations of notes (Catchpole and Rowell 1993; Osiejuk et al. 2012), but when songs are varied and complex objectively setting apart dialects is difficult and the subjective component becomes more important (Eens 1997; Podos and Warren 2007). In this paper, we apply a novel technique designed to objectively identify groups, the SIMPROF test (Clarke et al. 2008), to cope with the problem of detecting geographic variation in birdsongs. When significant different sets of songs according to this test are restricted to a continuous geographic area we have defined them as microdialects.

Methods

Study area

The study was carried out from March to June during 2011 and 2012 in Rincón de Ademuz (Valencia, eastern Spain; 40°N, 1°3′W, 1000 m a.s.l.). We selected the three largest populations (Hontanar, Losar and Pinar) constituting the bulk of the study area metapopulation, because the number of males in the other two occupied habitat patches in the area was too low for statistical analyses (Pérez-Granados and López-Iborra 2013). Patch size ranged from 193 to 200 ha and the distances between studied patches were 2.1 and 4.5 km. They were surrounded by a matrix of forests and some agricultural areas, habitats considered unsuitable for the species (Suárez 2010). Local population densities during the study period, calculated by dividing the number of males estimated each year by the mapping method (see below) by patch size, were very similar (2011/2012 densities: 0.58/0.80 males/10 ha in Hontanar; 0.60/1.03 males/10 ha in Losar; and 0.65/0.80 males/10 ha in Pinar). The vegetation was a shrub-steppe dominated by *Thymus* spp., *Genista scorpius* and *Rosmarinus officinalis*. In all plots, there are some dispersed trees, especially pines *Pinus* spp. and junipers (*Juniperus oxycedrus* and *J. communis*). More information on the study area can be found in Supporting information I and Pérez-Granados and López-Iborra (2013).

Recordings

The number of males per plot was estimated each year by the mapping method. Each plot was completely walked six times at dawn when song activity was most intense. Locations of all detected birds on different days were mapped, and we considered an area to be a territory when a male was detected on at least three different days. In late May and early June of each year, the song of each male was recorded from as close as possible (range 10–40 m), using a digital portable flash memory recorder Marantz PMD 661 and a Sennheiser ME 67 shotgun microphone with a K6 powering unit and MZW67 PRO-SW wind protection. Recordings were performed only on windless days. Recorded positions were taken with the use of a GPS device with at least 3–5 m accuracy. We recorded over 90 % of males present in each plot every year.

We recorded song vocalisations of each male for a variable time in 2011 (mean ± SD; 67 ± 30 s.) while the 2012 recordings were standardised to a length of two min to ensure that the repertoire size of each male was totally recorded during this year. In 2012, 33 males were recorded twice (4 min) and the rest once (2 min; mean ± SD; 198 ± 58 s.). To assess whether the shorter recordings obtained in 2011 could be used in this study, we first calculated the necessary time for recording the full repertoire of the males recorded in 2012. We obtained a mean value of 45 ± 32 s (range 10–83 s.). Thus, we only analysed 2011 recordings longer than this value. Mean duration of the 2011 recordings selected was 82 ± 23 s. All the recordings of a given male, cluster of territories and nearby clusters were obtained on the same day, thus avoiding problems of individual identification on successive days (Laiolo and Tella 2005).

Song analysis

Songs were subjected to a low-pass band filter set at 1 kHz to eliminate any low frequency background noise. Song types were identified by a single observer using visual inspection of sonograms with Avisoft-SASLab Pro Software v.5.02 (Avisoft Bioacoustics, Berlin, Germany). Spectrograms for each song were produced with a 512-pt fast Fourier transformation (frequency resolution 43 Hz, time resolution 2.90 ms, Flat Top window and 87.5 % overlap).

Dupont's Lark song includes a variable number of discrete song types (2–12 per individual), which are largely shared and repeated in the same order by neighbouring males in spring (Laiolo et al. 2008). A song type consists of a low number of song units (usually 2–4) that occur together in a specific sequence. More details can be found in Fig. 2 and a typical sequence recorded in the study area is available in Pérez-Granados (XC216990, on www.xeno-canto.org). First, we coded the different song types with a capital letter (e.g. A, B, etc.). When we found a song type that had been previously catalogued, we labelled it accordingly, and if it was a new song type it was

categorised with a new label and added to the catalogue. When a song type was similar to one previously catalogued, but differed slightly on the basis of timing, frequency or shape, we created a template spectrogram for each and compared them with a spectrogram cross-correlation algorithm (Specht 2007), for the purpose of determining whether or not it was a new song type or only a variation within a concrete song type. We used the Avisoft SASLab Pro "Scan for template spectrogram patterns" function with the following settings: high-pass cut-off frequency 1300 Hz, max frequency deviation 0 Hz, identification threshold 0.5–0.8 (depending on the quality of the recording), masking interval 0 s, margin 0.1 s (Petrusková et al. 2008, 2010). If the cross-correlation algorithm was <0.5, these song types were treated as distinctive song types in subsequent analyses. Once song types of all males were classified, we built a binary matrix (presence/absence) of males × song types. We defined song-type diversity as the number of song types occurring in a population, and male repertoire size as the number of distinct song types within the repertoire of individuals (Laiolo and Tella 2007). We estimated individual song rate as the total number of songs uttered per minute for each male (Garamszegi et al. 2007; Laiolo et al. 2008). We only used 2012 recordings, which were obtained during a longer time period, to calculate song rate, since we expected that song rate could be affected by recording length.

Statistical analysis

During the study period, we conducted a ringing programme using small spring-traps that allowed us to colour-ring 11 and 22 males in 2011 and 2012, respectively (Pérez-Granados and López-Iborra 2015). However, as we were not able to identify each male while recording at dawn, and some males could have been recorded in both years, we used generalised linear mixed models (GLMM) to analyse differences in male repertoire size among sites and years. Despite these limitations, four males colour-ringed in 2011 were recorded in 2012 and individually identified since they were the only males singing in the recording locations. A GLMM was fitted with repertoire size as a dependent variable, Plot as a fixed effect with three levels (Hontanar, Losar or Pinar) and the year as a random effect with two levels (2011 and 2012). We used the likelihood ratio test to test for the significance of the fixed effect. For this analysis, we used the glmer function in the R "lme4" package (Bates et al. 2014) and a Poisson distribution for errors since the dependent variable was a count. Song rate in 2012 was compared between plots using one-way ANOVA.

Song accumulation curves have been used to estimate the repertoire size of singing birds, since this estimate is dependent on sample size, and the more songs that are analysed the fewer new song elements will be added to the repertoire estimate (Garamszegi et al. 2002). The asymptote of a function fitted to the accumulation curve will be an estimate of repertoire size. Despite some problems, at least when this method is applied to bird species with a large song repertoire (Botero et al. 2008), accumulation curves have been used frequently to estimate repertoire size (Zamm and Dunstan 2008; Franco and Slabeekoorn 2009; Rendall and Kaluthota 2013; Sosa-López and Mennill 2014). The same rationale may be applied to estimate song type diversity at the population level, since the more males that are recorded the less likely it will be to detect new song types in a given population. This is analogous to sampling species in a community (Gotelli and Chao 2013), but in this case each recorded male may be considered as a sample of the song types existing in the population. Therefore, we estimated a song type accumulation curve as a function of the number of males recorded (both years pooled) for each locality using the program EstimateS 9.1 (Colwell 2013), a tool commonly used to estimate species richness. For this purpose, we built an incidence matrix of male (samples) × song types (species) for each locality and obtained the expected number of song types for a random set of t samples (Eq. 17 in Colwell et al. 2012; also known as 'Mao Tau' estimator in former versions of this software) and their 95 % confidence intervals. As the number of recorded males was, by chance, the same in two localities (Hontanar and Pinar) and the number of song types stabilised after a few males were recorded in the other (Losar), we only compared the final estimates of song type richness among localities. If 95 % CI did not overlap, the song type diversity of each population was considered significantly different ($p < 0.01$; MacGregor-Fors and Payton 2013).

To identify groups of males with distinctive song type repertoires, we calculated the Jaccard index of song similarity for each pair of males (see details in Petrusková et al. 2010), and performed a subsequent cluster analysis of the similarity matrix. We used the similarity profile permutation test procedure (SIMPROF, using Primer v6; Primer-E, Plymouth, UK) to detect significant groups of males in the resulting cluster. SIMPROF is an exploratory data analysis method that uses null hypothesis testing to identify structure in ecological communities or in any group of samples, characterised by multiple variables from which a similarity matrix may be calculated (Clarke et al. 2008). This method may be used to objectively discriminate significant groups resulting from agglomerative clustering methods, provided that standard settings in software for sound analysis are used by different researchers, thus avoiding the subjective interpretation of geographic variation in songs (Eens 1997; Podos and Warren 2007). Although diverse quantitative

methods have been recently used in birdsong study, most of them have been developed to evaluate the song structure and the similarity among song types (e.g. Lachlan et al. 2010, 2013; Wellock and Reeke 2012), to our knowledge, SIMPROF has not been used before to study song geographic variation but has the potential to provide a more objective definition of dialects or groups of similar songs than has been possible thus far.

The procedure ranks the observed similarities between samples (males in our case) from smallest to largest and plots them against their ranks. An iterative permutation procedure, based on randomly shuffling song types, in our case across males, generates a distribution of permuted profiles from which the mean profile and its confidence intervals may be calculated. This procedure is run for the samples clustered at each node of the dendrogram, such that failure to reject the null hypothesis at any node implies that finer divisions further down that branch of the dendrogram are not supported. This is called a type I SIMPROF (Somerfield and Clarke 2013). To ascertain the level of structure present in the branches formed by the dendrogram representing male similarity, a similarity profile routine was run with 1000 permutations and 999 simulations, and the stopping rule specified at the 5 % significance level (Clarke et al. 2008). We defined as microdialects song groups that are significantly different according to SIMPROF, that are restricted to a continuous geographic area and that include at least one song type not shared with other significant groups. Given the design of our study, we included the additional condition of including at least two males recorded within the same year, to avoid defining a microdialect by the grouping of the same male recorded in both years. SIMPROF procedures were performed to test the null hypothesis that song type structure does not exist in the Dupont's Lark population of males studied.

We analysed within- and between-patch song variation at the level of song composition using song types as discrete units. First, we calculated the corresponding matrices of geographic distances between pairs of males (ranging between 65 and 10,763 m in 2011 and between 56 and 10,918 m in 2012). Second, to quantify the variation of the degree of song sharing with distance, we compared the Jaccard index of song similarity between four types of males defined according to the distance between them: close males, i.e. familiar neighbours (<500 m, equivalent to the maximum dispersal distance within sites detected in the study area from ringed bird recaptures; unpublished data); males within a particular habitat patch (excluding pairs of males in the first group), i.e. local groups (500–2000 m); males located in closer patches, Hontanar and Losar (2000–6000 m); and males from the most distant populations, Hontanar and Pinar (>6000 m). We tested the

hypothesis that song similarity was higher between males distributed closer in space (neighbours) than between males from local groups and males living in different habitat patches. To do this, we compared the Jaccard index between pairs of males situated in a given distance categories with two-tailed exact permutation tests using the Monte Carlo method (see Briefer et al. 2010). Correlations between song type similarity and distance between males were analysed using a permuted correlation test (1000 permutations) for matrices containing similarities and geographic distances (Briefer et al. 2010), a test equivalent to the Mantel test, that overcomes the problem of the lack of independence of distance or similarity matrices. These tests were performed using IBM SPSS Statistics v.22.

Results

Repertoire size and composition

We visually inspected 417 song spectrograms from 27 males in 2011 (9 in Hontanar, 10 in Losar and 8 in Pinar; total recording time: 37 min), and 1601 spectrograms obtained from 51 males in 2012 (15 in Hontanar, 20 in Losar and 16 in Pinar; total recording time: 2 h 48 min). We identified 34 and 29 song types in 2011 and 2012, respectively, for a total of 37 song types for both years pooled (Table 1). We counted an average of 5.07 ± 0.87 (mean $\pm$ SD) and 5.31 ± 0.75 song types per individual in 2011 and 2012, respectively (range 2–8). Male repertoire size and song rate did not differ significantly between areas (Male repertoire size; GLMM: Likelihood Ratio Test $\chi^2 = 2.13$, $p = 0.345$; $df = 2$; Song rate; ANOVA: $F_{2,48} = 2.328$, $p = 0.109$; Table 1). The song type diversity of each plot was similar between years, but differed greatly between sites as shown by non-overlapping confidence intervals (Table 1). The Hontanar population had larger song type diversity than the other two populations and Pinar had a larger song type diversity than Losar (Table 1).

Microgeographic variation and degree of song sharing

We were able to identify seven distinct groups in the SIMPROF analyses, which converge in three main branches, according to the three sites where the birds were recorded (Fig. 1). According to the definition of microdialect that we used, three of these groups cannot be considered as such because they comprised only one male (group E), two males recorded in different years (group B) or we did not detect at least one group-specific song type, i.e. not shared with other groups (group F shared song types

Table 1 Male repertoire size (number of song types uttered per male) in Dupont's Lark (*Chersophilus duponti*), song-type diversity per year and accumulated (number of song types registered in each population), and 95 % confidence intervals (CI) for song type accumulation in the three habitat patches studied per year. Song rate (number of song types/minutes recorded) of each population is also shown. Number of males recorded per population are shown in parentheses

| | Male repertoire size | | Song-type diversity | | | | Song rate |
	2011	2012	2011	2012	2011 and 2012	CI 95 %	2012
Pinar	5.5 ± 1 (8)	5.2 ± 0.4 (16)	11 (8)	8 (16)	11 (24)	7.55–14.45	10.92 ± 2.11 (16)
Losar	4.7 ± 0.6 (10)	4.9 ± 0.3 (20)	5 (10)	5 (20)	5 (30)	5–5	8.95 ± 2.51 (20)
Hontanar	5.2 ± 0.8 (9)	6.0 ± 1.0 (15)	18 (9)	16 (15)	21 (24)	16.89–23.11	9.77 ± 3.48 (15)

Fig. 1 Cluster dendrogram of Dupont's Lark (*Chersophilus duponti*) males studied according to their song type structure. *Short horizontal lines* in the dendrogram represent clusters significant at the 95 % level using the SIMPROF test. *Vertical dashed lines* separate males recorded in each locality, whose names appear at the *bottom. Grey boxes* represent males recorded in 2011, while *black boxes* males recorded in 2012
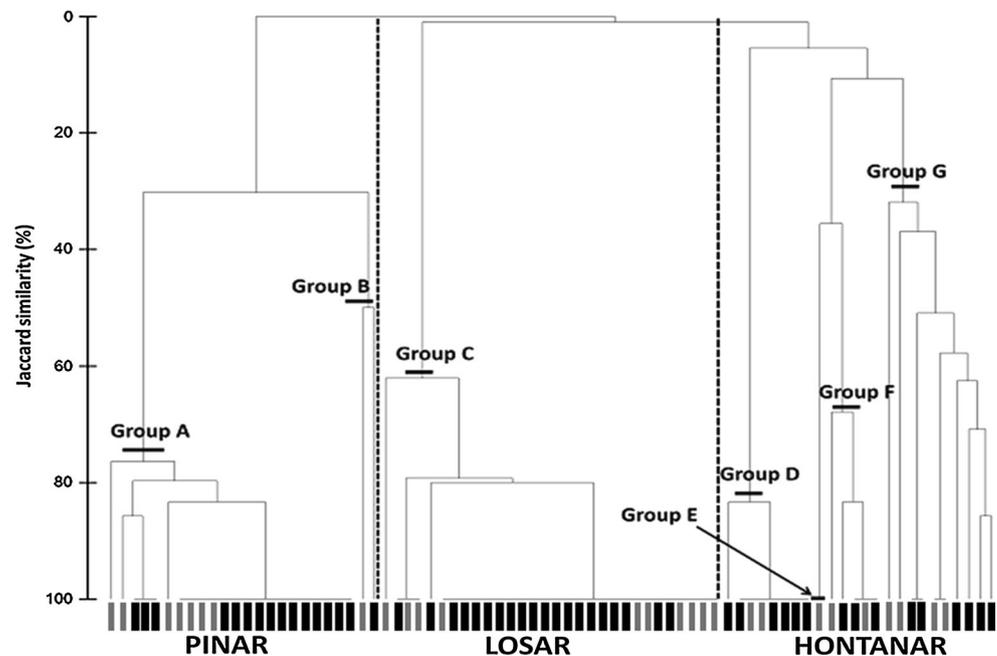
with males in groups E and G). Therefore, we identified four microdialects. Group A was defined by the presence of three unique song types only sung by these males (song types A, B and C; microdialect A). Group C was distinguished by another three unique song types (D, E, F, microdialect C); group D was characterised by four unique song types (G, H, I, J, microdialect D) and group G was also characterised by four exclusive song types (O, P, Q and R, microdialect G). Sonograms of the exclusive song types in the four Dupont's Lark microdialects found are shown in Fig. 2.

We found great differences within plots in terms of song sharing between males. In the main groups of Losar and Pinar, Jaccard similarity was >80 % in most cases (Fig. 3). However, in Hontanar, we found a greater variation in the degree of song sharing between males, to the point that some males of that population did not share any song type. In this locality, all males that composed group D were

recorded in the western half of the habitat patch, while the males of the other three groups (E, F and G) were located in the eastern half. Thus, we detected two microdialects within the same habitat patch (Supporting information I) sung by males separated by at least 500 m. All microdialects were sung in similar proportions of males between years (Table 2). We recorded a male in Hontanar in 2012 singing two song types (song type M and N) typical to Losar, representing the only case of song types shared among plots.

Song similarity decreased with distance (Fig. 3). The relationship was strong, significant and similar among years ($r_s = -0.74$ and -0.80 in 2011 and 2012, respectively; $p < 0.001$ in both cases; Fig. 3). The Jaccard similarity index for closer males (<500 m) was very high (approximately 0.82). For the males in the same habitat patch, but separated by more than 500 m, song similarity was still high (around 0.5–0.6; Fig. 3) but lower than for
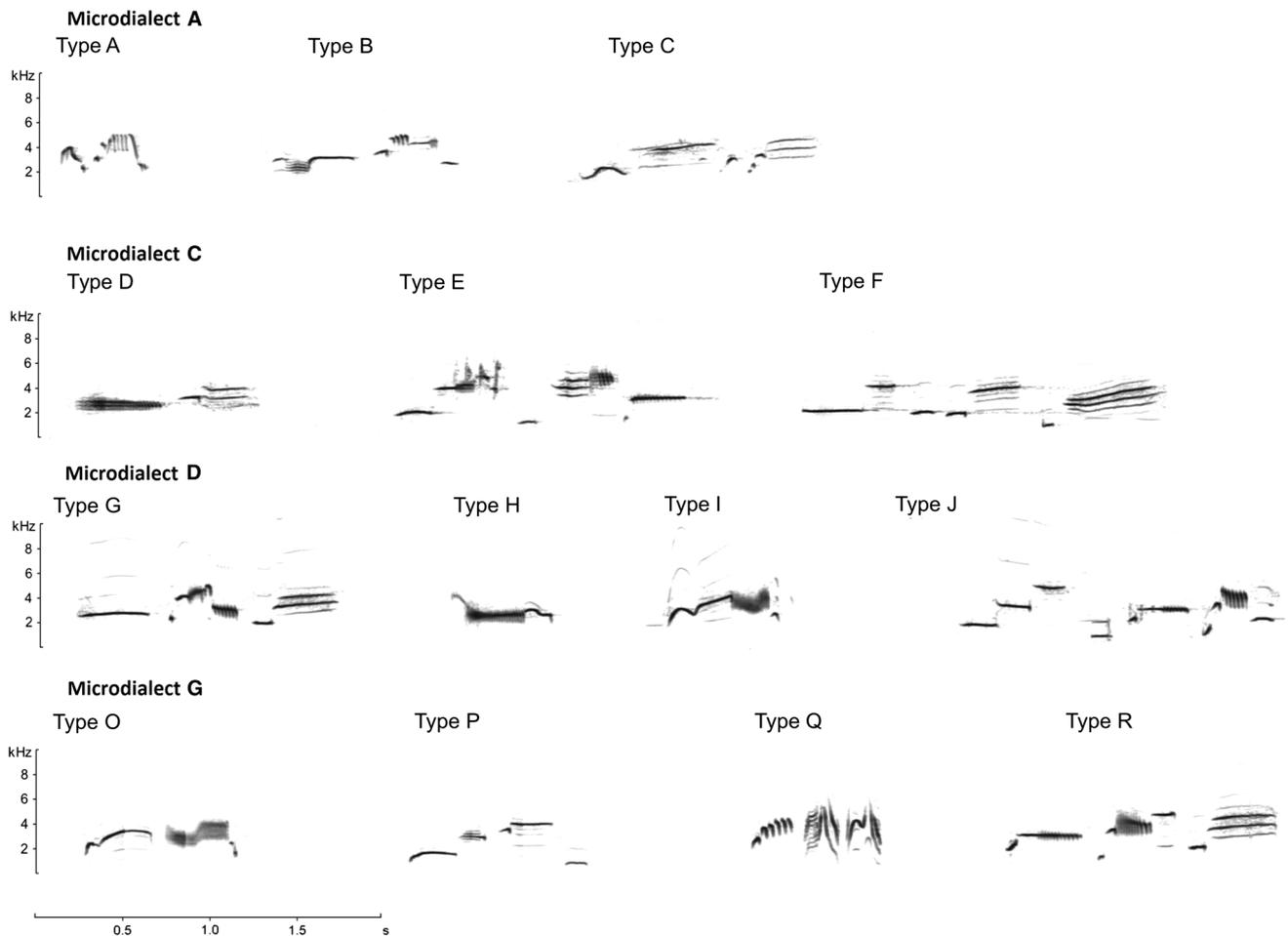
Fig. 2 Sonograms of the four microdialects found in the studied population of Dupont's Lark. Microdialects are named according to the group names identified by the SIMPROF analysis (Fig. 1). The recordings are shown in the Supporting information

closer males, especially in 2012 when the larger sample size led to the estimation of non-overlapping confidence intervals. On the other hand, this index was close to zero for males of different populations (distance >2000 m; Fig. 3).

Discussion

We found that song type diversity varies between sites, mainly due to differences in the degree of song sharing within populations. We found a microdialect in each population, and two microdialects in one population, which appear to be related to the effect of habitat fragmentation in the study area. There were no statistically significant differences in male repertoire size or song rate between populations, so the variation of song type diversity between populations cannot be explained by differences among individuals. The effect of year on microdialect spatial distribution was also small or nil, since males recorded in different years in the same patch converge in common SIMPROF groups. The consistency of the results across the two study years lends robustness to the conclusions of this study.

Contrary to our results, previous studies found differences among Dupont's Lark populations in male repertoire size (Laiolo 2008; Laiolo et al. 2008). In our study area, mean male repertoire size was lower than the value expected for habitat patches with an area similar to the studied populations (5.7 song types per male), but is within the range of variability found by Laiolo (2008). Likewise, we registered a maximum of eight different song types uttered per male when male repertoire sizes can reach values up to 12 song types in other populations (Laiolo et al. 2008). Thus, male repertoire size may be characterised as poor in the study area, in comparison with previous studies (Laiolo 2008; Laiolo et al. 2008), which might be due to the small number of males in the studied populations (15 males on average in the three patches). Several possible processes may contribute to explain
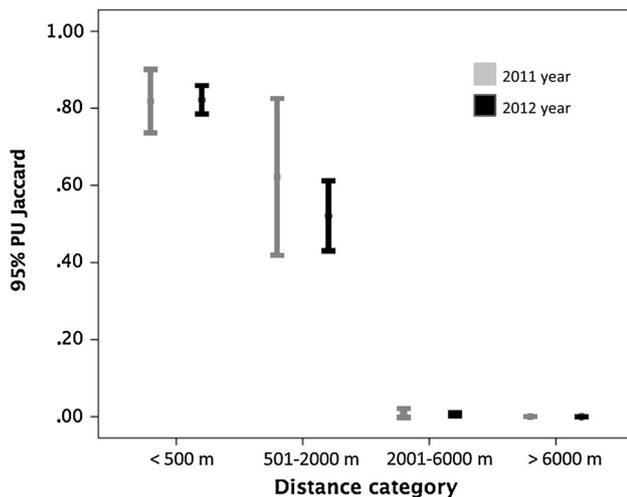
Fig. 3 Song type similarity measured by the Jaccard index among pairs of Dupont's Lark males from all study sites (1000 bootstraps). Means with 95 % confidence intervals summarise pairwise comparisons of all close neighbours within a site (distance <500 m), all local non-neighbouring males (distance between 501 and 2000 m), males from the two closest populations (distance between 2001 and 6000 m) and comparisons with all males from the most distant sites (distance >6000 m). The Jaccard index was calculated only for males recorded in the same year

smaller male repertoire size in small populations. On the one hand, a low number of Dupont's Lark males reduces the possibilities that immature birds will learn their songs through imitative singing (Laiolo and Tella 2007). Low male repertoire size and song type diversity may reduce the number of Dupont's Lark immigrants and thus the availability of new tutors for immature males (Laiolo and Obeso 2012). Finally, the isolation degree among populations and poor habitat quality may also affect male song repertoire, since inbred males or those with less genetic diversity tend to have smaller song repertoires than non-inbred males or those with higher genetic diversity (Marshall et al. 2003; Reid et al. 2005).

Song rate has been suggested as an indicator of male or territory quality in several passerine species (Gottlander 1987; Alatalo et al. 1990; Hoi-Leitner et al. 1995). In addition, other authors have provided convincing evidence that song rate is also related to escalated interactions among males (e.g. Searcy and Yasukawa 1990; Laiolo and Tella 2005). In our case, we did not find differences in song rate among plots, which may be due to the similar number and distance between neighbouring males in the study area or habitat quality among plots (Supporting information II). Finally, we should not forget that the studied populations have suffered an annual decline rate of around 7 % on average over the last several years (Pérez-Granados and López-Iborra 2013), and thus it is possible that the effects of population reduction on song diversity has been so powerful that we were not able to detect differences at an individual scale among populations. This correlation between group size decline and song diversity has been well documented in the study species (e.g. Laiolo and Tella 2007; Laiolo et al. 2008), as well as in mammals (Rendell and Whitehead 2003), including humans (Krause 1992).

At the population level, we found large differences in song type diversity among sites. Song type diversity was only five song types in Losar, but reached values up to 18 and 11 in Hontanar and Pinar, respectively. These values are within the expected range given by Laiolo and Tella (2007) for populations of similar patch size to those studied here. The larger number of song types registered in Pinar, in comparison with Losar, is explained almost entirely by the presence of two males with unique song types, who contributed up to four song types to the song type diversity of that population each year. These males may be immigrants from another population located 1 km away in the Cuenca Province (Pedro Izquierdo, 10 males; Suárez 2010) or may be survivors of two populations that were located 200 m away, which became extinct 4–7 years prior to data collection (Pérez-Granados and López-Iborra 2013).

The highest song type diversity found in Hontanar is explained by the presence of two microdialects within the population and by the higher song dissimilarity between the males of that population. One microdialect was located on the western side of the population, while the other was on the eastern side, demonstrating that neighbouring males

Table 2 Proportion of males (of the total recorded each year in each area) singing a particular microdialect

	Pinar		Losar		Western Hontanar		Eastern Hontanar	
	2011 (n = 8)	2012 (n = 16)	2011 (n = 10)	2012 (n = 20)	2011 (n = 2)	2012 (n = 6)	2011 (n = 7)	2012 (n = 9)
Microdialect A	100	94	0	0	0	0	0	0
Microdialect C	0	0	100	100	0	0	0	0
Microdialect D	0	0	0	0	100	100	0	0
Microdialect G	0	0	0	0	0	0	43	67

Number of males singing each microdialect was identified according to SIMPROF results

n Number of males recorded

located only a few hundred meters apart may independently develop and maintain their own songs, which significantly reduces the 5 km distance published by Laiolo (2008) to detect song variation between populations. The coexistence of two microdialects within the same population may be due to the existence of a narrow stretch of cereal crops in the middle of Hontanar. This field, with a mean width of 110 m and extending over 75 % of the patch area from north to south, was never occupied by the species during the study period and is a habitat type avoided by this lark (Seoane et al. 2006; Suárez 2010). Despite the presence of this cereal patch, both sides of Hontanar are connected through suitable habitat (small shrubs and flat relief), and there is no acoustic isolation between the two sides, since males of the western side are able to hear songs from the eastern side and vice versa. Previous studies have shown that the presence of elements of fragmentation in the habitat patches can affect the degree of song sharing in different passerine species (Cachtpole and Rowell 1993; Briefer et al. 2010), including the Dupont's Lark (Laiolo and Tella 2005). Our results show that land use changes, even at a reduced scale, may create insurmountable barriers for the communication system of the species between areas.

We found a high degree of song sharing between males within the same habitat patch but none among patches. These results may be explained by the species' behaviour and its song learning processes, which may be enhanced by habitat fragmentation in the study area (Briefer et al. 2010; Petrusková et al. 2010; Rivera-Gutierrez et al. 2010). Laiolo and Tella (2005) and Laiolo (2008) interpreted from their results that Dupont's Larks learn to sing from adult males after their dispersal movements, which mean that song transmission among individuals and populations depends on the dispersal of adult males. In the study area, adult mean breeding dispersal has been estimated at only 150 m ($n = 20$; own data), which may explain the high degree of song sharing found (Beecher et al. 1997; Hill et al. 1999). Likewise, the low adult dispersal movements could contribute to explaining the high consistency of song type diversity found across years and plots. Some adult Dupont's Larks recorded in their territories during 2012 had been ringed in a different plot as nestlings in 2011 (unpublished data). Despite this, we did not record song types of their natal sites in any case, which provide the first scientific evidence that immature Dupont's Lark males learn their songs from nearby adult males after dispersal movements (Laiolo 2008). Likewise, the fact that some males were recorded in their territories both years (identified by ringing and their territory location) and the high consistency across time of most of the song types seems to indicate that the species may be age-limited song learners or that they modify their songs only slightly from year to year.

Complete sharing of song repertoires within a whole habitat patch, as occurs in Losar, is unusual and is only relatively common in species in which each male has only a single song (Nelson and Marler 1994; Nelson 2000), but is in agreement with other studies developed with species of medium-repertoire size (Camacho-Schlenker et al. 2011). Previous studies in other Dupont's Lark populations reported an overall song-type sharing within localities of 16–54 % (Laiolo and Tella 2005). Interestingly, four census surveys were carried out in Losar during the breeding season in 2003 (Campos 2003) and none of them revealed the presence of Dupont's Lark in the area. Thus, it seems likely that the high degree of song sharing found in Losar may be a result of a recent founder effect since, if immature birds established their territories around the colonisers, they would have learnt to sing from them (Morton 1987; Holland et al. 1996; Lachlan and Slater 2003; Parker et al. 2012).

Song sharing was negatively related to distance, so that Dupont's Larks from nearby territories shared more song types than non-neighbouring birds, a pattern that held in the two study years. Song similarity among populations was close to null, despite being separated by only 2 and 4.5 km. Even we found high song dissimilarity between neighbouring groups of males within the same habitat patch. It is interesting that Laiolo and Tella (2005) found that 67 % of the song types registered (169 out of 253) were shared by at least two populations, in contrast to the meager 5 % (two out of 37) of song types that were shared between our studied populations. Our results are surprising given that the study by Laiolo and Tella (2005) covered most of the distribution range of the species in Spain and even included one population in Morocco. This may indicate that Dupont's Lark is severely isolated in our study area or that the degree of song sharing may differ greatly between regions due to unknown factors.

Previous studies proposed that the Dupont's Lark song plays a role in mate attraction (Laiolo and Tella 2008; Laiolo et al. 2008). However, there is no definitive data supporting this assumption (Laiolo et al. 2008). The relative stability of male repertoire size and song rate among males found in this study suggests that they are not targets for sexual selection by female choice in the study area. However, the poor repertoire sizes and extremely high degree of song sharing found may be due to a problem of cultural erosion, and thus females may not be able to select males on the basis of song traits (Laiolo et al. 2008). The high degree of song sharing found within neighbouring males and the fact that any adult bird crossed a microdialect boundary, despite some microdialects being separated by only hundreds of meters, suggest that male–male interactions may play a role in maintaining dialects through the supposed advantages of song sharing between neighbouring territorial males. This is reflected in the Dear

Enemy Hypothesis (Fisher 1954), which states that, by sharing neighbours' songs, males may be able to recognise them and show reduced aggression. Likewise, the female choice of a mate may also play a role in maintaining dialect populations, which could occur if females respond more positively to males singing the local dialects than alien ones (Baker 1982; Miyazaki and Nakagawa 2014). Experimental studies evaluating Dupont's Lark male responses to stranger and local songs will allow for the testing of the hypotheses about whether or not song sharing is a target for sexual selection by male competition or female choice in the species. This, combined with a quantification of gene-flow among populations with different song composition, may contribute to understanding the role that geographic song variation plays in creating reproductive barriers in the species.

The low values of male repertoire size, along with the high degree of song sharing within neighbouring males found in this study, are consistent with populations that are suffering problems of cultural erosion, which could be considered as the prelude to population extinction (Laiolo and Tella 2007; Laiolo et al. 2008). In general, our results confirm the typical pattern of isolation by distance, with microdialects differing between plots and a decline in song similarity with distance between males. The communication system of the species is disrupted by isolation and habitat fragmentation, even at a very small scale. These symptoms, and the fact that at least three populations have become extinct in the study area during the last 10 years (Pérez-Granados and López-Iborra 2013), lead us to recommend the rapid implementation of a conservation plan for the species in this area. This conservation plan may include the development of corridors and habitat management in potential habitat patches surrounding Dupont's Lark populations, which could act as stepping-stones, improving the connection between populations and thereby song transmission between patches. This study should stimulate future research to identify differential selection processes affecting Dupont's Lark songs in close populations, which may provide useful insights about song acquisition and conservation of the species.

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