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Preliminary acoustic analyses of the structure of Red-billed Leiothrix *Leiothrix lutea* (Scopoli, 1786) song samples from Northern Italy

Luca Baghino[1]* & Sauro Giannerini[2]

[1]Via P. Magretti 19/1, 16142 Genova, Italy
[2]Via A. La Rocca 77, 58051, Magliano in Toscana (GR), Italy

*corresponding author: luca.baghino@tiscali.it

LB 0000-0002-6849-0769, SG 0000-0001-9678-6412

Abstract - The authors present an introductory piece of research concerning the acoustic analysis of a set of song samples of Red-billed *Leiothrix Leiothrix lutea* from two regions of Northern Italy (Veneto, Liguria). By using an R package to analyze and find variations in the structure of the species' male songs, the count of the number of sound events detected, and the duration of pause event, show the most significant differences; moreover, the duration of signal events turns out to be different. The limited sample and the current lack of specific studies for comparison do not allow the authors to speculate whether the said variations are attributable to inter-individual variability or geographic isolation and habitat adaption: further research from wider geographic areas is no doubt needed, also using the methods we followed, to make the analysis replicable.

Keywords: acoustic analysis, exotic, Red-billed *Leiothrix Leiothrix lutea*, song, Northern Italy.

INTRODUCTION

The Red-billed Leiothrix *Leiothrix lutea* (Scopoli, 1786) is a polytypic species, distributed throughout a wide area ranging from southwestern Asia to southern China and northwestern India; it is naturalized in Japan, the Hawaiian Islands, Spain and France (Puglisi et al.2009). In Italy, the Red-billed Leiothrix is regarded as a resident bird (Baccetti et al. 2021), a naturalized breeding species whose populations belong to the subspecies *L. l. calypigia* (Brichetti & Fracasso 2010). It can be considered as such because its presence in the Italian peninsula consists of at least three self-sustaining populations (Verducci 2009). Its current distribution throughout Italy includes various populations scattered from Liguria in the Northwest (the region where some escaped birds formed the first breeding groups around the 1980s: Spanò et al. 2000), to Tuscany (Verducci 2009) and Lazio (Puglisi et al. 2011) in Central Italy. Fragmented populations are also known in the Veneto, Friuli-Venezia Giulia and in the Marche. Most of these settlements are believed to have originated around the 1990s or shortly before.

In neighboring Mediterranean France, the bird is widespread in the region of Nice (Belaud 2009) as well as the southwestern part of the country (Béarn: Basly 2007) and, further west, in the Iberian Peninsula (Pereira et al. 2019).In Italy, its breeding habitat consists of woodland with dense shrub undergrowth (Tuscany: Verducci, 2009), uncultivated

and abandoned crops with shrubland, and pine and holm oak thickets (Liguria: Spanò et al. 2000); in the Northeast (Veneto: Piva, 2019), the Red-billed Leiothrix preferably settles in fresh woods of chestnut and hornbeam, especially if close to streams.

To date, no analytical data is available on the species' song structure and the state of research on Red-billed Leiothrix vocalizations in Italy is poorly known. Although the species is described in some detail by Ramellini (2017), it is not yet sufficiently investigated; the Red-billed Leiothrix, whose song is generically defined as variable in structure (Collar et al. 2017), is considered a bird featuring a repertoire of high inter-individual variability (Farina et al. 2013).Three types of song have been described, which are of different duration or with quieter and subdued tones (Collar et al. 2017). It is a bird that can acoustically interact with different species in the community (Ramellini 2017) and enter into competition, probably impacting on the Eurasian Blackcap in eastern Liguria (Farina et al. 2013) and on the Common Nightingale in Veneto (Piva 2019).

Our work aimed to verify and analyze differences and kinds of variation in the structure of song samples along the Red-billed Leiothrix distribution range.

MATERIALS AND METHODS

During 2018 and 2020 we collected some recordings of songs performed by individuals from seven different sites in two regions of Northern Italy, fairly distant geographically (Veneto and Liguria, nearly 250 km apart), for which quality recordings were available.

In Veneto, where the species has been widespread since about 2000 (Brichetti & Fracasso 2010), recordings were obtained in two different sites, the (Piva 2019) and the Chiampo valley, a pre-alpine valley on the edge of the Lessini mountains, about 45 km apart.

In Liguria two areas were selected: one located in the eastern part of the region, along the Riviera di Levante with three sites, Mezzanego, Castiglione Chiavarese (Genoa) and Deiva Marina (La Spezia). Here a first dissemination center was reported in 1980s (Spanò et al., 2000) and the species' density was known to be so high (Baghino et al. 2013) that it turns out to be acoustically dominant in the local bird community (Farina et al. 2013). The other area lies 115 km away, in western Liguria, specifically in the westernmost part of the province of Savona. Here the Red-billed Leiothrix is known to have been present since the 2000s and is also able to perform some migratory movements (Chiusi 2011) in the region where the species' breeding range appears to be, if not separated, at least still discontinuous to date (Baghino & Fasano 2017).

We acquired song samples referable to the Type I song as it was described in Collar et al. (2017): a rather rapid and fluty warble of up to 15 notes, recalling the Eurasian Blackcap (*Sylvia atricapilla*). We collected the recordings from 15 April to 20 May, before the egg-laying phase of the species (Brichetti & Fracasso 2010) when the singing activity is most intense.

In all areas, 96kHz/24-bit digital stereo recorders (Sony PCM-M10, Zoom H1) coupled with parabolic microphones (Dodotronic Hi-Sound stereo) were used on sunny/non-rainy days with light wind (wind speed < 0.3 m s^{-1}) and in absence of appreciable background noise. The audio files were recorded at a 48kHz/24bit sample rate in WAV format to achieve high sound quality and they were run by an audio analysis and editing software (Adobe Audition 7.01). Fourteen files contain multiple songs of the same individual, while two files include multiple songs of two different individuals. The songs analyzed were individually selected from the recordings obtained with parabolic microphones.

We also carried out deeper analyses of the male song through the open-source program RStudio (RStudio, Inc., 250 Northen Ave., Boston, MA 02210 844-448-1212) featuring a set of integrated tools designed to interact with an R environment for syntax highlighting, while taking advantage in particular of the added-in Seewave package (Sueur et al. 2008), considered the most suitable for our case study.

For each song (*.WAV file) we computed the duration of syllable periods, of pause periods

between syllables, and their ratio (function: timer; Fig.1 and Fig. 2). We set an amplitude threshold of 15% applied on the Hilbert envelope with no time threshold and power factor. In order to smooth the time wave, and thus remove the residual noise, we also set msmooth, that is preferable for short (<60 s) sounds (Sueur 2018). The window length was a set of 512 samples without overlap. We analyzed the male songs considering three parameters: i) "s" as the duration of signal syllable (s) in seconds, ii) "p" as the duration of pause event among syllables in seconds (s) and iii) "n" as the count of the number of syllables detected.

To determine the distribution of all three parameters, we performed a Shapiro-Wilk test which did not show evidence of non-normality (parameter "s": W = 0.958, *P* = 0.068; "p": W = 0.976, *P* = 0.071; "n": W = 0.988, *P* = 0.570). Based on this outcome, we decided to use a parametric test. We calculated the mean and standard deviation of each parameter by site; furthermore, an analysis of variance (one-way ANOVA) was carried out to compare the difference between sites.

To analyze and find variations in the structure of species song we examined a batch of 100 songs. The duration of recordings is between 2 seconds and 7 seconds, with most samples coming from sites in the Veneto (*N* = 63) and Liguria (*N* = 37), of which 23 are from the eastern part of the region and 14 from the western part): in this species, the frequency of the male song ranges from 1500 to 3800 Hz (Farina et al. 2013).

In order to test the reliability of the sample obtained using the timer () function, we carried out the manual measure of a subsample (*N*=27) of songs. For each parameter considered (s, p, n) the difference in average of the two samples was tested by a paired t-Test. The results (parameter s: *t* = -1.622, *df* = 52, *P* = 0.110; parameter p: *t* = 0.413, *df* = 52, *P*= 0.681; parameter n: *t* = 0.278, *df* = 52, *P*= 0.781) allowed to accept the null hypothesis for each parameter, and therefore to consider the sample obtained with the timer () function as valid.

RESULTS

The summaries of results obtained from the analysis of song samples (minimum, maximum, mean, and standard deviation) by parameter were: "s" (min=0.01, max=0.17, mean 0.075±0.02), "p" (min=0.05, max=0.23, mean 0.13±0.03) and "n" (min=8, max=32, mean 16.03±4.65). The mean results by area are illustrated in Tab.1 and in the Fig. 3 and 4. All ANOVA tests were significant. All three parameters in the song samples of Red-billed Leiothrix differed significantly among the sites considered (one-way ANOVA: parameter "n": F = 7.16, P = 0.000; "p": F = 4.48, P <0.01; "s": F = 2.717, P<0.05). Our analyses showed differences in duration with a mean number of notes spanning wider than the 15 indicated by Collar et al. (2017); however, and in general, the current lack of research on the specific issue of song structure in the Red-billed Leiothrix allows a limited potential for comparisons and considerations.

DISCUSSION

A vast body of scientific literature has focused on the general topic of geographic variation in birdsong, which is most widespread among *Passeriformes*. Very distinctive intraspecific variations in syntax are found in a number of species and the microgeographic perspective primarily tends to focus on differences in phonetic variation (Mundinger 1982): genetic and cultural drift, natural, sexual and cultural selection are the causes called into question (Podos & Warren 2007). In the case of the Red-billed Leiothrix, a further source of variation may be the result of the species' ability to get adapted to the other species found in bird communities (Farina et al. 2013), and this might also have a role on a local scale.

The song repertoire of a species is affected by the environment and the density of individuals: the case of the Red-billed Leiothrix, an invasive and a new acoustically dominant species (Farina et al. 2013), a competitor with the power to impact on some native songbird species (Pereira et al. 2020) and a massive expansion throughout Western Europe (Herrando et al. 2010), can fit into this context.

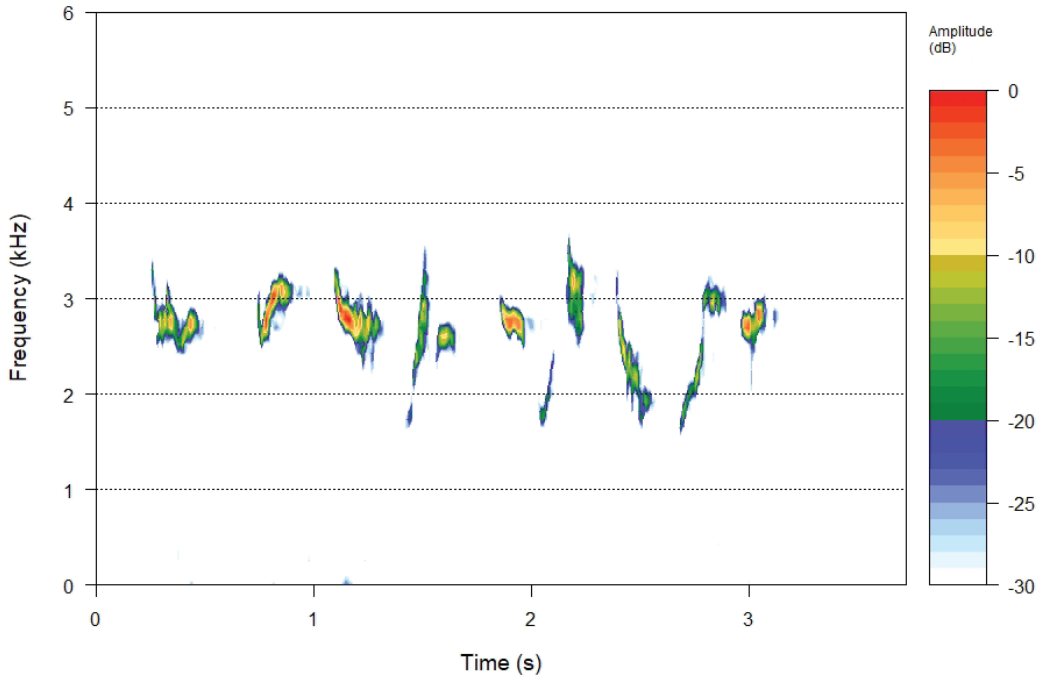

Figure 1. Spectrogram of male song obtained with "spectro ()" command. This function returns a two-dimension spectrographic representation of a time wave. The function corresponds to a short-term Fourier transform. We have run function using the default settings and modified the frequency Y-axis limits (in kHz), using flim = c (0.6).

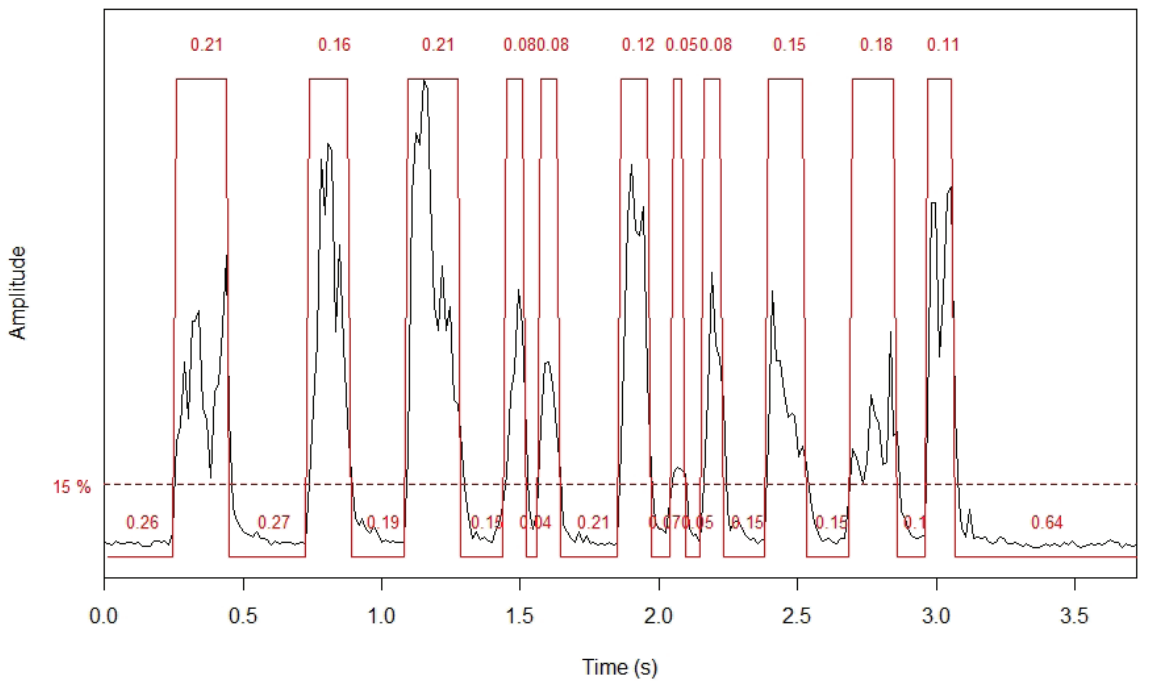

Figure 2. Plot resulting from the analysis of the parameters using the command "timer ()": this function computes and shows the duration of signal periods, the pause periods and their ratio. The amplitude threshold set for signal detection is 15%.

Table 1. Summary data of the song samples examined acoustically from recording sites of Northern Italy (V = Veneto; L = Liguria); the number of syllables, the duration of the emission and the duration of pauses are calculated with the mean ± standard deviation **(μ±SD)**; the measurement of time is in seconds.

Region	Site (province)	Geographic coordinates UTM WGS84	Habitat	Days/ session	Year	N strophes	N syllables (μ±SD)	Duration of the emission (μ±SD)	Duration of pauses (μ±SD)
V	Chiampo Valley (VI)	676325 5045795	Broadleaved woodland with thick undergrowth near a stream	1	2018	8	18.5±3.02	0.06	0.13±0.02
V	Colli Euganei, Torreglia (PD)	711950 5022070	Mixed woodland with predominant chestnut	1	2020	55	17.4±5.19	0.07±0.02	0.15±0.04
L	Deiva Marina (SP)	543370 4898100	Pinewood with thick undergrowth	1	2018	3	16.00±2.00	0.1±0.04	0.12±0.02
L	Mezzanego (GE)	534640 4914140	Shrubland with scattered patches of trees	2	2018	15	13.53±2.55	0.08±0.01	0.12±0.02
L	Castiglione Chiavarese (GE)	537770 4900750	Pinewood with thick undergrowth	1	2018	5	22.2±6.30	0.04±0.03	0.09±0.03
L	Casanova Lerrone (SV)	421230 4875700	Thermo-mesophilic woodland with undergrowth and ditches near a stream	1	2018	11	12.36±2.37	0.09±0.03	0.14±0.02
L	Garlenda (SV)	426600 4875100	Mixed woodland with thick and high undergrowth	1	2020	3	17.33±1.15	0.08±0.01	0.12±0.04

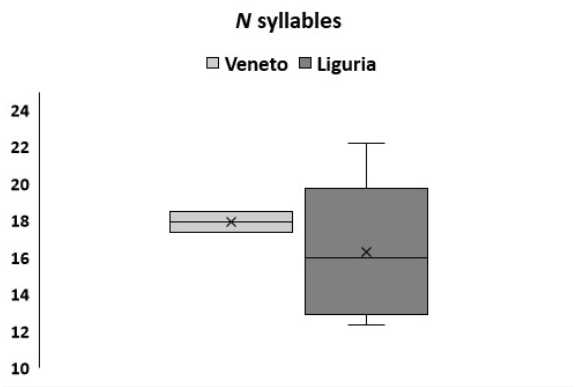

Figure 3. Number of syllables detected in the song samples from Veneto and Liguria regions.

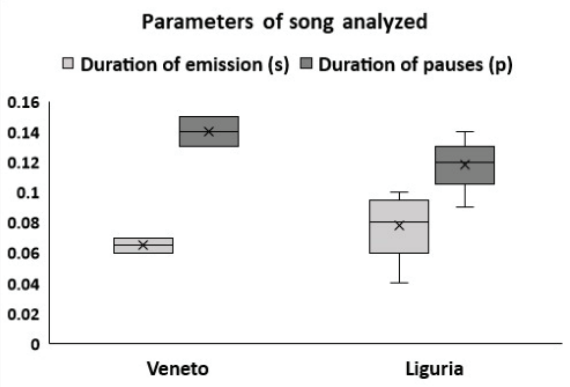

Figure 4. Parameters analysed (duration of emission and of pause in seconds) in the song samples from Veneto and Liguria regions.

The data collected represent an exploratory piece of research on this so far poorly known issue: our set of recorded samples with its differences in time duration is too numerically limited to argue if and how much such differences may be related to inter-individual variability or geographic isolation and habitat adaption (Lovell & Ross Lein 2013). And even as the song patterns of conspecific individuals from not very distant breeding areas of Northern Italy are different, we cannot make any assumptions about the various origins of these disjoint clusters of breeding birds.

Conversely, we were unable to expand the range of samples using materials available from the main website (www.xeno-canto.org) dedicated to sharing wild bird sounds. The recordings of species under pressure due to trapping are restricted, and it was not possible to download them.

Since bio-acoustic and eco-acoustic information is still largely lacking, further research is needed in regard to the variations of Red-billed Leiothrix vocalizations, with many more samples to be analyzed from wider geographical areas, both within and outside Italy.

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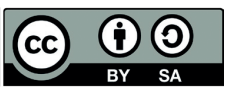

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