

# Current landscape attributes and landscape stability in breeding grounds explain genetic differentiation in a long-distance migratory bird

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## Keywords

gene flow; landscape genetics; landscape resistance; Normalized Difference Vegetation Index (NDVI); *Luscinia svecica azuricollis*; genetic differentiation; isolation.

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## Abstract

Numerous studies have highlighted a major role of isolation by both geographic distance and current landscape resistance in controlling bird population genetic differentiation. However, the importance of past landscape features or landscape temporal stability in shaping population genetic structure remains undervalued, particularly in birds. We assessed the role of isolation by landscape resistance – derived from current landscape attributes and measures of landscape stability –, in comparison to geographic isolation, as drivers of genetic differentiation of the Iberian bluethroat (*Luscinia svecica azuricollis*), a migratory bird whose populations breed in fragmented and dynamic landscapes affected by land use change. First, we characterized bluethroat genetic structure using microsatellite genotypes and evaluated genetic distances. Then, we built species distribution models using as a predictor a time series (two decades) of values of the Normalized Difference Vegetation Index as proxies of recent past landscape stability and current landscape features to ultimately generate landscape resistance values through different functions. Finally, we used maximum-likelihood population effects models to evaluate the relationships between genetic distances and both geographic and landscape resistance distances. We found a genetic structure of four clusters in the Iberian bluethroats populations, as well as a high level of genetic differentiation. Genetic structure was better associated with landscape resistance, rather than with geographic distance. The highest values of habitat suitability corresponded to areas where vegetation remained mostly stable during the two decades prior to bird surveys, with low annual precipitation and spring temperature, being the relationship between gene flow and presence of intervening habitat among populations linear or quasi-linear. Our results suggest that conservation policies and land management practices that promote the maintenance of semi-open pasture-shrub mosaics (e.g. through extensive livestock grazing) can strongly benefit Iberian bluethroat populations, improving gene flow and population connectivity.

## Introduction

In the Mediterranean region, centuries of complex interactions between nature and human activities, such as agriculture, forestry and livestock traditional farming practices, have shaped heterogeneous and dynamic landscape mosaics of semi-natural open habitats with high conservation value

(Fowler, 2003). Nowadays, the persistence of these human-shaped landscapes is compromised by two opposite trends of land use change: abandonment of traditional activities and agricultural intensification (Álvarez-Martínez *et al.*, 2014; Cervera *et al.*, 2016). Typically, secondary succession processes after land abandonment are leading to landscape homogenization in marginal lands, while land intensification

1 is occurring in the most productive areas, like the fertile val-  
2 ley bottoms (Kuemmerle *et al.*, 2016). As a consequence of  
3 these changes, species living in semi-natural open habitats  
4 are suffering progressive isolation due to fragmentation and  
5 loss of suitability of their habitats (Suárez-Seoane & Baudry,  
6 2002; Caplat & Fonderflick, 2009; Herrando *et al.*, 2014).

7 In heterogeneous landscapes, current landscape composi-  
8 tion (i.e. availability of suitable habitat) and configuration  
9 (i.e. spatial connectedness) determine species dispersal and  
10 gene flow, with habitat loss and fragmentation potentially  
11 leading to population size reduction and genetic differentia-  
12 tion, either through genetic drift or natural selection (Johnson  
13 *et al.*, 1992; Barton, 2001; Wang & Summers, 2010; Jackson  
14 & Fahrig, 2016). When landscapes are highly dynamic due  
15 to quick changes in land use/cover, the explicit consideration  
16 of landscape stability associated with habitat management  
17 actions is particularly valuable to understand population  
18 genetic patterns (Fuller *et al.*, 1995; Frankham, 1995, 2005).  
19 Recent past landscape changes (landscape legacies) can be  
20 related to extinction-colonization processes, including  
21 sequential founder events (Wade & McCauley, 1988), acting  
22 as potentially important evolutionary forces (Barr *et al.*,  
23 2008; Amos *et al.*, 2012, 2014; Athrey *et al.*, 2012).

24 Numerous studies have highlighted a major role of isola-  
25 tion by geographic distance (IBD) in controlling gene flow  
26 and, consequently, population genetic structure across the  
27 landscape (Wright, 1946; Spear *et al.*, 2010). However,  
28 thanks to recent technical advances, it has been demon-  
29 strated that, in heterogeneous land mosaics where the land-  
30 scape matrix might limit species dispersal (McRae, 2006;  
31 Peterman *et al.*, 2014), isolation by current landscape resis-  
32 tance is most correlated with gene flow (Dudaniec *et al.*,  
33 2016; Milanesi *et al.*, 2017). So far, relative fewer studies  
34 have used past landscape patterns (landscape stability) to  
35 evaluate the role of isolation by landscape resistance (but  
36 see Zeller, McGarigal, & Whiteley, 2012; Fant *et al.*, 2014;  
37 Bishop-Taylor, Tulbure, & Broich, 2017). Among the data  
38 sources available for evaluating the role of past landscape  
39 patterns in landscape genetics, multitemporal series of  
40 remote sensing data show the greatest potential (Manel  
41 *et al.*, 2003). Such data can be easily integrated with other  
42 ecoclimatic datasets, affording opportunities to test hypothe-  
43 sis at different temporal scales and develop new research  
44 paradigms (Porter *et al.*, 2009). Despite their usefulness,  
45 remote sensing data have been rarely used in landscape  
46 genetic studies, probably due to a gap of interdisciplinary  
47 integration between landscape ecologists and population  
48 geneticists (Storfer *et al.*, 2007; Epps & Keyghobadi, 2015;  
49 Balkenhol *et al.*, 2015).

50 In this study, we develop a species distribution modelling  
51 approach based on remote sensing data to evaluate the role  
52 of isolation by current landscape resistance and landscape  
53 stability, in comparison to isolation by geographic distance,  
54 as drivers of current patterns of genetic differentiation in Ibe-  
55 rian bluethroats (*Luscinia svecica azuricollis*). The Iberian  
56 bluethroat is a passerine breeding in fragmented and shifting  
57 semi-natural mosaics associated with young stages of the  
58 ecological succession (i.e. degraded holm oak shrublands,

heathlands and broom formations; Alda *et al.*, 2013), where  
the species benefits from abundant nesting and foraging  
opportunities (García-Tejero *et al.*, 2013; Arizaga, Alonso &  
Castro, 2017). The persistence of these mountain habitats in  
the species breeding range is threatened by land abandon-  
ment, which leads to a loss of open spaces and, subse-  
quently, to a landscape homogenization towards shrublands  
and woodlands (Morán-Ordóñez *et al.*, 2012) and a decrease  
in breeding habitat availability. Bluethroat habitat availability  
changes across the landscape rapidly due to disturbance  
events (i.e. fires and grazing) followed by secondary succes-  
sion (i.e. shrub encroachment), which results in a framework  
for habitat choice and local genetic divergence at relatively  
small scale (Guschanski *et al.*, 2008). In a previous study,  
Alda *et al.* (2013) investigated the genetic structure of  
*L. s. azuricollis* in its northernmost breeding grounds.  
Despite bluethroats being long-distance migrants with great  
mobility, Alda *et al.* (2013) inferred three highly divergent  
genetic groups in the Iberian subspecies. However, their  
results did not support the hypothesis that geographic dis-  
tance was the driver of this differentiation. Here, we expand  
the scope of that study by retesting the isolation by distance  
hypothesis across the whole distribution range of the Iberian  
subspecies, considering, as alternative hypotheses that land-  
scape stability (recent past landscape resistance) and/or cur-  
rent landscape resistance are the drivers of genetic  
differentiation. This work would provide insights into the  
effect of habitat connectedness changes resulting from large-  
scale habitat management activities on the genetic structure  
and diversity of a highly vagile species.

## Material and methods

### Study model

The bluethroat *Luscinia svecica* is a long-distance migratory  
passerine that breeds from Iberia to Eastern Siberia and  
Alaska, overwintering in the North of Africa and the South  
of the Iberian peninsula (Cramp, 1988). Bluethroats show  
great site fidelity to their breeding grounds (Arizaga &  
García, 2013), so the genetic diversity observed in Europe  
could be due to geographical isolation and local adaptation  
processes (Johnsen *et al.*, 2006). Ten subspecies have been  
described according to both male throat ornament and plu-  
mage patterns, although the taxonomic classification of this  
subspecies complex is not exempt from controversy (García  
*et al.*, 2017). The subspecies complex is associated with phe-  
notypic variations and life history. Bluethroats breed in a  
wide variety of habitats across their distribution range, from  
the sea level up to 4000 m a.s.l.: (1) In North and Central  
Europe, the species is mostly associated with alpine and sub-  
alpine low scrublands and wetlands; (2) in France, it breeds  
in salt meadows; and (3) in the Iberian peninsula, bluethroats  
nest in broom and holm oak shrublands (Cramp, 1988). The  
Iberian subspecies *L. s. azuricollis*, located at the southern-  
most edge of the species' range in Eurasia, is genetically  
and phenotypically well differentiated from the other sub-  
species (Johnsen *et al.*, 2006; Hogner *et al.*, 2013; García

*et al.*, 2017) and shows higher genetic differentiation than their north-European counterparts (Alda *et al.*, 2013).

### Bird sampling, DNA extraction and microsatellite genotyping

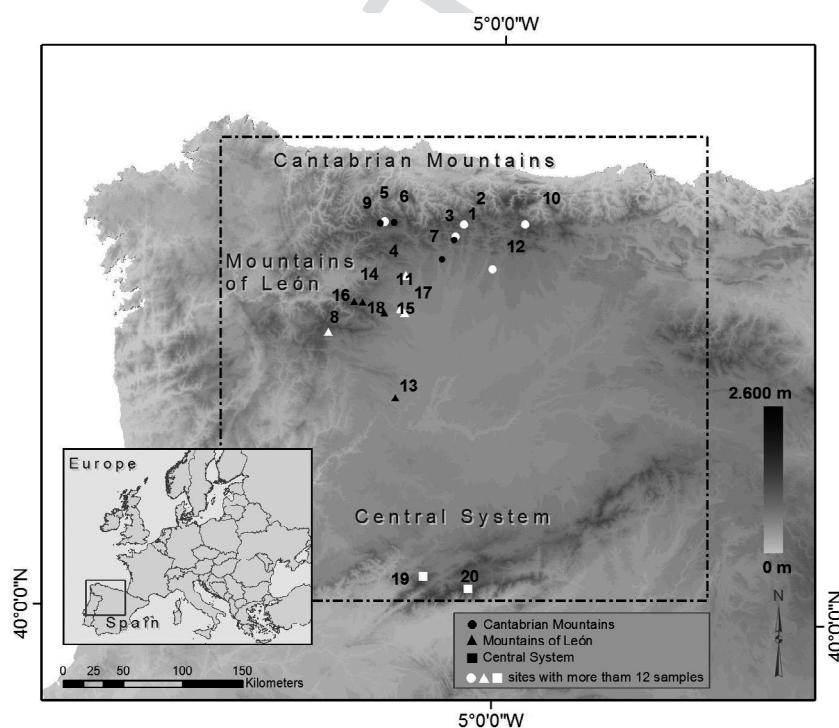
Between 2010 and 2011, we sampled 266 bluethroats in 20 breeding localities distributed all across the whole *L. s. azuricollis* range in the Iberian Peninsula: nine in the Cantabrian Mountains, nine in the Mountains of León and two in the Central System (Fig. 1; Supporting Information Tables 1S1 and 2S1 from Appendix S1). From this set of samples, 83 had been included in an earlier study (Alda *et al.*, 2013). Bluethroats were captured using tape-lured mistnets and claptraps baited with mealworms (Johnsen *et al.*, 2006). We extracted blood samples by venepuncture of the brachial or jugular vein, preserved them in absolute ethanol and stored at  $-20^{\circ}\text{C}$ .

Genomic DNA from blood was extracted and samples were genotyped for 12 microsatellite loci: Aar8, Ase19, C $\mu$ 4, C $\mu$ 10, Fhu2, Hru7, Mcy4, PAT MP 2-43, Pdo5, Phtr2, PmaC25 and Ppi2. Two of the 12 loci screened were removed from further analyses because Pdo5 showed strong evidence of null alleles and Aar8 was monomorphic. A more detailed description of DNA extraction and microsatellite amplification can be found in Alda *et al.* (2013).

### Genetic patterns and processes

We investigated the genetic structure of Iberian bluethroat breeding populations using GENELAND v. 4.0.4 (Guillot, Mortier, & Estoup, 2005) (See Supporting Information Appendix S2 for further information on GENELAND settings). The differentiation was evaluated using  $F_{ST}$  values, as a measure of pair-wise genetic distance (Weir & Cockerham, 1984), with the “hierfstat” R-package (Goudet, 2005). Bonferroni correction was applied on  $P$ -values for  $F_{ST}$ . We used ANOVA to test differences in genetic diversity among clusters and localities on the basis of the number of different alleles ( $N_a$ ), the observed heterozygosity ( $H_{obs}$ ) and the expected heterozygosity ( $H_{exp}$ ) that were calculated in GenAlEx v.6.5 (Peakall & Smouse, 2012) and FSTAT v.2.9.3 (Goudet, 1995).

The Inbreeding Fixation Index ( $F_{IS}$ ) was quantified for each cluster and locality using GenAlEx v.6.5. Additionally, the effective population size ( $N_e$ ) was estimated for each cluster to explore both current population size and population demographic history using LDNe v.1.31 software (Waples & Do, 2008). This analysis is based on the Burrow’s measure of linkage disequilibrium and assumes that genetic drift is the only process responsible for the signal in the data, instead of selection, mutation or migration processes. We excluded those alleles with a frequency lower than 0.02 and



**Figure 1** Limit of the study area, fixed considering the current breeding range of the subspecies in the Iberian Peninsula. The figure shows the localities sampled for genetic analyses. See Table 1 for correspondence between site numbers and names. Symbols represent different mountain regions within the breeding range of *Luscinia svecica azuricollis* (circles: Cantabrian Mountains, triangles: Mountains of León, squares: Central System). Symbols in white indicate sites with more than 12 samples. The dashed rectangle delimits the area where the habitat suitability model was fitted.

1 calculated 95% confidence intervals using a jack-knife  
2 approach.

3 Finally, we tested for genetic signals of a rapid reduction  
4 in population size at both cluster and locality levels using  
5 the program BOTTLENECK 1.2.02 (Piry, Luikart, & Cornuet,  
6 1999). We assumed a two-phase model of multistep  
7 mutation, accounting for 5%, 10% and 20% of all mutations.  
8 Significance of heterozygosity excess was determined using  
9 the Wilcoxon signed-rank test, which provides the most  
10 powerful statistical results for tests of bottlenecks in datasets  
11 with small samples sizes and less than 20 loci. Following  
12 statistical recommendations for the locality-level analyses  
13 (Kalinowski, 2005; Peery *et al.*, 2012), we only included  
14 those sites where 12 or more individuals were genotyped (12  
15 sites; Supporting Information Table 1S2).

### 17 **Current and recent past landscape** 18 **patterns: Habitat suitability modelling** 19 **analysis**

21 We modelled habitat suitability for Iberian breeding blue-  
22 throats using MaxEnt 3.3.3k (Phillips, Anderson, & Schapire,  
23 2006), which is a machine learning method designed for evalu-  
24 ating the species–environment relationship on the basis of spe-  
25 cies presence-only data (Elith *et al.*, 2006, 2011). Since our  
26 focus was on identifying areas holding suitable habitat  
27 between the existing populations, rather than predicting the  
28 potential distribution patterns over the entire Iberian Peninsula,  
29 the extent of the study area was fixed as a squared window  
30 including the current breeding range of the subspecies in the  
31 Iberian Peninsula (120 000 km<sup>2</sup>; Fig. 1).

32 Bluethroat occurrence data used for model calibration  
33 included locations covering the whole breeding range of the  
34 subspecies that were sourced from own fieldwork during the  
35 period 2010–2011, local publications, museum collections  
36 and local ornithologists. A total number of 796 field surveys  
37 were conducted following a point transect sampling method  
38 (Bibby *et al.*, 2000) during the breeding season (from 1st  
39 May to 15th June, 2010 and 2011), 3 h after sunrise and  
40 under good weather conditions. At each sampling point, spe-  
41 cies presence was recorded during 5 min by both visual and  
42 hearing contacts. After removing duplicates (we kept only  
43 one observation per 200 m grid cell, the spatial resolution of  
44 the analysis), the number of records available to fit the mod-  
45 els was of 262 (Fig. 2a). The background dataset was a sam-  
46 ple of 10,000 random points collected across the study area,  
47 encompassing the prediction locations and informing on the  
48 distribution of the covariates in the landscape.

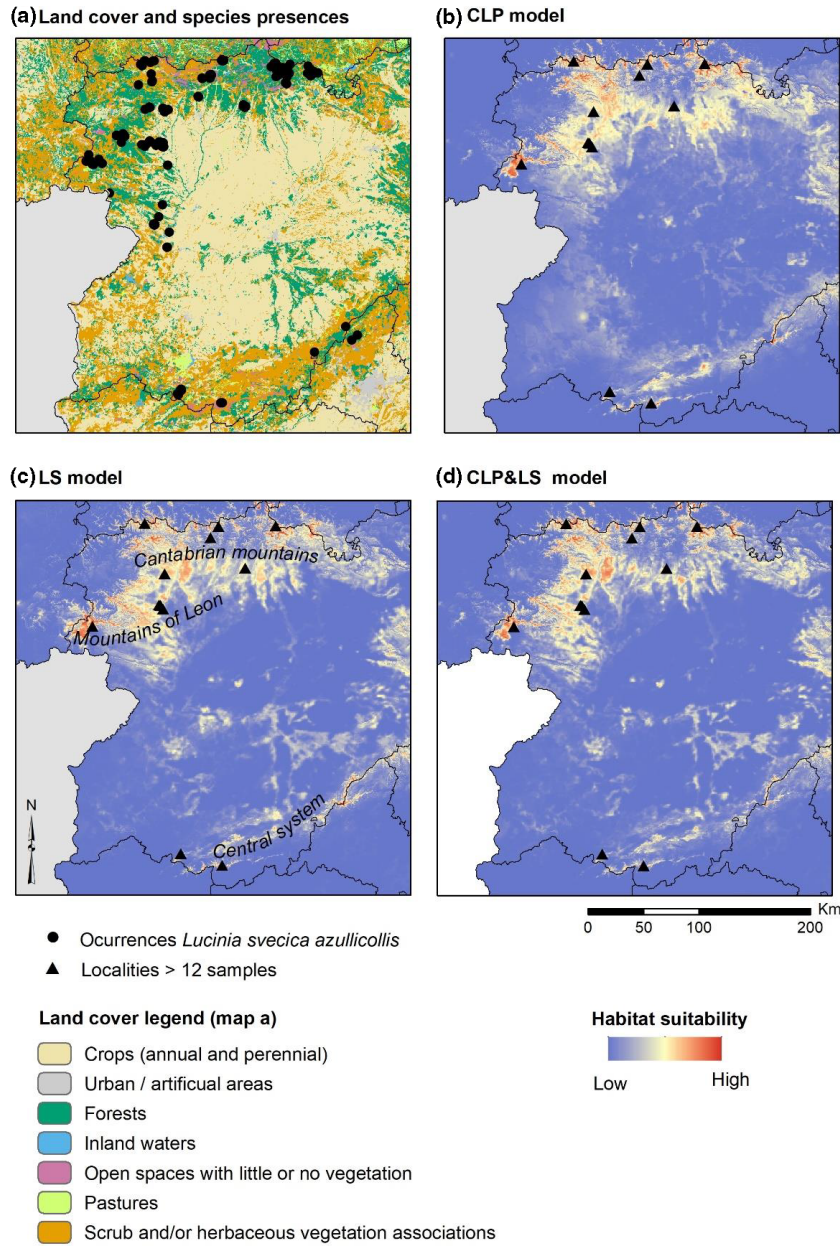
49 We selected a set of environmental variables informing on  
50 climate, topography and vegetation that are likely to influ-  
51 ence breeding habitat suitability for bluethroats (Table 1): (1)  
52 Climatic variables included the average mean and minimum  
53 temperatures of spring (March, April and May; correspond-  
54 ing to species arrival to breeding sites, mating and nesting)  
55 and summer (June, July and August; corresponding to fledg-  
56 ing and post-breeding months prior to migration), the annual  
57 seasonality (standard deviation of monthly temperature as a  
58 measure of continentality) and the total annual rainfall. These

predictors were derived from the Climatic Digital Atlas of  
the Iberian Peninsula (Ninyerola, Pons, & Roure, 2005) that  
was built at 200 m of spatial resolution based on climatic  
data for the period 1951–1999. (2) Topographic factors  
included slope and southness (as a measure of aspect), both  
extracted from a 200-m digital elevation model (Spanish  
Geographic Institute), as well as annual total radiation. (3)  
Vegetation was estimated using a temporal series of Nor-  
malised Difference Vegetation Index (NDVI; Rouse *et al.*,  
1973) values derived from NOAA-AVHRR satellite images  
at 1 km resolution for the period 1987–2010. NDVI is a  
simple and easily interpretable index that combines visible  
and near-infrared reflectance measurements. It is considered  
as a proxy of net primary production and vegetation vigour,  
emerging as a powerful indicator to explore the link between  
animal distribution and available resources (Pettorelli *et al.*,  
2011). Satellite images were daily collected and geometri-  
cally and atmospherically corrected using standard calibration  
coefficients (Rao & Chen, 1999) at the Spanish Remote  
Sensing Laboratory of Valladolid University. After eliminat-  
ing the clouds, radiometric errors were reduced using the  
monthly maximum value composite method (Holben, 1986).  
The annual mean of monthly NDVI composites for 2010  
(contemporary to bluethroat sampling; NDVI<sub>2010</sub>) for each  
pixel was then used to estimate spatial variation on current  
vegetation condition. We also calculated the coefficient of  
variation of annual series of NDVI values collected across  
the period 1987–2009 (more than two decades before the  
year of species sampling; NDVI<sub>CV</sub>), and used it as an esti-  
mate of the recent past landscape stability, vegetation com-  
position and vigour.

We fitted three models accounting for breeding habitat  
suitability across the landscape: (1) A CLP-Current Land-  
scape Patterns – model based on climate, topography and  
current NDVI values (NDVI<sub>2010</sub>); (2) a LS- Landscape Sta-  
bility – model based on climate, topography and the  
NDVI<sub>CV</sub> as a measure of recent past landscape stability; and,  
(3) a CLP&LS-Current Landscape Patterns and Landscape  
Stability – model accounting for the additive effect of cur-  
rent landscape and recent past landscape stability (NDVI<sub>2010</sub>  
and NDVI<sub>CV</sub>, respectively), along with climatic and topo-  
graphic variables. To minimize multicollinearity problems,  
we evaluated the Pearson's pair-wise correlations between all  
candidate predictors on a sample of 50000 points randomly  
distributed across the study area. When two or more vari-  
ables were strongly correlated ( $r_{\text{Pearson}} > 0.7$ ; Tabachnick &  
Fidell, 1996), we kept the variable with a more direct inter-  
pretation from the point of view of the ecology of the spe-  
cies. Variables retained for fitting the models are detailed in  
Table 1 and Supporting Information Fig. 1S3. The logistic  
output of the models can be interpreted as a habitat suitabil-  
ity index ranging from 0 to 1 (García *et al.*, 2007). A value  
near 1 represents sites where the habitat is optimal for the  
bird; a value near 0 corresponds to unsuitable sites.

We controlled the complexity of the response shapes by  
allowing only for linear, quadratic and product features.  
Models with these restricted feature types are smoother than  
those fitted with the default settings, less prone of being

LOW RESOLUTION COLOR FIG



**Figure 2** (a) Occurrence data (black points) of *Lucinia svecica azullicollis* used to fit the MaxEnt model in the Iberian Peninsula. The map also shows the main land cover types present in the study area (source: CORINE land cover 2006 – CLC2006: <https://land.copernicus.eu/pa-n-european/corine-land-cover/clc-2006>). Note the large extension of agricultural crops in between the mountain ranges where the species breeds. (b) Habitat suitability map based on current landscape patterns (CLP model output). (c) Habitat suitability map based on recent past landscape stability (LS model output). (d) Habitat suitability map considering the additive effect of current landscape patterns and landscape stability (CLP&LS model output). Maps (b–d) show the localities where more than 12 samples were collected (used for both genetic and connectivity analyses).

fitted to data idiosyncrasies and potentially better at predicting new places (Merow *et al.*, 2014). Model predictive performance (discrimination ability; Guillera-Arroita *et al.*, 2015) was assessed using the area under the receiver-operator characteristic curve (AUC; Hanley & McNeil, 1982), adapted for its use with presence-background samples (Phillips, Anderson, & Schapire, 2006). AUC values range from

0 to 1, where 1 indicates perfect model discrimination and 0.5 a model no better than random (Elith *et al.*, 2006). To reduce bias in the assessment of model performance, we performed statistical resampling, where AUC values were calculated by 10-fold cross-validation. This procedure is used to avoid overfitting by testing predictive accuracy on withheld portions of the data (Phillips *et al.*, 2009). We also

**Table 1** List of predictors accounting for climate, topography and vegetation that have been considered for modelling the habitat suitability of Iberian bluethroats

Variable	Description	Resolution (m)
Climate		
*TEMP <sub>SPRING</sub>	Average mean temperature in spring (March, April and May)	200
TEMP <sub>SUMMER</sub>	Average mean temperature in summer (June, July and August)	200
TMIN <sub>SPRING</sub>	Averaged minimum temperatures of spring (March, April and May)	200
TMIN <sub>SUMMER</sub>	Averaged minimum temperatures of summer (June, July and August)	200
*BIO4	Seasonality: Annual temperature variation based on the standard deviation of monthly temperature averages	200
*RAI	Total annual rainfall	200
Topography		
*SLO	Slope expressed in degrees from a digital elevation model (Spanish Geographic Institute)	200
*SOUTH	Southness. Measure of the aspect normalized to a -1 to 1 scale. Calculated as: $\text{Cos}(((\text{aspect})180^\circ)/\text{DIV degrees})$	200
*RAD	Total annual solar radiation	200
Land cover		
*NDVI <sub>2010</sub>	Annual Mean NDVI values for 2010 derived from a series of monthly Maximum Value Composites of Normalised Difference Vegetation Index images (NDVI-MVC)	1000 <sup>a</sup>
*NDVI <sub>CV</sub>	Coefficient of variation of NDVI values of an annual series of Maximum Value Composites of Normalised Difference Vegetation Index (NDVI-MVC) for the period 1987–2009	1000 <sup>a</sup>

Low correlated variables ( $r_{\text{Pearson}} < 0.7$ ) that were retained for modelling analyses are preceded by the symbol\*

<sup>a</sup>NDVI values were assumed constant within all 200 m cells contained in each km.

calculated the Akaike Information Criterion (AIC; Burnham & Anderson, 2002) value for each Maxent model using the package ENMeval (Muscarella *et al.*, 2014), in order to evaluate which model best fit the observed data.

### Landscape resistance surfaces

We converted the habitat suitability maps achieved from CLP, LS and CLP&LS models into resistance surfaces using different transformation functions to test different hypotheses about the potential resistance of landscape attributes to species dispersal (Supporting Information Fig. 2S3 from Appendix S3).

At one end, we assumed that the larger amount and connectiveness of suitable habitat between two localities, the smaller the genetic distance (i.e. a linear relationship between habitat suitability  $h$  and landscape resistance  $R$ , where  $R = 1-h$ ). At the other end, we assumed that landscape resistance is minimum at mid to high values of habitat suitability, but very high when habitat suitability is low (i.e. a negative exponential function  $R = h^{-1}$ ). Between these two extremes, following Trainor *et al.* (2013), we used the Equation 1 to estimate resistance surfaces that asymptotically approached both the linear and negative exponential extremes depending on the value of a single rescaling parameter  $c$ .

$$R = 100 - 99 \frac{1 - \exp(-c \times h)}{1 - \exp(-c)} \quad 1$$

Considering different values of  $c$ , from 0.25 to 16, we generated seven additional resistance layers for each habitat suitability model. Values in all resistance layers ranged from 1 to 100, representing minimum and maximum resistance values, respectively.

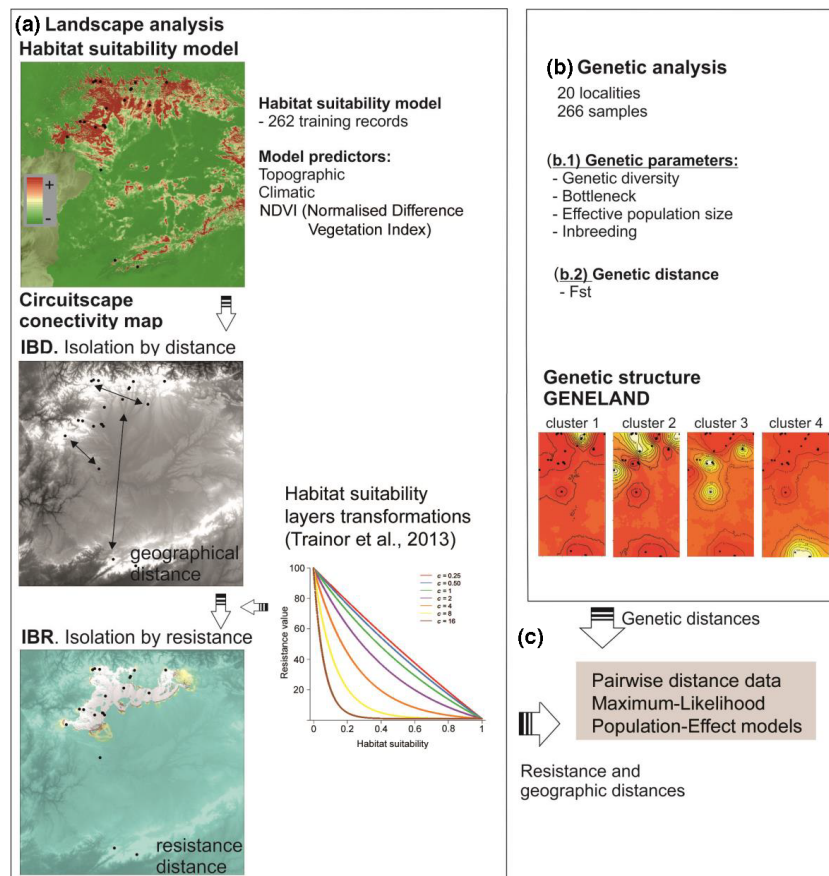
We also created a resistance layer assuming homogeneous resistance to movement across the territory ( $R = 1$ ) to test for the effect of geographic distance on bluethroat genetic differentiation (IBD hypothesis).

### Connectivity modelling analysis

We used the eight-neighbour cell-connection scheme settings in CIRCUITSCAPE v.4.0 (McRae, 2006; McRae & Shah, 2011) to evaluate pair-wise distances between localities with more than 12 samples based on: (1) IBD; (2) isolation by landscape resistance derived from current landscape patterns (IBCR), isolation by landscape resistance derived from landscape stability (IBLSR) and isolation by landscape resistance derived from both current landscape and stability (IBC&LSR). The IBCR, IBLSR and IBC&LSR hypotheses were evaluated using the multiple resistance layers derived from CLP, LS and CLP&LS models, respectively. CIRCUITSCAPE is a tool that incorporates the principles of the electric circuit theory to model gene flow and differentiation in plant and animal populations (McRae *et al.*, 2008). It estimates a unique value of pair-wise resistance between two locations by integrating dispersal through grid cells with different resistances to species movement over all potential dispersal paths. It has been successfully used to model genetic connectivity between populations in heterogeneous landscapes (Phillipsen & Lytle, 2012; Amos *et al.*, 2014).

### Effect of geographic and landscape resistance on genetic differentiation

To assess the relationship between genetic distance ( $F_{ST}$  values) and landscape resistance to species dispersal (IBD, IBCR, IBLSR and IBC&LSR), we fitted Maximum-Likelihood Population Effects models (MLPE) accounting for the non-independence of pair-wise distance data (Clarke, Rothery, & Raybould, 2002; Van Strien, Keller, & Holderegger,



**Figure 3** Flow chart showing the methodological steps carried out in the study: (a) Landscape permeability analysis included: habitat suitability modelling, generation of resistance surfaces, Circuitscape connectivity assessment based on Isolation-By-Distance and Isolation-by-Resistance (IBD and IBR, respectively) and pair-wise distances calculation. (b) Genetic analysis encompassed genetic parameters estimation and quantification of genetic distances among populations based on  $F_{ST}$  values. (c) Correlations of pair-wise landscape resistance and geographic distance with genetic distance were evaluated with Maximum-likelihood population-effect models.

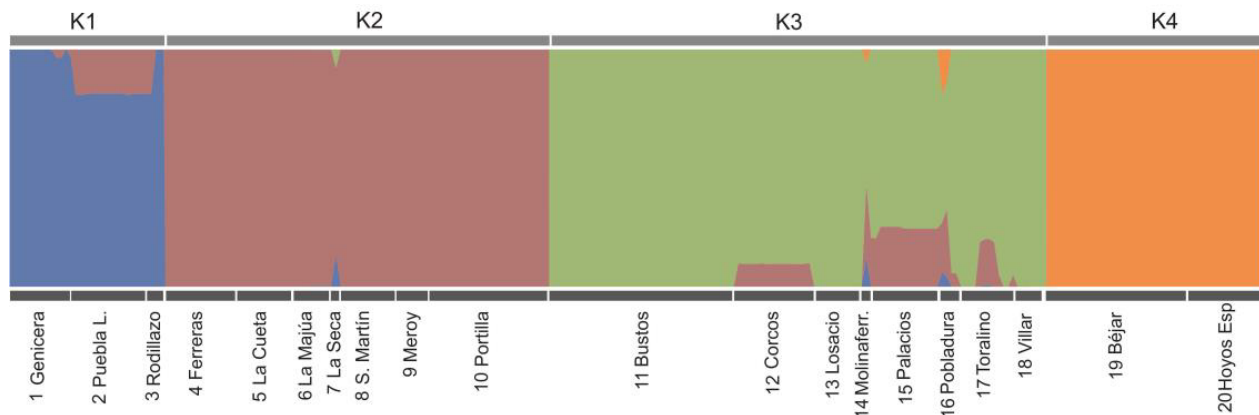
2012). These models perform well in landscape genetics model selection approaches when compared to other methods able to accommodate pair-wise distances, such as Mantel tests (Shirk, Landguth, & Cushman 2017). Models were fitted using the *ResistanceGA* R Package (Peterman, 2018), setting `REML = FALSE` in the `mlpe_rga()` function. This results in an unbiased Akaike Information Criterion (AIC; Burnham & Anderson, 2002) score fit on maximum likelihood estimates. Candidate models were compared using the  $AIC_{RMLE}$  score and two  $R^2$  estimates (marginal  $R^2_{GLMM(m)}$  and conditional  $R^2_{GLMM(c)}$ ), which measure the variance explained by the fixed effects parts of the MLPE model only (i.e. the pair-wise IBD, IBCR, IBLSR or IBC&LSR resistance distance matrices) or by both the fixed and random effects, respectively (Nakagawa & Schielzeth, 2013; Johnson, 2014; Nakagawa, Johnson, & Schielzeth, 2017).  $R^2$  estimates were calculated using the `r.squaredGLMM` function in the R package *MuMin* (Barton, 2019). We compared models based on a single predictor, either IBD or the multiple variations of IBCR, IBLSR and IBC&LSR resistance matrices (Fig. 3).

## Results

### Genetic patterns and processes

The GENELAND Bayesian clustering analysis evidenced an optimal genetic structure of four clusters (Fig. 4; Supporting Information Fig. 1S2 from Appendix S2). Genetic differentiation was significant among localities after Bonferroni adjustment (global  $F_{ST} = 0.022$ ,  $P = 0.0002$ ) in 41 of 66 pair-wise comparisons (62%, significant  $F_{ST} = 0.002$ –0.091; Supporting Information Table 1S2 from Appendix S2). Genetic diversity parameters, however, did not differ significantly among genetic clusters or localities (ANOVA,  $P > 0.05$ ) (Table 2 and Supporting Information Table 2S2 from Appendix S2).

The estimates of the inbreeding coefficient ( $F_{IS}$ ) differed among clusters ( $P = 0.0013$ , 800 randomizations). The local inbreeding coefficient ( $F_{IS}$  value over all loci) was positive and significantly different from zero in clusters K1 ( $F_{IS} = 0.071$ ,  $P = 0.0300$ ) and K4 ( $F_{IS} = 0.072$ ,  $P = 0.0075$ ), as well as in a locality sited in the Central System



**13** **Figure 4** Individual Bayesian assignment of 20 sampling localities and 266 individuals to each GENELAND cluster.

**Table 2** Genetic diversity in the 12 localities with 12 or more individuals genotyped and BOTTLENECK results testing the null hypothesis of mutation-drift equilibrium under the two-phase model, using the Wilcoxon test (TPM, 95% single-step mutations and 5% multiple-step mutations)

Locality	Genetic diversity			Bottleneck			
	Na	$H_{obs}$	$H_{exp}$	$F_{IS}$	One tail, heterozygosity deficiency	One tail, heterozygosity excess	Two tail, both outcomes
01 Genicera	4.5	0.594	0.613	0.073	0.903	0.116	0.232
02 Puebla de Lillo	4.9	0.623	0.642	0.060	0.947	0.065	0.131
04 Ferreras de Cepeda	4.9	0.624	0.626	0.034	0.984	<b>0.042</b>	0.084
05 La Cueta	4.6	0.607	0.612	0.048	0.997	<b>0.005</b>	<b>0.010</b>
08 San Martín de Castañeda	4.9	0.716	0.625	-0.103	0.722	0.313	0.625
10 Portilla de la Reina	5.2	0.626	0.638	0.039	0.958	0.053	0.106
11 Bustos	5.9	0.644	0.648	0.020	0.947	0.065	0.131
12 Corcos	5.2	0.601	0.600	0.031	0.615	0.423	0.846
15 Palacios de la Valduerna	5.2	0.626	0.634	0.049	0.813	0.216	0.432
17 Toralino	4.9	0.669	0.638	-0.012	0.991	<b>0.012</b>	<b>0.024</b>
19 Béjar	5.8	0.596	0.619	0.055	0.688	0.348	0.695
20 Hoyos del Espino	4.8	0.507	0.542	<b>0.101</b>	0.278	0.754	0.557

Na, Average number of alleles per locus;  $H_{obs}$  and  $H_{exp}$ , average observed and expected heterozygosity;  $F_{IS}$ , inbreeding coefficient. Figures in bold indicate  $F_{IS}$  values significantly different from zero and rejection of null hypothesis of mutation drift equilibrium ( $P < 0.05$ ).

( $F_{IS} = 0.101$ ,  $P = 0.0098$ ).  $F_{IS}$  values were significantly positive for three loci in K1, one locus in K2, three loci in K3 and two loci in K4 (Table 2; Supporting Information Tables 2S2 and 3S2 from Supporting Information Appendix S2). The mean effective size ( $N_e$ ) of the breeding population of Iberian bluethroats was 522 individuals (Jackknife 95% CI = 304–1371). No significant excess of heterozygosity was detected in any of the genetic clusters, indicating no evidence of recent bottlenecks. However, a significant heterozygosity excess was found in three localities ( $P < 0.05$ , Table 2).

### Habitat suitability models

The predictive performance of CLP (AUC mean  $\pm$  SD = 0.948  $\pm$  0.015), LS (AUC mean  $\pm$  SD = 0.951  $\pm$  0.009) and CLP&LS (AUC 0.957  $\pm$  0.011) habitat suitability models was high, indicating good model discrimination. Spring

temperature, annual rainfall and vegetation (NDVI) explained more than 85% of the variance in all models (Table 3). Despite the temperature being the most relevant variable in all cases, vegetation played a major role in defining breeding habitat suitability, explaining 30% of the variance in CLP model. Topographic predictors (slope, radiation and southness) and seasonality were relatively unimportant in the models (contribution < 6%). The highest values of habitat suitability for the focal species were found at the Northern and Eastern part of the study area (Fig. 2b–d). These correspond to areas with low/medium NDVI coefficient of variation (LS and CLP&LS models: areas where vegetation has remained mostly stable during two decades prior to bird surveys), low annual precipitation and low spring average mean temperatures (see Supporting Information Fig. 3S3, 4S3 and 5S3 in Appendix S3 for the response curves of the predictors). The AIC values for the CLP, LS and CLP&LS models were 7037, 7011 and 6951, respectively.



**Table 3** Permutation importance of each environmental variable in Maxent models. The values of each environmental variable are randomly permuted, in turn, on training presence and background data

Variable	CLP model	LS model	CLP&LS model
TEMP <sub>SPRING</sub>	<b>47.0</b>	<b>55.2</b>	<b>43.5</b>
BIO4	6.0	5.7	6
RAI	<b>21.6</b>	<b>17.6</b>	<b>16.2</b>
SLO	2.4	3.5	2.7
SOUTH	0.6	0.6	0.5
RAD	0.1	0	0.2
NDVI <sub>2010</sub>	<b>22.4</b>	-	<b>11.6</b>
NDVI <sub>CV</sub>	-	<b>17.4</b>	<b>19.2</b>

The model is re-evaluated on the permuted data and the resulting drop in training AUC is shown in the table, normalized to percentages. Variables are as follows: average temperature in spring (TEMP<sub>SPRING</sub>), annual seasonality (BIO4), total annual rainfall (RAI), slope in degrees (SLO), southness (SOUTH), total annual radiation (RAD), annual mean NDVI for 2010 (NDVI<sub>2010</sub>) and coefficient of variation of NDVI for the period 1987–2009 (NDVI<sub>CV</sub>). CLP model: model accounting current landscape attributes; LS model: model accounting for recent past landscape stability; CLP&LS model: model accounting for both current landscape and recent past landscape stability. In bold variables contributing more than 10%.

These values suggest that CLP&LS model, considering the additive effect of current attributes and temporal stability of landscape, was the best at explaining bluethroat occurrence.

### Effect of geographic and landscape resistance on genetic differentiation

Genetic distance pattern was better supported by landscape resistance than by Euclidean distance ( $\Delta AIC_{\text{Euc}} > 5$ ; Table 4). IBCR, IBLSR and IBC&LSR models explained between 62% and 76 % of the variation in genetic distances, with small differences among models, depending on the transformation function considered. The best performing transformation functions were those assuming a linear or quasi-linear relationship ( $c = 0.25$ – $2$ ; Supporting Information Fig. 2S3 from Appendix S3) between habitat suitability and landscape resistance. The negative exponential and the transformation functions approximating the negative exponential ( $c = 8, 16$ ) performed only slightly better than the Euclidean function. This suggests that subtle differences in intermediate- or high-quality habitat strongly influence species dispersal.

### Discussion

This study showed evidences of a significant genetic structure in Iberian breeding bluethroats that correlated better landscape resistance than to geographic distance. Regardless geographic distance, functional connectivity (and, hence, gene flow) between breeding areas was higher when the intervening landscape among populations consisted of temporally stable patches of highly suitable breeding habitat,

being the relationship between gene flow and presence of intervening habitat linear or quasi-linear. These results suggest that despite the high vagility of bluethroats, structural habitat connectedness between landscape patches showing low temporal variability in vegetation condition play an important role at facilitating Iberian bluethroat breeding dispersal. However, it should be highlighted that our models explained only a part of the genetic variation (<72% in all cases). A reason could be that dispersal is not only related to landscape resistance or geographic distance but is also associated with other factors not considered in this study, including behavioural traits (mating systems and philopatry), demographic parameters (effective population size and variations in breeding success between populations), physiological tolerance or dispersal ability. All these factors can interact and shape genetic structure at different spatiotemporal scales (Anderson *et al.*, 2010; Athrey *et al.*, 2012). For instance, strong site fidelity could explain the small rates of gene flow of populations found at low to mid latitudes (FitzSimmons *et al.*, 1997; Van Bekkum *et al.*, 2006) in comparison with the northernmost grounds, where breeding site fidelity is much lower (Both & Visser, 2001; Martin & McKay, 2004). The interaction among breeding site fidelity, habitat fragmentation and small population size might result in an increase in genetic drift (Rousset, 1997). The fact that the model considering the potential additive effect of current landscape patterns and recent past landscape stability did not explain more genetic variation than models based on each of these terms separately (current *vs.* recent past landscape stability) also supports this idea. Another explanation may relate to the fact that we used NDVI values as surrogates of vegetation composition and vigour, may show limitations to capture completely what makes a particular site especially suitable for the species in terms of habitat conditions. Likely, NDVI resolution (1 km) did not allow to detect subtle differences in the within-pixel grassland availability, where bluethroats require less foraging effort (the species forages for food on the ground). Moreover, climatic predictors explained most of the variance in all habitat suitability models (CLP, LS and CLP&LS; 60%; Supporting Information Appendix S3), which might partly explain why all models explain similar amounts of genetic variation.

The maintenance of suitable breeding habitat for bluethroats depends on both human activities and natural processes, such as secondary succession after disturbance (Calvo, Tárrega, & de Luis, 2002). The structure of these habitats is inherently variable and, consequently, their population carrying capacity changes constantly. In fact, many landscape patches holding suitable habitat may disappear because of cutting and burning or intensive cattle farming (Morán-Ordóñez *et al.*, 2013). Following disturbance, patches may rapidly recover due to auto-succession processes and, then, be re-occupied by bluethroats in an estimated period of 5–10 years (authors, unpublished data). At the other end, current patches of suitable habitat may become permanently unsuitable for the focal species in the short mid-term if shrublands evolve into forest habitats, as a result of secondary succession, a prevailing process in the mountain

**Table 4** Results of the maximum-likelihood population effects models (MLPE) for the relationship between genetic distances ( $F_{ST}$ ) and each of the species dispersal hypotheses tested: “Current” for isolation by current landscape resistance (IBCR), “Landscape stability” for isolation by landscape resistance derived from landscape stability (IBLSR), “CurrentLS” for isolation by landscape resistance derived from both current landscape and stability (IBC&LSR) and “Euclidean distance” for isolation by distance (IBD)

Landscape	Resistance	AIC	$\Delta AIC_{null}$	$\Delta AIC_{Euc}$	$R^2$ GLMM(m)	$R^2$ GLMM(c)
Current	2	127.72	-61.12	-16.69	0.66	0.76
Landscape stability	2	127.90	-60.95	-16.51	0.66	0.73
Landscape stability	1	128.05	-60.80	-16.36	0.65	0.73
Current	1	128.18	-60.66	-16.23	0.66	0.75
Landscape stability	0.5	128.47	-60.38	-15.94	0.65	0.72
Landscape stability	Linear	128.72	-60.12	-15.69	0.65	0.72
Current	0.5	128.76	-60.08	-15.65	0.65	0.74
Landscape stability	0.25	128.77	-60.08	-15.64	0.65	0.72
Current	4	129.08	-59.76	-15.33	0.66	0.76
Current	0.25	129.14	-59.71	-15.27	0.65	0.74
Current	Linear	129.18	-59.66	-15.23	0.65	0.74
Landscape stability	4	129.54	-59.31	-14.87	0.65	0.73
CurrentLS	2	131.86	-56.99	-12.55	0.64	0.72
CurrentLS	1	132.21	-56.63	-12.20	0.63	0.72
CurrentLS	0.5	132.65	-56.19	-11.76	0.63	0.71
CurrentLS	4	132.70	-56.15	-11.71	0.64	0.73
CurrentLS	0.25	132.94	-55.91	-11.47	0.63	0.71
CurrentLS	Linear	132.98	-55.87	-11.43	0.62	0.71
Current	8	134.08	-54.77	-10.34	0.64	0.74
Landscape stability	8	134.32	-54.53	-10.09	0.63	0.73
CurrentLS	8	135.91	-52.94	-8.50	0.63	0.72
CurrentLS	Exp <sup>-1</sup>	136.64	-52.21	-7.78	0.62	0.72
Current	16	137.00	-51.85	-7.41	0.62	0.72
Landscape stability	Exp <sup>-1</sup>	137.32	-51.53	-7.10	0.62	0.72
Landscape stability	16	137.57	-51.27	-6.84	0.62	0.72
Current	Exp <sup>-1</sup>	137.83	-51.01	-6.58	0.62	0.72
CurrentLS	16	137.98	-50.87	-6.43	0.62	0.72
Euclidean		144.41	-44.44	0.00	0.54	0.61

The field “resistance” indicates the rescaling approach used to convert habitat suitability values into resistance distances (linear, negative exponential –  $Exp^{-1}$  – or values of the  $C$  rescaling factor of 0.25, 0.5, 1, 2, 4, 8 and 16 in Equation 1: Figure 2S3 in Appendix S3). AIC is the Akaike’s Information Criterion generated for each MLPE model.  $\Delta AIC_{null}$  is the difference between the AIC value generated for each MLPE model and the AIC of a ‘null’ model with the same structure of random effects and no explanatory fixed predictors.  $\Delta AIC_{Euc}$  is the difference of AIC between landscape resistance and Euclidean distance models.  $R^2_{GLMM(m)}$  and  $R^2_{GLMM(c)}$  are the marginal and conditional  $R^2$  values of the fitted MLPE model respectively.

systems under study (Morán-Ordóñez *et al.*, 2013; Álvarez-Martínez *et al.*, 2014). This problem is especially relevant for the bluethroat populations breeding at the foothills of the Cantabrian Mountains and Mountains of León, where the abandonment of livestock farming is more pronounced and the populations of wild ungulates are less numerous. Nevertheless, this situation can be rapidly reversed by disturbance processes like fire that, in the study area, is a management tool widely used by local farmers to facilitate the regeneration of pastures supporting extensive grazing (Viedma, Moreno, & Rieiro, 2006).

Iberian breeding populations are isolated from each other (especially those in the Central System) and also from other European populations (note that the closest populations are over 500 km away, in France, and correspond to the subspecies *L. s. namnetum*; Johnsen *et al.*, 2006). Peripheral populations often present low levels of genetic diversity and high inbreeding in comparison with populations located at

the core of the species distribution range (Hoffman & Blows, 1994; Hampe & Petit, 2005). This is the case, for example, of *L. s. namnetum* and *L. s. azuricollis*, whose breeding areas are the most geographically isolated, showing the lowest allelic richness and heterozygosity and the highest inbreeding coefficients within the subspecies complex (Johnsen *et al.*, 2006). We found significant values of inbreeding for Iberian populations, being consistent with isolation and small population size. In these situations, mating is non-random and occurs among close relatives, resulting in an increase in the rate of homozygotic descendants in a few generations. This fact may have severe implications for subspecies persistence, as it would reduce its capacity to respond against future environmental changes (Keller & Waller, 2002; Hoffman & Sgrò, 2011). Indeed, the estimated effective population size was very small for the subspecies as a whole, as well as for the genetic clusters. In some cases, it was lower than 500 individuals, the minimum value considered necessary to

1 guarantee long-term genetic integrity (Franklin & Frankham,  
2 1998). The bottleneck episodes detected in three of the eval-  
3 uated localities, together with the reduced effective popula-  
4 tion size in some of the genetic clusters suggest recent  
5 declines in the Iberian bluethroat breeding population size, at  
6 least locally. The explicit consideration of bottleneck epi-  
7 sodes is of great relevance because they may lead to a high  
8 expression of recessive alleles, reducing individual fitness,  
9 genetic diversity and population viability, which make popu-  
10 lations less capable to adapt against environmental changes  
11 (Bouzat, 2010; Velando, Barros & Morán, 2015).

### 12 **Implications for conservation**

13 Since the second half of the last century, the Iberian mountains  
14 where bluethroats inhabit is being affected by rural abandon-  
15 ment that leads to vegetation encroachment, landscape homog-  
16 enization and afforestation (Herrando *et al.*, 2014; Lasanta  
17 *et al.*, 2016). These changes are reducing the availability of  
18 semi-natural breeding habitats in mountain areas of the Iberian  
19 Peninsula, which, according to the results of our study, may  
20 have severe consequences on bluethroat genetic structure and  
21 connectivity. However, within the subspecies range, there are  
22 still many unoccupied suitable areas, which suggests that habi-  
23 tat suitability is not enough to understand the genetic composi-  
24 tion and structure of this subspecies and that managing  
25 bluethroat populations still needs improved knowledge on the  
26 behavioural traits of this subspecies. However, with the current  
27 knowledge, habitat management is the main action that can be  
28 practically implemented to conserve bluethroat populations  
29 across the study area.

30 Current habitat management policies related to shrublands  
31 and heathlands, as those related to livestock farming, wild  
32 herbivorous pressure, wildfire prevention or land abandon-  
33 ment, can have a significant impact on the Iberian bluethroat  
34 population dynamics in the mid and long term. Our results  
35 suggest that promoting the spatial connectedness and tempo-  
36 ral stability of suitable habitat (shrub/heath – pasture  
37 mosaics) in heterogeneous human-shaped landscapes would  
38 improve gene flow and, consequently, species persistence.  
39 These management interventions should be prioritized in the  
40 areas with the highest risk of habitat loss (due to either land  
41 abandonment and natural succession or to habitat removal  
42 for intensification) and higher potential impact for the sub-  
43 species. In particular, our results suggest especial conserva-  
44 tion efforts should be made to preserve the Central System  
45 bluethroat population, which is located at the southernmost  
46 limit and highest altitude of the subspecies range, and shows  
47 the highest genetic and phenotypic divergence and isolation,  
48 as well as the smallest population size. On the other hand,  
49 in this area there is a high risk of habitat loss associated  
50 with tourist pressure (skiing and mountain tourism) and wild-  
51 fires. As it is not possible to implement measures to improve  
52 functional connectivity between this and the populations in  
53 the north of the Peninsula, due to distance (150 km) and the  
54 lack of suitable intervening habitat (Fig. 2), management  
55 should be focused on promoting habitat connectivity between  
56 localities.

Management actions that could prove beneficial to  
increase suitable habitat between localities include, for exam-  
ple, the maintenance of extensive livestock farming because  
livestock maintain open spaces where prey diversity is high-  
est (García-Tejero *et al.*, 2013) and limit forest expansion  
and landscape homogenization (Lasanta *et al.*, 2016; Gua-  
dilla-Sáez, Pardo-de-Santayana, & Reyes-García, 2019).  
Also, the maintenance of other frequent low-intensity distur-  
bances in the landscape is also a key to maintain bluethroat  
habitat: experimental treatments in mountain areas in the  
north of the Iberian Peninsula showed that shrubland com-  
munities recover their original state 9 years after burning and  
clearing, with trees starting to encroach shrublands 15 years  
after the disturbances (Calvo, Tárrega & de Luis, 2002).  
Thus, in areas with presence of bluethroat populations where  
extensive farming has been abandoned, conservation inter-  
ventions such as frequent shrub clearing with machinery or  
controlled-burning promoting the maintenance of shrubland-  
pasture mosaics (Morán-Ordóñez *et al.*, 2013) should be ade-  
quately planned and implemented if the species is to be pre-  
served. Investments in the promotion of traditional  
management (e.g. through subsidies to extensive livestock  
practices) to improve the conservation status of bluethroats  
could be more cost effective if, besides improving the condi-  
tion of the subspecies habitat, the maintenance of semi-nat-  
ural mosaics can also contribute to achieve other environ-  
mental goals such as reduce fire risk, mitigate soil  
erosion, increase pasture quality or improve wildlife habitat  
(Lasanta *et al.*, 2016). For example, in the study area, man-  
agement for promoting semi-natural shrub/heath-pasture  
mosaics will also benefit other species of EU Community  
Conservation Interest (Birds Directive 2009/147/EC; Anon,  
2009), such as the grey partridge *Perdix perdix* subsp. *his-*  
*panensis* or the broom hare *Lepus castroviejoi*. Along with  
the bluethroat, these are the flagship species of the species  
community inhabiting these semi-open shrub/heath-pasture  
mosaics in northernmost Iberian mountain system.

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## Authors' contributions

J.G., J.T.G. and S.S.-S. designed the study. J.G., A.M.-O and S.S.-S. wrote the first manuscript. J.G. collected bird data, J.S. provided satellite data and J.G. and A. M.-O carried out the statistical analyses with input from J.T.G., M.C.-R and S.S.-S. All authors contributed critically to the drafts and gave final approval for publication.

## Data availability statement

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## Supporting information

Additional supporting information may be found online in  
the Supporting Information section at the end of the article.

**Appendix S1.** XXXX.

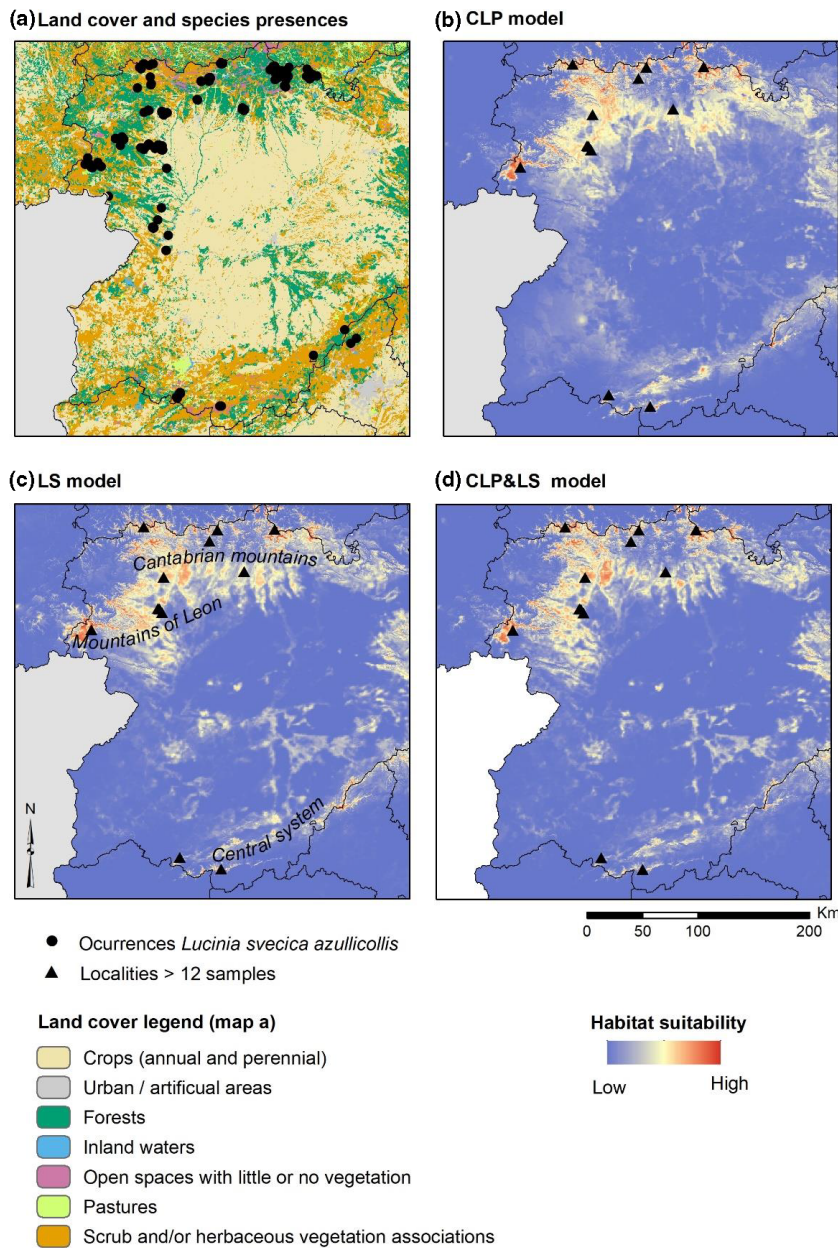
**Appendix S2.** Genetic patterns and processes.

**Appendix S3.** Habitat suitability model.

7

# Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main article.



The importance of past landscape features or landscape temporal stability in shaping population genetic structure remains undervalued, particularly in birds. We found a genetic structure of four clusters in the Iberian bluethroats populations, as well as a high level of genetic differentiation. Genetic structure was better associated with landscape resistance, rather than with geographic distance. Our results suggest that conservation policies and land management practices that promote the maintenance of semi-open pasture-shrub mosaics (e.g. through extensive livestock grazing) can strongly benefit Iberian bluethroat populations, improving gene flow and population connectivity.