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3   **Morphological differentiation in a migratory bird across geographic gradients in**

4   **mountains of southern Europe**

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6   Running tittle: Morphological differentiation across gradients

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8   Javier García[1, 2,]*, Juan Arizaga[3], José Ignacio Rodríguez[2], Daniel Alonso[3] and Susana

9   Suárez-Seoane[4]

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11   *****Corresponding author

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13   [1]Department of Biodiversity and Environmental Management, University of León,

14   Campus de Vegazana s/n, 24071 León, Spain.

15   [2]Iberian Ringing Group, C/ Daoiz y Velarde, 16. León, Spain.

16   [3]Department of Ornithology, Aranzadi Society of Sciences, C/ Zorroagagaina 11, 20014

17   Donostia-San Sebastián, Spain.

18   [4]Department of Organisms and Systems Biology (BOS, University of Oviedo) and

19   Biodiversity Research Institute (IMIB; CSIC - Oviedo University - Principality of

20   Asturias). Oviedo, Mieres (Spain).

21

30   **ABSTRACT**

31   **Aim**

32   In temperate mountain ranges, sharp spatial variations in habitat heterogeneity and

33   climate provide a perfect study setup to assess genetic and phenotypic differentiation in

34   bird populations. In this paper, we analyzed morphological divergence patterns across

35   geographic and environmental gradients, in correlation with genetic differentiation and

36   geographic isolation, in the breeding grounds of a long-distance migratory passerine.

37   **Location**

38   Northwestern Iberian mountains.

39   **Major taxa studied**

40   The Iberian bluethroat, *Luscina svecica azuricollis.*

41   **Methods**

42   We collected a sample of 625 Iberian bluethroats across their whole breeding range to

43   measure body weight and tarsus length, as well as wing length and pointedness.

44   Morphological differentiation across geographic (latitude and elevation) and

45   environmental (climate and vegetation) gradients was assessed using generalized linear

46   mixed models. Additionally, the role of genetic distance and geographic isolation as

47   drivers of morphological differentiation was evaluated with Mantel tests.

48   **Results**

49   Bird morphology varied significantly with latitude and elevation, but not with climate or

50   vegetation. In the case of latitude, morphological differences in body size were contrary

51   to Bergmann's rule statements. All biometric and morphometric variables diverged

52   among localities. A similar trend was found for genetic clusters, except for wing shape.

53   Body weight and wing length were both correlated to geographic distance, while only

54   the former varied with genetic differentiation. The greatest genetic and phenotypic

differentiation was detected in the southernmost mountain range, that holds the most

geographically isolated genetic group.

Main conclusion

Evidence suggests that the strong morphological differentiation observed in the Iberian

breeding bluethroat across geographic gradients results from the combination of

unambiguous deterministic forces, such as ecological selection mechanisms, and other

factors, such as geographic isolation, that can be either deterministic, stochastic or both,

all acting at different scales.

Key words: Bergmann's rule, Bluethroat, body size, genetic distance, geographic

isolation, *Luscinia svecica* subsp. *azuricollis*, wing pointedness.

INTRODUCTION

The assessment of the factors underlying spatial patterns of morphological differentiation is useful to test theoretical and applied questions in ecology and conservation (Morgan, 2004), including adaptation to migration (Mönkkönen, 1995), microhabitat food selection, new habitat colonization or survival rate (Forstmeier & Keßler 2001; Hall, Ryttman, Fransson & Stolt, 2004). Morphological differentiation is a complex phenomenon in which stochastic phenomena (i.e., genetic drift that is particularly relevant in small and isolated populations; Wright, 1931; Bolnick et al., 2011), deterministic selective forces (i.e., adaptations to environment and social context; Hughes, 2000; Millien et al., 2006) and other ecological processes that cannot be unambiguously treated as being deterministic or stochastic (i.e., dispersal and diversification; Zhou & Ning, 2017) may participate simultaneously (Coyne & Orr 2004; Orsini, Vanoverbeke, Swillen, Mergeay & De Meester, 2013). Therefore, the joint analysis of morphological and genetic patterns is relevant to decipher whether differentiation occurs solely due to geographic or functional isolation or whether other evolutionary forces, such as dispersal, philopatry or habitat selection (Slatkin, 1985), are at work (Clegg et al., 2002). In this sense, it should be considered that morphological and genetic differentiation frequently do not show identical patterns and processes, as they might be driven by different factors (e.g. local environmental conditions) acting in different ways, which may impact on the parameters under assessment with a lack of congruency. For instance, a strong habitat selection might be associated to a high morphological differentiation level and a low adaptation capability to different environments. Therefore, in changing environments phenotypic, where selective pressures often vary, plasticity could be a suitable strategy (Millien et al., 2006; Siepielski, DiBattista & Carlson, 2009).

Mountain ranges in temperate regions are ideal scenarios for exploring diversification processes. On the one hand, they frequently give rise to highly variable topographic and climatic conditions operating at macro and micro-scale level which, hence, promote the existence of a high richness of habitats. On the other hand, they were a refuge for many species during the last glaciation, many of them remaining currently isolated in heterogeneous habitats with island-like conditions (Hewitt, 1996). Consequently, mountain populations may show morphological adaptations that vary soundly across geographic and environmental gradients, even at relatively small scale. In the case of mountain birds, body size and wing shape (pointedness) are two main morphological traits that may change substantially at intra-specific level and, therefore, constitute a good tool to investigate diversification processes.

Differences in animal body size across geographic gradients were first codified in Bergmann's rule, which postulates that body size enlarges with latitude (Bergmann, 1847; Mayr, 1956; Lindsey, 1966; Huston & Wolverton, 2011) and elevation (Teplitsky & Millien, 2014), as a consequence of thermoregulation (i.e., larger bodies increase heat conservation in cold environments because of the surface-volume ratio; Rodríguez, López-Sañudo & Hawkins, 2006). The rule was originally defined inter-specifically (Blackburn, Gaston & Loder, 1999), but it was commonly applied afterwards to different populations within single species (Berke, Jablonski, Krug, Roy, & Tomasovych, 2013). Despite the occurrence of deviations due to phylogenetic history, species range size, reproductive factors or competition (Watt, Mirchell & Salewski, 2010; Huston & Wolverton, 2011), Bergmann's rule has held true for many taxa throughout the world (Hamilton, 1961; Blackburn & Gaston 1996; Gaston & Blackburn 1996; Ashton, 2002). In fact, it has been demonstrated for endotherms of different size (e.g. 72% of birds and 65% of mammals showed a trend of increasing body size with

latitude; Meiri & Dayan, 2003; Millien et al., 2006) and, to a lesser extent, for

ectotherms (temperature-size rule; Atkinson, 1994). Thus, Bergmann's rule is currently

considered as a valid ecological pattern. Nevertheless, heat conservation is no longer

accepted as a general explanation since, contrary to endotherms, ectotherms cannot

maintain a stable body temperature (Ashton, Tracy, & De Queiroz 2000). Alternatively,

other mechanisms have been proposed for Bergmann's body size clines, such as

resource availability (Blackburn, Gaston & Loder, 1999), which is mediated by climate,

productivity, competition and predation (Yom-Tov & Geffen, 2011). Since all these

factors vary at different spatial scales, body size clines may be expected to occur not

only at large (global), but also at small (regional) scale. In this sense, the processes

underlying regional patterns of animal body size still remain uncertain (Blackburn &

Gaston, 1996; Gaston & Blackburn, 1995; Greve et al., 2008). Thus, more research

needs to be done, particularly at fine taxonomic scales (e.g. different populations within

a subspecies), to fully understand phenotypic variability in body size at different spatial

scales (Meiri et al., 2007).

Wing shape is a major driver of reproductive isolation and speciation, as it is directly

linked to migratory behaviour (Winker, 2010). In fact, this morphological trait may be

considered as a proxy of energy-efficient flight and migratory movement magnitude.

This relationship was systemized as the Seebohm's rule (Seebohm, 1901; Mönkkönen,

1995; Voelker, 2001; Forschler & Bairlein, 2011), which establishes that migrants have

relatively longer and more pointed wings than resident birds. More pointed wings allow

for greater flight efficiency, reduce the induced drag at the wing and produce a larger

forward component in flight (Rayner, 1988; Lockwood, Swaddle & Rayner, 1998).

Wing shape has also been related to breeding ground elevation (Bears, Drever &

Martin, 2008), habitat use and foraging behaviour (Marchetti, Price & Richman, 1995;

143   Forstmeier & Keβler, 2001), density of obstacles (vegetation) (Alatalo, Gustafsson &

144   Lunderg, 1984), sexual selection (Hedenström & Møller, 1992) and predation risk

145   (Swaddle & Lockwood, 1998).

146   In this study, we aim to assess morphological differentiation patterns in the breeding

147   grounds of a long distance migratory passerine, the bluethroat *Luscinia svecica*, across

148   geographic and environmental gradients, in correlation with genetic differentiation and

149   geographic isolation. This is a small (14-20 cm) polytypic passerine breeding from

150   Iberia to Alaska (Cramp, 1988; Meijer & Stastny, 1997), with the westernmost

151   European populations overwintering in southwestern Europe, northerwestern Africa and

152   western Sahelian belt (Arizaga et al., 2015). The phenotypic (Arizaga, Campos &

153   Alonso, 2006; Hogner et al., 2013; García, Johnsen, Fuertes, & Suárez-Seoane, 2017)

154   and genotypic (Johnsen et al., 2006, 2007) variation of this species constitutes a

155   complex subspecific mosaic associated to differences in geographic morphs and life

156   history, where the Iberian breeding bluethroat *L. s. azuricollis* is considered among the

157   most ancestral forms (Johnsen et al., 2006). This subspecies is distributed across strong

158   geographic and environmental gradients in northwestern Iberian mountains, where it

159   shows a moderate degree of genetic differentiation (Alda, García, García & Suárez-

160   Seoane, 2013; García et al., 2020). Iberian bluethroats breed along an altitudinal

161   gradient ranging from 750 to 2400 m a.s.l., under considerably different climatic

162   conditions (i.e. mean annual temperature spans from 0ºC to 12 ºC and mean annual

163   rainfall from 400 to 1800 mm). Habitats are fragmented and geographically isolated and

164   consist of different types of shrublands: from degraded holm oak shrublands (*Quercus*

165   *rotundifolia* and *Cistus* spp.), in the lowest areas, to heathlands (*Erica* spp. and *Calluna*

166   *vulgaris*) and brooms (*Cytisus* spp. and *Genista* spp.), in the uppermost lands (García et

167   al., 2000; Arizaga, García & Suárez-Seoane. 2011, Alda et al., 2013). Across this

168 gradient, the prey assemblage that constitute the bluethroat diet also varies substantially

169 (García-Tejero et al., 2013). Iberian bluethroats winter in different areas of the Iberian

170 Peninsula and tropical Africa (Arizaga et al., 2006, 2015), although there is no accurate

171 information about the specific wintering quarters of each population.

172 We hypothesize that: (i) Birds living in the highest and northernmost areas would have

173 the largest body size, in accordance with Bergmann's rule. (ii) Wing pointedness would

174 differ among populations, according to the existent knowledge on migratory routes and

175 phenology (Delmore, Kenyon, Germain & Irwin, 2015; Arizaga et al., 2015; Lomas et

176 al. 2019). (iii) Genetic and morphological differentiation patterns would be correlated in

177 response to local adaptation, among other evolutionary forces, since bluethroat

178 populations occupy highly fragmented and geographically isolated habitats.

179

180 **METHODS**

181

182 **Data collection**

183 A total of 625 Iberian bluethroat breeding males were sampled for morphological

184 evaluation between 1998 and 2012. They were captured using mist nets and spring traps

185 throughout 23 localities of the Cantabrian Mountains, Mountains of León (with

186 foothills) and Central System (Figure 1; Table S1, Supplementary Material), covering

187 the whole altitudinal and environmental range where the subspecies is known to breed

188 in Spain. Since sampling (March-September) partially coincided with the migration

189 period of European populations included in other subspecies, we applied a habitat use

190 criterion to ensure that all captured individuals corresponded to the local subspecies.

191 Therefore, we avoided sampling in habitats exploited by bluethroat migrants, i.e. wet

192 areas with dominant halophytic communities, that are not used by local birds (Bermejo

193   & De la Puente, 2004; Arizaga et al., 2006; Arizaga, Barba, Alonso & Vilches, 2010;

194   Arizaga & Tamayo, 2013). Measurements were taken by a team of three skilled ringers

195   that used the same protocols. All data were standardized to a single observer.

196   Recaptures were excluded to avoid pseudo-replication (only data from the first capture

197   event of each bird were used).

198   For each bird, we measured a set of biometric variables: body weight ($\pm$ 0.1 g, measured

199   with a digital balance), wing length (maximum chord) ($\pm$ 0.5 mm), primary feathers

200   length (from the base to the tip of the feather, $\pm$ 0.5 mm; P2 to P9, numbered

201   descendently) and tarsus length ($\pm$ 0.1 mm, measured with a digital caliper). The

202   measurement of the total length of the P2 to P9 feathers provides more statistical

203   consistency than the distance between the feather tips, as it allows for lower

204   measurement error (distances are much shorter than primary lengths) and higher

205   repeatability (Lessels & Boag, 1987; Swaddle & Witter, 1994). The length of P2 to P9

206   feathers -in descending order- was entered into a size-constrained component analysis

207   (SCCA), which is a method widely used to estimate wing morphology. The second axis

208   (C2) of the SCCA can be interpreted as a morphometric index of wing

209   roundness/pointedness (Lockwood et al., 1998). Low values of C2 correspond to high

210   wing pointedness (Arizaga, Campos, & Alonso, 2006).

211   Additionally, we determined birds' sex and age, classifying individuals as either first-

212   /second-year bird (FY/SY, EURING code 3 and 5) or adult (AD, EURING code 4 and

213   6). FY birds undergo a partial moult after fledging, before the autumn migration, whilst

214   AD do a complete moult. Therefore, the existence of a moult limit, which is normally

215   found in great coverts, reveals that a bird is a FY or a SY (if captured during its second

216   year of life, before its first complete moult after breeding for the first time). Otherwise,

217   the bird is classified as AD (no moult limit).

To account for geographic and environmental variation, we recorded, for each sampling

locality: (i) latitude and elevation; (ii) dominant vegetation type, i.e. brooms (*Cytisus*

spp. and *Genista* spp), heathlands (*Erica* spp. and *Calluna vulgaris*) or holm oak

shrublands (*Quercus rotundifolia* and *Cistus* spp.); and, (iii) averaged mean temperature

and rainfall of spring (March, April and May; corresponding to species arrival to

breeding sites, mating and nesting) and summer (June, July and August; corresponding

to fledging and post breeding months prior to migration). Climatic data were retrieved

from the Climatic Map of the Iberian Peninsula (Ninyerola et al., 2005), which provides

data for the period 1950–1999 at 200 m of spatial resolution. In order to draw the

general climatic pattern of each locality, variables were entered into a Principal

Component Analysis (PCA), where the first principal component accounted for 74.75%

of the variance.

Genetic divergence: microsatellite genotyping, genetic structure and differentiation

A subsample of 266 bluethroats, from the whole set of individuals captured for

morphological assessment, was considered for genetic analysis. Genomic DNA was

extracted from blood samples, all being genotyped for 12 microsatellite loci: Aar8,

Ase19, Cul4, Cul10, Fhu2, Hru7, Mcy4, PAT MP 2-43, Pdo5, Phtr2, PmaC25 and Ppi2.

The loci Pdo5 and Aar8 were dismissed due to strong evidence of null alleles and

monomorphism, respectively. This dataset was used to evaluate: (i) genetic

differentiation on the basis of F_{ST} values, as a measure of pair-wise genetic distance;

and, (ii) genetic structure by means of R- GENELAND v. 4.0.4 (Guillot, Mortier &

Estoup, 2005), a package that applies a Bayesian clustering method to infer a number of

clusters (K) of individuals. The achieved four clusters (Figure 1) were already

considered in a previous study (García et al., 2020) dealing with the genetic

243 differentiation of the Iberian bluethroat. Further methodological information can also be

244 found in Alda et al. (2013).

245

246 **Morphological differentiation across geographic and environmental gradients**

247 To evaluate the morphological differentiation of the Iberian bluethroat across

248 geographic and environmental gradients and test the Bergmann's rule, we calculated an

249 index of body size that was made of the first Principal Component ($PC1_{morph}$) of a PCA

250 based on body weight, wing length and tarsus length (Freeman & Jackson 1990;

251 Schauble 2004; Milá, Wayne & Smith, 2008). $PC1_{morph}$ showed high and positive factor

252 loading for all the input variables and accounted for 54.3% of the variance.

253 Thereafter, we ran Generalized Linear Mixed Models (GLMM) using, in turn, the

254 indices of body size ($PC1_{morph}$) and wing pointedness achieved in each locality, as

255 object variables, and the geographic (latitude and elevation) and environmental (climate

256 and vegetation) variables, as predictors. The locality was included in the models as a

257 random effect and the age of the birds (juveniles *vs*. adults) as a fixed effect, because it

258 may likely affect morphometric traits (Arizaga et al., 2006). GLMM is an appropriate

259 method for modelling clustered and autocorrelated data (Pinheiro & Bates, 2000) and,

260 therefore, for dealing with spatial pseudorreplication. Prior to the analysis, we evaluated

261 the Pearson's pairwise correlations between all predictors in order to minimize further

262 multi-collinearity problems. When two or more explanatory variables were strongly

263 correlated (r> 0.7), we kept the variable with a more direct interpretation from the point

264 of view of the ecology of the species. The most parsimonious models were selected by

265 means of Akaike's Information Criterion (Burnham & Anderson 2002) using the dredge

266 function of MuMIn R library. All statistical tests were implemented in R, version 3.6.3

267 (R Core Team, 2020).

268

Geographic isolation and genetic distance as drivers of morphological differentiation

First, we tested differences in biometric (body weight, wing length and tarsus length) and morphometric (wing pointedness) traits among genetic clusters and localities using ANOVA and post-hoc Tukey test. Then, we evaluated whether the differentiation of these traits was driven by geographic isolation and /or by genetic differentiation by means of Mantel tests (Mantel, 1967). Morphological and genetic differentiation, as well as geographic isolation, were calculated as Euclidean distances between all pairs of sampling localities.

RESULTS

Body size varied significantly across the geographic gradient present in the Iberian bluethroat breeding range. Nevertheless, no body size differentiation was found through environmental gradients of climate and vegetation. The best model within the set of candidate models included age, latitude and elevation. As expected, body size was positively related to elevation. In contrast, it was inversely correlated to latitude, birds located at the southernmost grounds being the largest. This latitudinal trend in body size is on the opposite direction to that expected according to Bergmann's rule. Wing shape (pointedness) also varied significantly across the geographic gradient. Specifically, the best model included age and latitude, the wing shape index being negatively related to latitude (i.e. higher latitude, higher wing pointedness) (Table 1).

As a trend, body weight, wing length and tarsus length varied significantly among genetic clusters (Table S2, Supplementary Material). According to the Tukey-HSD post hoc analyses, the strongest differences were found among the Central System (genetic

293 cluster K4) and the rest of breeding nuclei (genetic clusters K1, K2 and K3) (Table 2).

294 In fact, bluethroats breeding in the southernmost mountains of the study area had the

295 largest body mass, wings and tarsus, in respect to other individuals evaluated across the

296 subspecies' range (Table S2). Regarding wing shape, birds from the northernmost

297 mountain clusters (K1 and K2) had more pointed wings than those at lower elevation

298 (K3) and further south, in the Central System (K4) (Figure S1, Supplementary

299 Material). Additionally, body weight ($F_{22, 579}=5.57$, $p<0.001$), wing length ($F_{22,629}=8.24$,

300 $p<0.001$), wing pointedness ($F_{20, 426}=6.01$, $p<0.001$) and tarsus length ($F_{22,595}=2.21$,

301 $p<0.01$) diverged among sampling localities.

302 Morphological differentiation in body weight and wing length was significantly

303 correlated to geographic distance. However, this relationship was not found in the case

304 of wing shape and tarsus length (Figure 2). Only body weight was driven by genetic

305 distance (Figure 3).

306 Table 3 shows a summary of the main results found in this research.

307

308 **DISCUSSION**

309 Our results suggest a strong morphological differentiation in the Iberian bluethroat that

310 may be related to geographic isolation and ecological selection mechanisms. Regardless

311 geographic factors, differences in body size across the latitudinal gradient were clear,

312 but contrary to what Bergmann's rule states. Although this rule is applicable to a

313 considerable number of species (Hamilton, 1961; Blackburn & Gaston, 1996), there are

314 many other species or populations in which it is not (see, for example, Salewski,

315 Hochachka & Fiedler, 2010). Morphological differences not explained by latitude are

316 usually attributable to local variations in productivity that depend on soil fertility, water

317 availability and temperature, as well as to other factors as phylogenetic history,

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318   elevation gradients, species range size or competition (Strickland & Demarais, 2008;

319   Huston & Wolverton, 2011; Maestri et al., 2016). In our case, the fact that Bergmann's

320   rule was not apparently fulfilled might be explained by the effect of elevation. In fact,

321   Iberian bluethroats nesting in the southernmost mountain range, the Central System,

322   were found at the highest elevation. This mountain system also has the highest

323   Mediterranean influence among the target mountain ranges, thus birds need to search

324   for resources at higher elevation, where climatic conditions and available resources are

325   more favorable.

326   We found no correlation between morphological and genetic differentiation for wing

327   length and shape or tarsus length, neither between morphological divergence and

328   geographic isolation for wing shape and tarsus length. This lack of correlation between

329   genetic divergence and morphological differentiation could be associated in this case to

330   different factors. First, the isolation process is recent and the time elapsed might be

331   actually insufficient for a reproductive isolation to be reflected in neutral molecular

332   markers, such as the microsatellites used in this study (Nice & Shapiro, 1999). This

333   genetic delay is usually more obvious when values for morphologic divergence are

334   compared with genetic divergence calculated via mitochondrial DNA, given that the

335   mutation rate is lower than for microsatellites (Ortego, Aguirre & Cordero, 2012).

336   Second, if strong selective forces intervene, they could act more or less intensively over

337   adaptation processes to specific local conditions, for instance through differences in diet

338   and habitat selection (Fiedler, 2005; Förschler & Bairlein, 2011). Third, perhaps the

339   different factors and selective forces are acting antagonistically, thus masking

340   differentiation. In any case, wing and tarsus traits represent a trade-off between a variety

341   of conflicting and strong selective pressures. For instance, wing length has been linked,

342   in the case of the bluethroat, to different migration distances (Winkler & Leisler, 1992;

343 Pérez-Tris & Tellería, 2001; Arizaga et al., 2006), habitat use and foraging behaviour

344 (Marchetti et al., 1995; Forstmeier & Keβler 2001) or sexual selection (Hedenström &

345 Møller, 1992). Indeed, as the bluethroat forages on the ground, tarsus length, like other

346 morphological characters, could change rapidly when is subjected to natural selection

347 (Salewski, et al., 2014). The pattern described for bluethroats is consistent with that of

348 other species, as the dark-eyed junco (*Junco hyemalis*) that inhabits changing

349 environments affected by perturbations and is able to colonize new areas (Yeh & Price

350 2004) or remain and adapt to those changes via phenotypic plasticity or small

351 microevolutionary changes (González, Ronce, Ferriere, & Hochberg, 2013).

352 The findings derived from the analyses of biometric and morphometric variables

353 amongst genetic clusters strengthened the idea that morphological differentiation is not

354 only due to stochastic factors. Individuals from the Central System (K4) are noticeably

355 the largest, which is reflected in all biometric variables considered. Nevertheless, for the

356 case of wing pointedness, this trend does not occur, with individuals from the Northern

357 Mountains (K1 and K2) having the smallest, but most pointed wings. Such differences

358 in wing morphology could be related to differences in migration strategy but,

359 unfortunately, there is no accurate information on the overwintering grounds of Iberian

360 populations and even less on the existence of differentiated areas for each population. In

361 any case, the relationship between wing morphology and migration strategy might be

362 indirect, as this variable also varies according to other factors that may not be associated

363 to overwintering areas, but to selective pressures as a flight tactic against predation

364 (Mönkkönen, 1995). Furthermore, the assessment of the effect of geographic factors on

365 body size and wing shape indicate that there is no evolutionary confluence. On the one

366 hand, the largest bluethroats were found at the highest elevation and lowest latitude;

367 and, on the other hand, birds occupying territories at high elevation with similar

368 phenology in spring territory occupation did not have similar wing morphology. In this

369 sense, the migration phenology has been highlighted as a notable selective force

370 (Delmore et al., 2015), and the divergent migratory behavior as a potential source of

371 genetic and phenotypic divergence (Jones et al., 2005; Irwin, 2009; Winkler & Leisler

372 2005). This may be a basic question in genetic differentiation, as it could act as a barrier

373 to genetic exchange among populations. Iberian bluethroat populations supposedly do

374 not share overwintering areas, their arrival at breeding areas and reproductive

375 phenology not being coincident in time (Casagrande, Dell'Omo, Costantini &

376 Tagliavini, 2006). Differences in the arrival date from winter quarters to Iberian

377 breeding grounds are remarkable, with birds reaching the foothills of the Northern

378 Mountains towards the end of February or beginning of March, but arriving to the

379 highest elevation areas (over 2000 m a.s.l.) of these mountain ranges by May. A

380 significant variation in wing morphology has been also found within the subspecific

381 complex of the bluethroat, among southern (*L. s. namnetum, L .s. azuricollis*) and

382 central Europe (*L. s. cyanecula*) populations (Arizaga, Campos & Alonso, 2006), that

383 overwinter in the Iberian Peninsula and Africa, and Scandinavian populations (*L. s.

384 svecica),* that overwinter in Indo-European areas (Lislevand et al., 2015). Differences in

385 wing shape in correlation to migratory distance (Förschler & Bairlein, 2011) have also

386 been observed for other species including both migratory and sedentary populations.

387 This is the case of the dark-eyed junco or the blackcap (*Sylvia atricapilla*) (Mulvihill &

388 Chandler, 1990; Pérez-Tris & Tellería, 2001), with migrating populations tending to

389 have more pointed wings (Mulvihill & Chandler, 1990; Senar, Lleonart & Metcalfe,

390 1994; Fiedler, 2005). Nevertheless, other species, as the European blackbird (*Turdus

391 merula*), did not show significant wing morphology variation across migratory and

392 sedentary populations, indicating that differentiation may be restricted by other selective

17

forces, such as habitat (Fudickar & Partecke, 2012). Indeed, Förschler & Bairlein (2011) found that, among the four evaluated subspecies of the northern wheatear (*Oenanthe oenanthe*), the largest subspecies flying the longest distances across large stretches of open sea, presented the strongest morphological adaptations. Nevertheless, the smallest subspecies, distributed in breeding areas in the northernmost latitudes in Siberia, showed similar adaptations to long distance migration.

In conclusion, evidence suggests that the strong morphological differentiation found for Iberian bluethroat breeding populations across the geographic gradient is driven by a combination of unambiguous deterministic forces, such as ecological selection mechanisms, and other factors, such as geographic isolation, that can be either deterministic, stochastic or both, all acting at different scales. Indeed, the results point to local adaptations related to migratory strategies in the biometric variables of greater selective pressure. The study of the morphological differentiation in populations that inhabit fragmented habitats across geographic and environmental gradients is key to understand the early stages of speciation processes.

DECLARATIONS

Conflicts of interest – We do not have any conflict of interest to declare.

Author contributions – JG, SSS and JA designed the study and wrote the manuscript; NR and DA performed fieldwork.

Data availability statement – The data that support the findings of this study are openly available in the Dryad Digital Repository at https://doi.org/10.5061/dryad.msbcc2fz5

REFERENCES

Alatalo, R. V. Gustafsson, L., & Lunderg, A. (1984). Why do young birds have shorter wings than older birds? *Ibis,* 126, 410–415.

Alda, F., García, J., García, J. T., & Suárez-Seoane, S. (2013). Local genetic structure on breeding grounds of a long-distance migrant passerine: the Bluethroat (*Luscinia svecica*) in Spain. *Journal of Heredity*, 104, 36–46.

Arizaga, J., Alonso, D., Cortés, J. A., Eggenhuizen, T., Foucher, J., Franz, D., García, J., Koning, F., Leconte, M., Rguibi, H, Valkenburg, T., Vera, P., & Hobson, K. A. (2015). Migratory Connectivity in european bird populations: feather stable isotope values correlate with biometrics of breeding and wintering bluethroats *Luscinia svecica*. *Ardeola*, 62, 255–267.

Arizaga, J., Campos, F., & Alonso, D. (2006). Variations in wing morphology among subspecies might reflect different migration distances in Bluethroat. *Ornis Fennica*, 83, 162–169.

Arizaga, J., Barba, E., Alonso, D., & Vilches, A. (2010). Stopover of Bluethroats (*Luscinia svecica cyanecula*) in northern Iberia during the autumn migration period. *Ardeola*, 57, 69–85.

Arizaga, J., & Tamayo, I. (2013). Connectivity patterns and key non-breeding areas of white-throated Bluethroat (*Luscinia svecica*) European populations. *Animal Biodiversity and Conservation*, 36, 69–78.

Arizaga, J., García, J., & Suárez-Seoane, S. (2011). Ruiseñor pechiazul. *Luscinia svecica*. In Salvador, A., & Bautista, L. M. (eds.), *Enciclopedia Virtual de los Vertebrados Españoles*. http://www.vertebradosibericos.org/ Madrid. Museo Nacional de Ciencias Naturales.

441   Ashton, K. G. (2002). Do amphibians follow Bergmann's rule? *Canadian Journal of*

442   *Zoology*, 80, 708–716.

443   Ashton, K.G., Tracy, M.C., De Queiroz, A. (2000). Is Bergmann's rule valid for

444   mammals? *American Naturalist*, 156, 390-415.

445   Atkinson, D. (1994). Temperature and organism size - a biological law for ectotherms.

446   *Advances in Ecological Research*, 25, 1-58.

447   Bears, H., Drever, M. C., & Martin, K. (2008). Comparative morphology of dark-eyed

448   juncos *Junco hyemalis* breeding at two elevations: a common aviary experiment.

449   *Journal of Avian Biology*, 39, 152–162.

450   Bergmann, C. (1847). Ueber die Verhältnisse der Wärmeökonomie der Thiere zu ihrer

451   Grösse. *Gottinger Studien*, 3, 595–708.

452   Berke, S. K., Jablonski, D., Krug, A. Z., Roy, K., & Tomasovych, A. (2013). Beyond

453   Bergmann's rule: size–latitude relationships in marine Bivalvia world-wide. *Global*

454   *Ecology and Biogeography*, 22, 173–183.

455   Bermejo, A., & De la Puente, J. (2004). Wintering and migration of Bluethroat *Luscinia*

456   *svecica* in central Spain. *Ardeola*, 51, 285–296.

457   Blackburn, T. M., & Gaston, K. J. (1996). Spatial patterns in the body sizes of bird

458   species in the New World. *Oikos*, 77, 436–446.

459   Blackburn, T. M., Gaston, K. J., & Loder, N. (1999). Geographic gradients in body size:

460   a clarification of Bergmann's rule. *Diversity and Distributions*, 5, 165–174.

461   Bolnick, D. I., Amarasekare, P., Araújo, M. S., Bürger, R., Levine, J. M., Novak, M.,

462   Rudolf,V. H. W., Schreiber, S. J., Urban, M. C., & Vasseur, D. A. (2011). Why

463   intraspecific trait variation matters in community ecology. *Trends in Ecology &*

464   *Evolution*, 26, 183-192.

465   Burnham, K. P., & Anderson, D. R. (2002). *Model Selection and Multimodel Inference:*

466   *a practical information-theoretic approach*. Springer-Verlag.

467   Casagrande, S., Dell'Omo G, Costantini D., & Tagliavini J. (2006). Genetic differences

468   between early- and late-breeding Eurasian kestrels. *Evolutionary Ecology Research*,

469   8, 1029–1038.

470   Clegg, S. M., Degnan, S. M., Moritz, C., Estoup, A., Kikkawa, J., & Owens, I. P. F.

471   (2002). Microevolution in island forms: the roles of drift and directional selection in

472   morphological divergence of a passerine bird. *Evolution*, 56, 2090–2099.

473   Coyne, J. A., & Orr, H. A. (2004). *Speciation*. Sinauer Associates, Sunderland.

474   Cramp, S. (ed.) (1988). *The Birds of the Western Palearctic. Handbook of Birds in*

475   *Europe, the Middle East and Africa*, Vol V. Oxford University Press. Oxford.

476   Delmore, K. E., Kenyon H. L., Germain, R. R., & Irwin, D. E. (2015). Phenotypic

477   divergence during speciation is inversely associated with differences in seasonal

478   migration. *Proceedings of the Royal Society B: Biological Sciences*, 282, 20151921.

479   Freeman, S., & Jackson, W. M. (1990). Univariate metrics are not adequate to measure

480   avian body size. *The Auk*, 107, 69–74.

481   Fiedler, W. (2005). Ecomorphology of the external flight apparatus of blackcaps (*Sylvia*

482   *atricapilla*) with different migration behaviour. *Annals of the New York Academy of*

483   *Sciences*, 1046, 253–263.

484   Förschler, M. I., & Bairlein, F. (2011). Morphological Shifts of the External Flight

485   Apparatus across the Range of a Passerine (Northern Wheatear) with Diverging

486   Migratory Behaviour. *PloS ONE*, 6, e18732.

487   Forstmeier, W., & Keßler, A. (2001). Morphology and foraging behaviour of Siberian

488   *Phylloscopus* warblers. *Journal of Avian Biology*, 32, 127–138.

489 Fudickar, A. M., & Partecke, J. (2012). The Flight Apparatus of Migratory and

490   Sedentary Individuals of a Partially Migratory Songbird Species. *PLoS ONE*, 7,

491   e51920.

492 García, J., Fuertes, B., & Juan, M. (2000). Diferencias biométricas relacionadas con el

493   sexo y la edad en una población ibérica de pechiazul (*Luscinia svecica cyanecula*).

494   *Butlletí Grup Català d´Anellament*, 17, 11–16.

495 García, J., Johnsen, A., Fuertes, B., & Suárez-Seoane, S. (2017). Evolutionary

496   divergences in *Luscinia svecica* subspecies complex–new evidence supporting the

497   uniqueness of the Iberian Bluethroat breeding populations. *Ornis Fennica*, 94, 141–

498   149.

499 García, J., Morán-Ordóñez, A., García, J., Calero-Riestra, M., Alda, F., Sanz, J., &

500   Suárez-Seoane, S. (2020). Current landscape attributes and landscape stability in

501   breeding grounds explain genetic differentiation in a long-distance migratory bird.

502   *Animal Conservation*, 24, 120-134

503 Gaston, K. J., & Blackburn, T. M. (1996). Global scale macroecology: interactions

504   between population size, geographic range size and body size in Anseriformes.

505   *Journal of Animal Ecology*, 65, 701–714.

506 González, A., Ronce, O., Ferriere, R., & Hochberg M. E. (2013). Evolutionary rescue:

507   an emerging focus at the intersection between ecology and evolution. *Philosophical

508   Transactions of the Royal Society B: Biological Sciences*, 368, 20120404.

509 Greve, M., Gaston, K. J., Van Rensburg, B. J., & Chown, S. L. (2008). Environmental

510   factors, regional body size distributions and spatial variation in body size of local

511   avian assemblages. *Global Ecology and Biogeography*, 17, 514-523.

512 Guillot, G., Mortier F., & Estoup, A. (2005). Geneland: A program for landscape

513   genetics. *Molecular Ecology Notes*, 5, 712–715.

514 Hall, K. S., Ryttman, H., Fransson, Th., & Stolt, B. O. (2004). Stabilising selection on

515   wing length in reed warblers. *Journal of Avian Biology*, 35, 7–12.

516 Hamilton, T. H. (1961). The adaptive significances of intraspecific trends in wing

517   length and body size among bird species. *Evolution*, 15, 180–195.

518 Hedenström, A., & Møller, A. P. (1992). Morphological adaptations to song flight in

519   passerine birds: A comparative study. *Proceedings of the Royal Society of London,*

520   *Series B*, 247, 183–187.

521 Hewitt, G. M. (1996). Some genetic consequences of ice ages, and their role in

522   divergence and speciation. *Biological Journal of the Linnean Society*, 58, 247–276.

523 Hogner, S., Laskemoen, T., Lifjeld, J. T., Pavel, V., Chutný, B., García, J., Eybert, M.

524   C., Matsyna, E., & Johnsen, A. (2013). Rapid sperm evolution in the Bluethroat

525   (*Luscinia svecica*) subspecies complex. *Behavioral Ecology and Sociobiology*, 67,

526   1205–1217.

527 Hughes, L. (2000). Biological consequences of global warming: is the signal already

528   apparent? *Trends in Ecology & Evolution*, 15, 56–61.

529 Huston, M. A., & Wolverton, S. (2011). Regulation of animal size by eNPP,

530   Bergmann's rule, and related phenomena. *Ecological Monoghraps*, 81, 349–405.

531 Irwin D. E. (2009). Speciation: new migratory direction provides route towards

532   divergence. *Current Biology*, 19, 1111–1113.

533 Johnsen, A., Andersson, S., García Fernández, J., Kempenaers, B., Pavel, V., Questiau,

534   S., Raess, M., & Lifjeld, J. T. (2006). Molecular and phenotypic divergence in the

535   Bluethroat (*Luscinia svecica*) subspecies complex. *Molecular Ecology*, 15, 4033–

536   4047.

537 Johnsen, A., Fidler, A. E., Kuhn, S., Carter, K. L., Hoffmann, A., Barr, I. R., Biard, C.,

538   Charmantier, A., Eens, M., Korsten, P., Siitari, H., Tomiuk, J., & Kempenaers, B.

539   (2007). Avian Clock gene polymorphism: evidence for a latitudinal cline in allele

540   frequencies. *Molecular Ecology*, 16, 4867–4880.

541   Jones, J., Gibb, C. E., Millard, S. C., Barg, J. J., Katharine Girvan, M. Veit, M. L.,

542   Friesen, V. L., & Robertson, R. J. (2005). Multiple selection pressures generate

543   adherence to Bergmann's rule in a neotropical migratory songbird. *Journal of*

544   *Biogeography*, 32, 1827–1833.

545   Lessells, C. M., & Boag, P. T. (1987). Unrepeatable repeatabilities: a common mistake.

546   *Auk*, 104, 116–121.

547   Lindsey, C. C. (1966). Body sizes of poikilotherm vertebrates at different latitudes.

548   *Evolution*, 20, 456–465.

549   Lislevand, T., Chutný, B., Byrkjedal, I., Pavel, V., Briedis, M., Adamik, P., & Hahn, S.

550   (2015). Red-spotted Bluethroats *Luscinia s. svecica* migrate along the Indo-

551   European flyway: a geolocator study. *Bird Study*, 62, 508–515.

552   Lockwood, R., Swaddle, J. P., & Rayner, M. V. (1998). Avian wingtip shape

553   reconsidered: wingtip shape indices and morphological adaptations to migration.

554   *Journal of Avian Biology*, 29, 273–292.

555   Lomas, M., Willemoes, M., Arizaga, J., Onrubia, A., Cuenca, D., Alonso, D., Torralvo,

556   C., Tøttrup, A. P., & Thorup, K. (2019). Migration strategies of Iberian breeding

557   White-Rumped Swifts *Apus caffer*, Rufous-Tailed Scrub-Robins *Cercotrichas*

558   *galactotes* and Bluethroats *Cyanecula svecica*. *Ardeola* 66, 51–64.

559   Maestri, R., Fornel, R., Gonçalves, G. L., Geise, L., Freitas, T. R. O., & Carnaval, A. C.

560   (2016). Predictors of intraspecific morphological variability in a tropical hotspot:

561   Comparing the influence of random and non-random factors. *Journal of*

562   *Biogeography*, 43, 2160– 2172.

563 Mantel, N. A. (1967). The detection of disease clustering and a generalized regression
564   approach. *Cancer Research*, 27, 209–220.

565 Marchetti, K., Price, T., & Richman, A. (1995). Correlates of wing morphology with
566   foraging behavior and migration distance in the genus *Phylloscopus*. *Journal of*
567   *Avian Biology*, 26, 177–181.

568 Martí, R., & Del Moral, J. C. (eds). (2003). *Atlas de las aves reproductoras de España*.
569   DGCN-SEO/BirdLife, Madrid.

570 Mayr, E. (1956). Geographical character gradients and climatic adaptation. *Evolution,*
571   10, 105–108.

572 Meijer, R., & Stastny, K. (1997). *Bluethroat*. En: Hagemeijer E. J. M., & Blair M. J.
573   (eds). *The EBCC Atlas of European Breeding Birds: Their Distribution and*
574   *Abundance*. T. & A. D. Poyser.

575 Meiri, S., & Dayan, T. (2003). On the validity of Bergmann's rule. *Journal of*
576   *Biogeography*, 30, 331–351.

577 Meiri, S., Yom-Tov, Y., & Geffen, E. (2007). What determines conformity to
578   Bergmann's rule? *Global Ecology and Biogeography*, 16, 788–794.

579 Milá, B., Wayne, R. K., & Smith, T. B. (2008). Ecomorphology of migratory and
580   sedentary populations of the yellow-rumped warbler (*Dendroica coronata*). *Condor,*
581   110, 335–344.

582 Millien, V., Lyons, S. K., Olson, L., Smith, F. A., Wilson, A. B., & Yom-Tov, Y.
583   (2006). Ecotypic variation in the context of global climate change: revisiting the
584   rules. *Ecology Letters*, 9, 853–869.

585 Mönkkönen, M. (1995). Do migrant birds have more pointed wings? A comparative
586   study. *Evolutionary Ecology*, 9, 520–528.

587 Morgan, J. H. (2004). Remarks on the taking and recording of biometric measurements

588   in birds ringing. *Ring*, 26, 71–78.

589 Mulvihill, R. S., & Chandler, C. R. (1990). The relationship between wing shape and

590   differential migration in the Dark-eye Junco. *Auk* 107, 490–499.

591 Nice, C. C., & Shapiro, A. M. (1999). Molecular and morphological divergence in the

592   butterfly genus *Lycaeides* (Lepidoptera: *Lycaenidae*) in North America: evidence of

593   recent speciation. *Journal of Evolutionary Biology*, 12, 936- 950.

594 Orsini, L., Vanoverbeke, J., Swillen, I., Mergeay, J., & De Meester, L. (2013). Drivers

595   of population genetic differentiation in the wild: isolation by dispersal limitation,

596   isolation by adaptation and isolation by colonization. *Molecular Ecology*, 22, 5983–

597   5999.

598 Ortego, J., Aguirre, M. P., & Cordero, P. J. (2012). Genetic and morphological

599   divergence at different spatiotemporal scales in the grasshopper *Mioscirtus wagneri*

600   (Orthoptera: Acrididae). *Journal of Insect Conservation*, 16, 103–110.

601 Pérez-Tris, J., & Tellería, J. L. (2001). Age-related variation in wing-shape of migratory

602   and sedentary Blackcaps *Sylvia atricapilla*. *Journal of Avian Biology*, 32, 207–213.

603 Pinheiro, J. C., & Bates, D. M. (2000). *Mixed-Effects Models in S and SPLUS*. Springer

604 R Core Team. (2020). *R: A language and environment for statistical computing R*

605   *Foundation for Statistical Computing*, Vienna. Austria. URL http://www.R-

606   project.org/

607 Rayner, J. M. (1988). Form and function in avian flight. *Current Ornithlogy*, 1, 1-66.

608 Rodríguez, M. Á., López-Sañudo, I. L., & Hawkins, B. A. (2006). The geographic

609   distribution of mammal body size in Europe. *Global Ecology and Biogeography*, 15,

610   173–181.

611 Romano, A., Séchaud, R., & Roulin, A. (2021). Generalized evidence for Bergmann'

612 rule: body size variation in a Cosmopolitan owl genus. *Journal of Biogeography*, 48,

613 51–63.

614 Salewski, V., Hochachka, W. M., & Fiedler, W. (2010). Global warming and

615 Bergmann's rule: do central European passerines adjust their body size to rising

616 temperatures? *Oecologia*, 162, 247–260.

617 Salewski, V., Siebenrock, K. H., Hochachka, W. M., Woog, F., & Fiedler, W. (2014)

618 Morphological Change to Birds over 120 Years Is Not Explained by Thermal

619 Adaptation to Climate Change. *Plos ONE*, 9, e101927.

620 Salewski, V., & Watt, C. (2017). Bergmann's rule: a biophysiological rule examined in

621 birds. *Oikos*, 126.

622 Schauble, C. S. (2004). Variation in body size and sexual dimorphism across

623 geographical and environmental space in the frogs *Limnodynastes tasmaniensis* and

624 *L. peronii*. *Biological Journal of the Linnean Society*, 82, 39–56.

625 Seebohm, H. (1901). *Birds of Siberia*. Murray.

626 Senar, J. C., Lleonart, J., & Metcalfe, N. B. (1994). Wing-shape variation between

627 resident and transient wintering Siskins *Carduelis spinus*. *Journal of Avian Biology*,

628 25, 50–54.

629 Siepielski, A., DiBattista J., & Carlson S. (2009). It's about time: the temporal

630 dynamics of phenotypic selection in the wild. *Ecology Letters*, 12, 1261–1276.

631 Slatkin, M. (1985). Gene flow in natural populations. *Annual Review of Ecology and

632 Systematics*, 16, 393–430.

633 Strickland, B. K., & Demarais, S. (2008). Influence of landscape composition and

634 structure on antler size of white-tailed deer. *Journal of Wildlife Management*, 72,

635 1101–1108.

636 Swaddle, J. P., & Lockwood. R. (1998). Morphological adaptations to predation risk in

637   passerines. *Journal of Avian Biology*, 29, 172–176.

638 Swaddle, J. P., & Witter, M. S. (1994). Food, feathers and fluctuating asymmetry.

639   *Proceedings of the Royal Society of London, Series B*, 255, 147–152.

640 Teplitsky, C., & Millien, V. (2014). Climate warming and Bergmann's rule through

641   time: is there any evidence? *Evolutionary Applications*, 7, 156–168.

642 Voelker, G. (2001). Morphological correlates of migratory distance and flight display in

643   the avian genus *Anthus*. *Biological Journal of the Linnean Society*, 73, 425–435.

644 Watt, C., Mirchell, S., & Salewski, V. (2010). Bergmann's rule; a concept cluster?

645   *Oikos*, 119, 89–110.

646 Winker, K. (2010). On the origin of species through heteropatric differentiation: a

647   review and a model of speciation in migratory animals. *Ornithological Monographs*,

648   69, 1–30.

649 Winkler, H., & Leisler, B. (1992). On the ecomorphology of migrants. *Ibis*, 134, 21–28.

650 Winkler, H., & Leisler, B. (2005). *To be a migrant: ecomorphological burdens and*

651   *chances*. In: Greenberg, R., & Marra, P. P. (eds) *Birds of two worlds: the ecology*

652   *and evolution of migration*. John Hopkins University Press, Baltimore, pp 79–86

653 Wright, S. (1931). Evolution in Mendelian populations. *Genetics*, 16, 97–159.

654 Yeh, P. J., & Price T. D. (2004). Adaptive phenotypic plasticity and the successful

655   colonization of a novel environment. *The American Naturalist*, 164, 531–542.

656 Yom-Tov, Y., & Geffen, E. (2011). Recent spatial and temporal changes in body size of

657   terrestrial vertebrates: probable causes and pitfalls. *Biological Reviews*, 86, 531-541.

658 Zhou, J., & Ning, D. (2017). Stochastic community assembly: does it matter in

659   microbial ecology? *Microbiology and Molecular Biology Reviews*, 81, e00002-17.

BIOSKETCH

Dr Javier García carries out multidisciplinary research, combining genetic tools with ecological modelling and applied conservation approaches in the framework of landscape genetics. This paper is part of his PhD research at the University of León (Spain).

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Table 1. Best models tested by Mixed Generalized Linear Models including geographic and environmental drivers of biometric (body size index, $PC1_{morph}$) and morphological (wing pointedness) differentiation. Significant factors ($P < 0.05$) are shown in boldface type in each model.

Model/explanatory variable	Estimate	SE	Significance	ΔAIC
BODY SIZE				
Age + **Latitude** + **Elevation**				0.00
Intercept	0.4689	0.0923	p<0.001	
Age (young)	-0.2015	0.1138	p=0.0775	
Latitude	**-0.7784**	**0.0701**	**p<0.001**	
Elevation	**0.1415**	**0.0715**	**p=0.0487**	
Latitude + Elevation				1.09
Latitude				1.72
Age + **Latitude**				1.87
Age				3.40
Age + Elevation				4.16
WING SHAPE				
Age + **Latitude**				0.00
Intercept	1.1420	0.0147	p<0.001	
Age (young)	**0.0521**	**0.0137**	**p<0.001**	
Latitude	**-0.0388**	**0.0064**	**p<0.001**	
Age + **Latitude** + **Elevation**				1.91
Age + **Latitude** + Vegetation				2.46
Age + **Latitude** + Vegetation + Elevation				2.51
Age				4.79
Age + Elevation				6.84

Table 2. P-values of Tukey-HSD post hoc analysis of wing length, tarsus length, body mass and wing pointedness. K1-Central Cantabrian Mountains. K2- Cantabrian Mountains (except central area) and Mountains of León. K3-Foothills of Cantabrian Mountains and Montes of León, K4-Central System.

	K1-K2	K1-K3	K1-K4	K2-K3	K2-K4	K3-K4
Body mass	0.338	0.896	***	0.307	***	***
Wing length	0.841	0.703	*	*	***	***
Wing pointedness	0.979	*	***	*	***	0.210
Tarsus length	0.600	0.694	0.750	0.963	**	***

(*p<0.05, **p<0.01, ***p<0.001)

Table 3. Main findings and examples of other bird species or bluethroat subspecies where a similar result has been found.

Trait	Finding	Examples in other bluethroat subspecies or bird species
Body size	Body size varies across the geographic gradient. It increases with elevation, but decreases with latitude.	Hamilton (1961); Blackburn & Gaston (1996); Romano, Séchaud & Roulin (2021)
Body weight	Body weight shows significant variation at intra-subspecific level. This pattern is driven by genetic structure (clusters), genetic distance and geographic isolation.	Fiedler (2005)
Wing pointedness	Wing pointedness increases with latitude. It varies at intra-subspecific level in correlation to genetic structure (significant differences among some clusters)	Mulvihill & Chandler (1990); Pérez-Tris & Tellería (2001); Arizaga, Campos & Alonso (2006); Förschler & Bairlein (2011)
Wing length	Wing length exhibits intra-subspecific variation driven by genetic structure and genetic distance.	Fiedler (2005); Förschler & Bairlein (2011); Arizaga et al. (2015); Romano, Séchaud & Roulin (2021)
Tarsus length	Tarsus length change at intra-subspecific level, according to genetic structure.	Förschler & Bairlein (2011)

Figure captions

Figure 1. Map of the study area. Black grid represents the breeding range of the bluethroat *Luscinia svecica azuricollis* in the Iberian Peninsula, according to Martí & Del Moral (2003). Dots correspond to capture localities (n=23) where morphological measurements and genetic data were collected. Color indicates the genetic cluster identified for each locality: K1-Central Cantabrian Mountains. K2- Cantabrian Mountains (except central area) and Mountains of León. K3-Foothills of Cantabrian Mountains and Montes of León, K4-Central System. See Table S1 in the Appendix for more information about the sampling localities. The EPSG 25830 (ETRS89, UTM zone 30 N) was used as the coordinate reference system.

Figure 2. Mantel correlations between morphological trait differentiation of Iberian bluethroats and geographic distance (Euclidean distance) among localities.

Figure 3. Mantel correlations between phenotypic and genetic differentiation (F_{ST} values based on neutral molecular markers) of Iberian bluethroats among localities.
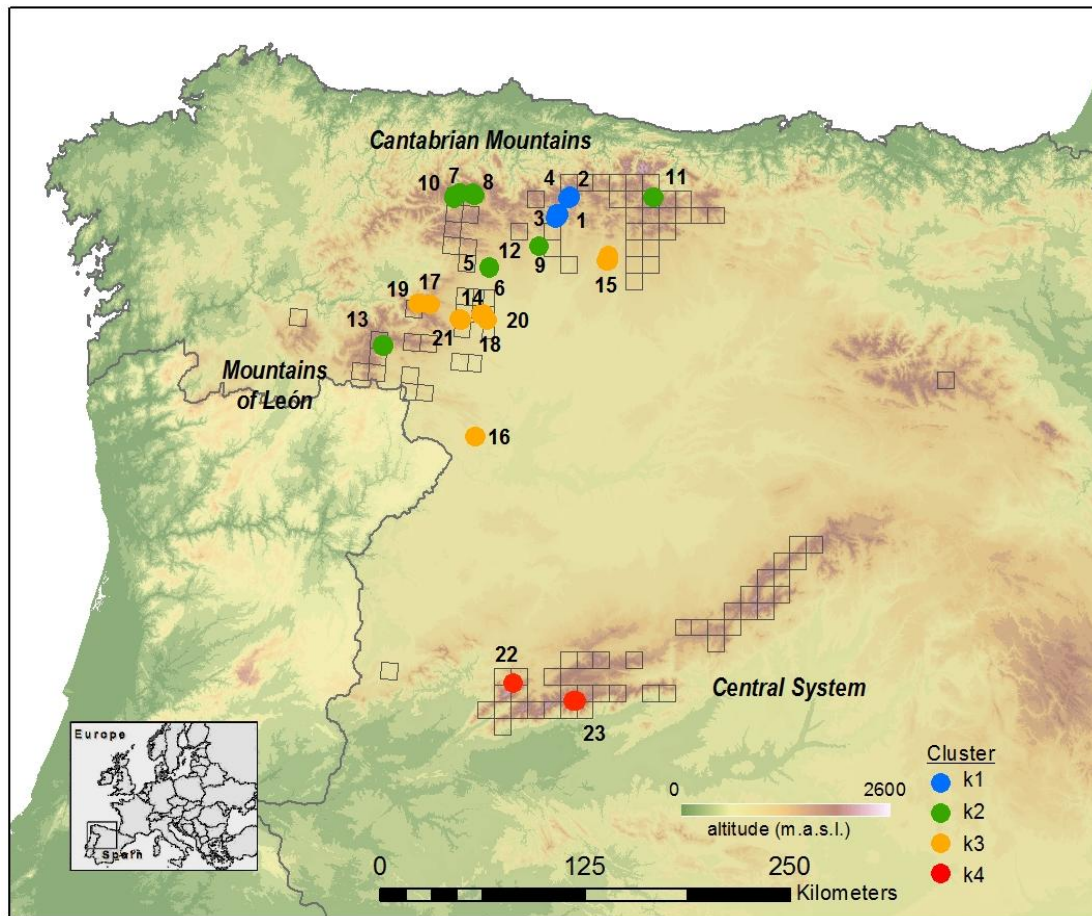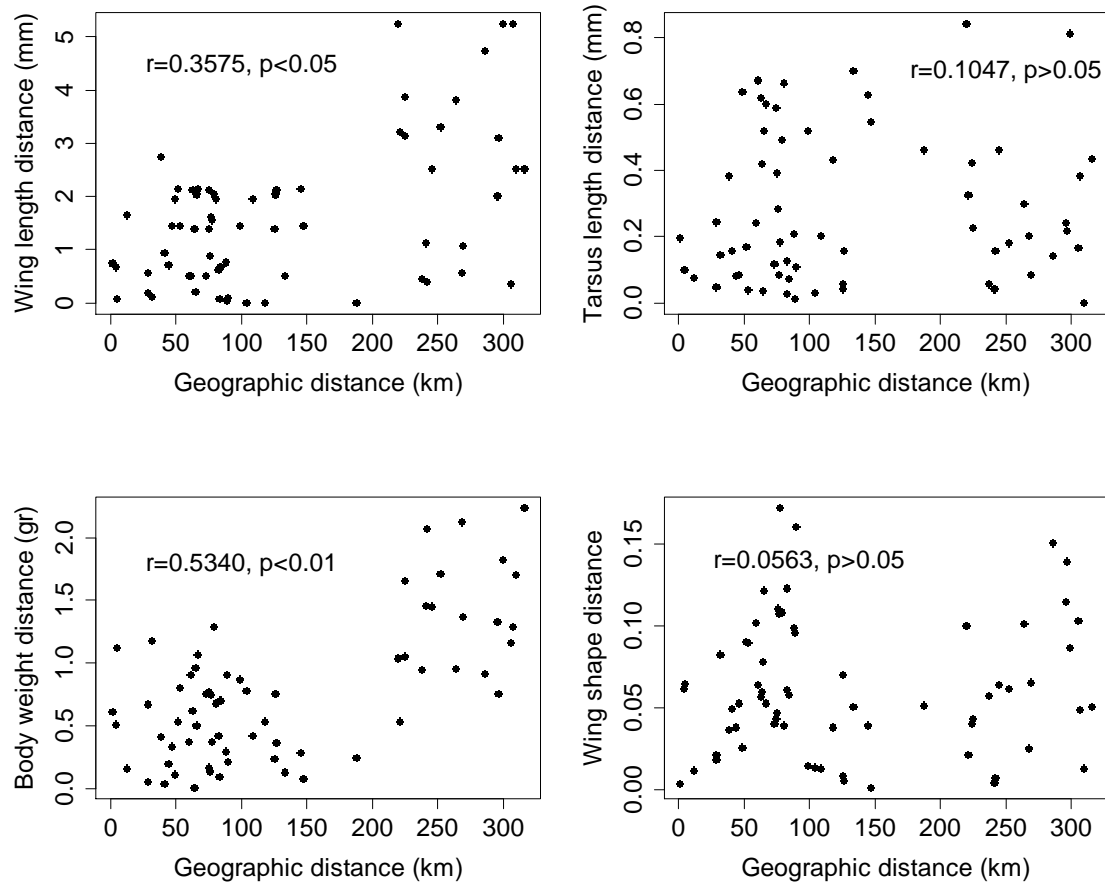
Figure 1

Figure 2

Figure 3