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Ceresa, F.; Belda, E.; Kvist, L.; Kajanus, M.; Monrós González, JS. (2018). Genetic differentiation between insular and continental populations of migratory and resident warblers, the Great Reed Warbler *Acrocephalus arundinaceus* and the Cetti's Warbler *Cettia cetti* *Journal of Ornithology*. *Journal of Ornithology* (Online). 159(3):703-712.
<https://doi.org/10.1007/s10336-018-1543-2>



The final publication is available at

<https://doi.org/10.1007/s10336-018-1543-2>

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Additional Information

Genetic differentiation between insular and continental populations of migratory and resident warblers, the Great Reed Warbler *Acrocephalus arundinaceus* and the Cetti's Warbler *Cettia cetti*

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1 **Abstract**

2

3 Island populations are frequently smaller than continental populations, have lower genetic diversity,
4 are more inbred and show genetic differentiation from the mainland. However, sufficient numbers
5 of immigrants may reduce the differentiation of insular populations and moderate the effects of
6 genetic drift. In this study, we compared insular and continental populations of Cetti's Warbler
7 *Cettia cetti* and Great Reed Warbler *Acrocephalus arundinaceus*. We assessed the degree of genetic
8 differentiation between insular and continental birds, their demographic history and genetic
9 diversity. We compared the results, taking into account the differences in migration strategy and
10 morphology of the two warblers. We found slightly lower genetic diversity in the insular
11 populations than in the continental birds, possibly because of the lower population size or reduced
12 immigration. The genetic differentiation between island and mainland birds was low, but higher in
13 the Cetti's Warbler than in the Great Reed Warbler, suggesting differences in the species' capability
14 of crossing the sea. We found evidence for a past bottleneck in both the insular and continental
15 populations of the Cetti's Warbler, while for the Great Reed Warbler we found no signs of past
16 population reductions. High dispersal capability of the Great Reed Warbler may have allowed high
17 gene flow, which may explain the observed interspecific differences in the demographic history.

18

19 **Keywords** Dispersal; Dispersal barrier; Gene flow; Insularity; Microsatellites

20 **Introduction**

21

22 Compared to mainland populations, island populations are often smaller, have a lower genetic
23 diversity and are more inbred (Frankham et al. 2010). These characteristics imply a higher
24 extinction risk, in fact most of the recently documented extinctions were of insular species, even
25 though they represent a minority of all species (Frankham et al. 2010). In species with sufficient
26 dispersal capability, island populations may receive immigrant individuals from the mainland. If
27 such immigration leads to sufficient gene flow, it may reduce genetic differentiation between insular
28 and mainland populations and moderate effects of genetic drift (Madsen et al. 1999; Hogg et al.
29 2006; Ortego et al. 2008; Frankham et al. 2010). Birds have generally high dispersal ability (Koenig
30 et al. 1996), but there are large differences among species, apparently related to characteristics such
31 as migration strategy, population size and habitat preference (Paradis et al. 1998; Sutherland et al.
32 2000). The environmental characteristics of areas, which separate suitable breeding habitats, also
33 influence the rate of gene flow (Frankham et al. 2010). Dispersal between islands and mainland
34 imply crossing a more or less wide water body, which may represent for some bird species a more
35 effective dispersal barrier than a similarly wide extension of land (Hodges and Krementz 1996;
36 Matchans et al. 1996; Ceresa et al. 2015).

37 Knowledge of the degree of connectivity between insular and continental bird populations may be
38 important for conservation purposes (e.g., Agudo et al. 2010) and for obtaining information about
39 species' capability to disperse and cross geographical barriers. Furthermore, comparing species with
40 different ecological and morphological characteristics may help to better understand the general
41 dispersal patterns of birds.

42 In this study, we compare insular and continental populations of two passerine birds, the
43 Cetti's Warbler *Cettia cetti* and the Great Reed Warbler *Acrocephalus arundinaceus*, based on their
44 population genetic structure, genetic diversity and demographic history. The Cetti's Warbler breeds

45 in a wide range from Western Europe and North-Western Africa to Central Asia. European
46 populations are mostly resident and breed in a variety of moist habitats such as marshes, lake- and
47 riversides, reedbeds, and sometimes also in dry scrublands (Kennerley and Pearson 2010). The
48 Great Reed Warbler is widely distributed across Europe, breeding mainly in inundated reedbeds
49 (Kennerley and Pearson 2010). It migrates over long distances and overwinters in sub-Saharan
50 Africa (Kennerley and Pearson 2010).

51 Both species breed on the island of Mallorca, which is one of the Balearic Islands located
52 about 150 km from the Spanish and 250 km from the Algerian coast. The Great Reed Warbler is
53 presumed to have higher dispersal capability than the Cetti's Warbler, given its longer migration
54 distance and more pointed wings (Kennerley and Pearson 2010), which allow higher flight
55 efficiency (Norberg et al. 1990). We therefore expect a weaker impact of insularity in the Mallorcan
56 populations of the Great Reed Warbler than in the Cetti's Warbler.

57

58 **Methods**

59

60 **Sampling and DNA extraction**

61 We obtained blood samples from 35 Cetti's Warblers and 44 Great Reed Warblers, mist-netted
62 during the breeding season of 2012-2013 (18 May – 30 July of 2012 and 9 May – 5 July of 2013) at
63 four marshland areas. For the Cetti's Warbler, we obtained samples from S'Albufera de Mallorca
64 (39°47' N - 3°06' E; extension ~1700 hectares) in Spain and Larache (35°08' N - 6°05' W; ~3600
65 ha) in Morocco (Fig 1). For the Great Reed Warbler, we obtained samples from the Pego-Oliva
66 Natural Park (38°51' N - 0°03' W; ~1250 ha) and Villafranca de los Caballeros (39°27' N - 3°19' W;
67 ~200 ha), both located in the Spanish mainland, S'Albufera de Mallorca and Larache (Fig 1).
68 Sampling took place late enough to exclude any possible wintering/migrating Cetti's Warblers,
69 while during May both local and migratory Great Reed Warblers can occur at the same time in our

70 study areas (Kennerley and Pearson 2010; Clement 2017). However, all Great Reed Warblers
71 sampled in May were breeding birds according to the development of the incubation patch or the
72 cloacal protuberance (in females and males, respectively; Svensson 1992). Therefore, occurrence of
73 non-local individuals in our sample is extremely unlikely. From Larache, we were able to sample
74 only two Great Reed Warblers, thus we did not include this locality in site-specific statistical
75 analyses (see below). Sample sizes of each site are given in Table 1. Blood samples (5-15 μ l) were
76 obtained by puncturing the brachial vein and stored in 96% ethanol. DNA was extracted using
77 UltraClean™ Blood Spin™ Kit (MoBio Laboratories) according to the manufacturer's protocol.

78

79 DNA amplification

80 We amplified 13 polymorphic microsatellite loci from the Cetti's Warblers: Cup28 (Gibbs et al.
81 1999, *Catharus ustulatus*), Gf05 (Petren 1998, *Geospiza fortis*), FhU2 (Ellegren 1992, *Ficedula*
82 *hypoleuca*), Ppi2 (Martínez et al. 1999, *Pica pica*), Zl54 (Frentiu et al. 2003, *Zosterops lateralis*),
83 Pca3 (Dawson et al. 2000, *Cyanistes caeruleus*), Hru6 (Primmer et al. 1995, *Hirundo rustica*),
84 Pdo μ 1 (Neumann and Wetton 1996, *Passer domesticus*), Aar4 and Aar8 (Hansson et al. 2000,
85 *Acrocephalus arundinaceus*), Esc3 and Esc6 (Hanotte et al. 1994, *Emberiza schoeniclus*) and Ase64
86 (Richardson et al. 2000, *Acrocephalus sechellensis*). From the Great Reed Warblers, we amplified
87 12 polymorphic loci: Cup28, FhU2, Pdo5 (Griffith et al. 1999, *P. domesticus*), Ppi2, Zl54, Pca3,
88 Aar4, Aar5 (Hansson et al. 2000, *Acrocephalus arundinaceus*), Aar8, Esc6, Ase34 (Richardson et al.
89 2000, *A. sechellensis*) and Ase64. We performed the Polymerase Chain Reaction (PCR) in 10- μ l
90 volumes using 1 μ l of DNA template, 1 μ l of PCR-buffer, 1 μ l of dNTPs (2 mM), 0.8 μ l of primers,
91 0.06 μ l of DNA-polymerase (Biotools) and 0.5 μ l of MgCl₂ (0.4 μ l for HrU6, 0.6 μ l for Esc3). The
92 PCR procedure consisted of an initial denaturation for 5 min (94°C), 35 cycles of denaturation for
93 30 s (94°C), annealing for 30 s (50°C) and synthesis for 45 s (72°C), and a final synthesis for 7 min
94 (72°C). Annealing temperatures for the primers were 48°C for Pdo5 and Gf05, 52°C for Ppi2 and

95 Ase64, 54°C for Cuμ28 and ZL54, and a touchdown from 50° to 45°C for Esc6, Hru6 and Esc3,
96 from 63°C to 53°C for Aar5, FhU2, Pca3 and Pdoμ1, form 54°C to 48°C for Aar4 and Ase34, and
97 form 60°C to 50°C for Aar8. We ran the PCR products on an ABI PRISM 3730 DNA Analyzer
98 (Applied Biosystems) and scored the alleles with GeneMapper 4.0. We checked the data with the
99 program MICROCHECKER 2.2.3 (van Oosterhout et al. 2004) for possible genotyping errors (null
100 alleles, scoring errors due to stuttering, large allele dropouts).

101

102 Statistical analysis

103 We used GENEPOP 4.2 (Raymond and Rousset 1995; Rousset 2008) to assess possible deviations
104 from the Hardy-Weinberg and linkage equilibrium separately for each sampling site and for the total
105 sample of both species. We calculated the expected heterozygosity (H_e) using Arlequin 3.5.1
106 (Excoffier and Lischer 2010), and inbreeding coefficient (F_{IS}) and allelic richness (A) with FSTAT
107 2.9.3 (Goudet 1995).

108 To investigate the genetic population structure within our samples, we used the Bayesian
109 program STRUCTURE 2.3.4 (Pritchard et al. 2000; Falush et al. 2003), which allows estimation of
110 the most probable number of distinct genetic clusters (K) in the data set. We adopted a model with
111 population admixture and correlated allele frequencies (Falush et al. 2003) and carried out the
112 analysis both with and without prior spatial information of sampling sites (i.e. sampling locality).
113 We performed ten independent runs for each value of K between 1 and 10, with a burn-in period of
114 50000 iterations and 500000 Markov chain Monte Carlo (MCMC) replications. Starting from the
115 STRUCTURE results, we also calculated the *ad hoc* statistics ΔK , which estimates the second order
116 rate of change of K -values between the consecutive numbers of genetic clusters. The highest value
117 obtained is inferred as the best estimator of the actual number of clusters (Evanno et al. 2005).
118 Although the program STRUCTURE is widely used to investigate the genetic structure of wild
119 populations, using and comparing more statistical methods is recommended, especially in case of

120 weak population structuring or complex phylogeographic patterns (e.g., Frosch et al. 2014, Kraus et
121 al. 2013, 2016, Wang et al. 2017). ~~Therefore, i~~In order to obtain further insights about population
122 structuring, we carried out also the discriminant analysis of principal components (DAPC), using
123 package adegenet (Jombart 2008) version 2.1.0 in R 3.3.2 (R Core Team 2016). This method
124 identifies genetic clusters through the K-means clustering algorithm, using the Bayesian
125 Information Criterion (BIC). To obtain more reliable assignments of individuals to clusters, we used
126 the *optim.a.score* function with 25 simulations to determine the optimal number of principal
127 components to be used in the DAPC. Furthermore, to estimate the genetic differentiation within and
128 among sampling locations, we performed an analysis of molecular variance (AMOVA) with the
129 program Arlequin, which was used also to calculate the pairwise F_{ST} values between the sampling
130 sites of the Great Reed Warbler. To obtain further details about gene flow, the program MIGRATE-
131 N [3.6.11](#) (Beerli and Felsenstein 1999, 2001) was used to perform a maximum likelihood estimation
132 of migration rates among sampling sites and among the genetic clusters identified by STRUCTURE
133 and DAPC. We applied the stepwise mutation model, ~~ran~~ 10 short and one long chain, recorded
134 every 100 steps of 50 000 or 500 000 genealogies, respectively and discarded 10000 trees from each
135 chain as a burn-in.

136 To investigate the demographic history of our insular and continental samples, we looked for
137 past bottlenecks using the program BOTTLENECK 1.2.02 (Cornuet et al. 1996; Piry et al. 1999),
138 which tests for heterozygosity excess caused by a recent reduction of the effective population size
139 (Piry et al. 1999). We used the Wilcoxon test under the two-phase mutation model with 95% single-
140 step mutations. We also estimated the Garza-Williamson index that compares the mean ratio of the
141 number of alleles to the range in allele size (Garza and Williamson 2001) with Arlequin, in order to
142 search for signs of population bottlenecks in a more remote past. This index decreases in a
143 bottlenecked population. For the Cetti's Warbler, we found weak population structuring between
144 samples from Mallorca and Larache and evidence for bottlenecks (see Results). Therefore, we used

145 the program DIY ABC 2.0.3 (Cornuet et al. 2008) to estimate the timing and severity of population
146 size reductions, as well as to further assess their occurrence. DIY ABC allows to compare different
147 competing historical/demographic scenarios, and for both Mallorca and Larache, we contrasted
148 three alternative scenarios: the first with no population size changes, the second describing
149 population growth and the third including a bottleneck (hereafter, scenario 1, 2 and 3, respectively).
150 We used the default range of priors for the time of population size changes (10-10000 generations)
151 and set priors for the effective population sizes to 10-10000 for Mallorca and 10-100000 for
152 Larache. We applied the default Generalized Stepwise Mutation model (Estoup et al. 2002) and four
153 default summary statistics. With these settings, a total of 3000000 simulated data sets were
154 calculated (1000000 per scenario). Among them, the 10000 sets calculated for the most supported
155 scenario and closest to the observed data according to the summary statistics were used for
156 parameter estimations.

157

158 **Results**

159

160 Cetti's Warbler

161 Using MICROCHECKER, we found possible null alleles in loci Esc6 and Hru6. Therefore, we
162 excluded these loci from calculations of the Hardy-Weinberg equilibrium, linkage disequilibrium
163 and inbreeding coefficient, and also from BOTTLENECK analysis, while for the other analyses we
164 used all 13 loci. We found no evidence of large allele dropouts or scoring errors in the data set. The
165 total sample was not in Hardy-Weinberg equilibrium ($\chi^2_{40} = 92.95$, $p < 0.001$), nor was Larache
166 ($\chi^2_{22} = 49.05$, $p < 0.001$) or Mallorca ($\chi^2_{18} = 43.90$, $p < 0.001$). Linkage disequilibrium was detected
167 in loci FhU2 - Pca3 and FhU2 - Pdoµ1 from both sampling sites and the total sample and in Pca3 -
168 Pdoµ1 from Mallorca and the total sample. After Bonferroni correction for multiple testing, linkage
169 disequilibrium only between FhU2 and Pca3 remained significant (adjusted significant p-values:

170 Mallorca, $p < 0.0033$; Larache and total sample $p < 0.0009$). Both FhU2 and Pca3 were anyway
171 included in the analyses. Expected heterozygosity and allelic richness were higher in Larache than
172 in Mallorca (Table 1a).

173 In the analysis without geographical information of the sampling sites, program
174 STRUCTURE attributed the highest likelihood to $K = 1$, while by adding geographical information
175 (LOCPRIOR model) the highest likelihood for $K = 2$ was obtained, also supported by ΔK . Using
176 the LOCPRIOR model, the assignment of individuals to two clusters corresponding to Mallorca and
177 Larache is clearly seen in the bar plot produced by STRUCTURE. Without spatial information the
178 difference between the two sites was less clear (Fig 3a). These results indicate weak population
179 genetic structuring between the samples from Mallorca and Larache. The DAPC confirmed the
180 occurrence of two genetic clusters (Fig. 4a), but in the continental sample some individuals were
181 assigned to the ‘insular cluster’, in a clearer way than depicted by STRUCTURE analysis (Fig 5a);
182 it is anyway possible that the DAPC is overconfident when assigning individuals to clusters (see
183 Frosch et al. 2014). According to the AMOVA analysis, genetic differentiation between the two
184 sampling sites was significant ($F_{ST} = 0.064$, $p < 0.001$). The migration rate calculated with
185 MIGRATE-N was higher from the continental to the insular sampling site ($M = 7.643$; CI 95%
186 7.299 - 8.012) than in the opposite direction ($M = 7.344$; CI 95% 7.011 - 7.675), but the 95%
187 confidence intervals of the two values overlapped widely.

188 We found signs of recent population reduction in Mallorca (Wilcoxon test, heterozygosity
189 excess, $p = 0.01$; shifted allele frequency class mode), but not in Larache (Wilcoxon test,
190 heterozygosity excess, $p = 0.91$; normal L-shaped allele frequency distribution). For both sampling
191 sites, the Garza-Williamson index was lower than 0.68 (Table 1a), which indicates past bottlenecks
192 (Garza and Williamson 2001). Past bottlenecks were indicated also by the DIY ABC analysis for
193 both Mallorca and Larache. In Mallorca, scenario 3 (bottleneck) obtained the highest support
194 according to the posterior probabilities calculated through both the direct (0.456) and the logistic

195 approach (0.467), while the second best scenario described a constant population size (scenario 1;
196 direct approach: 0.406; logistic approach: 0.427). According to scenario 3, the bottleneck took place
197 between 5060 (CI 95% 979 - 9510) and 1900 (CI 95% 560 - 6360) generations ago. This would
198 translate to approximately 18700 - 7000 years ago, assuming a generation length of 3.7 years
199 (BirdLife International 2016). The effective population size was 6820 (CI 95% 2580 - 9730) before
200 the bottleneck, 1330 (CI 95% 194 - 3460) during and 3480 (CI 95% 712 - 8580) after it. The model
201 checking performed for scenario 3 showed consistency between the posterior distributions and the
202 observed data for two of the four summary statistics, while significant deviations ($0.01 > p > 0.001$)
203 were found in the mean M index (Garza and Williamson 2001) and in the mean allele size variance
204 across loci. For Larache, the bottleneck scenario (scenario 3) was strongly supported according to
205 the posterior probabilities calculated through the direct and the logistic approach (0.946 and 0.973,
206 respectively). The estimated bottleneck timing was between 3630 (CI 95% 638 - 8550) and 693 (CI
207 95% 28 - 3030) generations ago, i.e. approximately between 13400 and 2500 years ago. The
208 effective population size was 73900 (CI 95% 34800 - 98200) before the bottleneck, 1320 (CI 95%
209 279 - 3000) during and 40800 (CI 95% 4150 - 93100) after it. The model checking supported also
210 the scenario 3, there were no significant deviations between the posterior distributions and the
211 observed data of the four summary statistics.

212

213 Great Reed Warbler

214 MICROCHECKER did not detect null loci, large allele dropouts or scoring errors in the data set.
215 There were no deviations from the Hardy-Weinberg equilibrium. Linkage disequilibrium was found
216 between loci Cuu28 - Zl54, Pdo5 - Pca3, Cuu28 - Aar8 and Aar8 - Ase34 in Pego-Oliva, Zl54 -
217 Aar8 in Mallorca, Zl54 - Esc6 in Villafranca and Aar8 - Ase34 from combined data. However, after
218 Bonferroni correction for multiple testing, none of these linkage disequilibria resulted to be
219 significant (adjusted significant p-values: Mallorca, $p < 0.0009$; Pego-Oliva, Villafranca and total

220 sample, $p < 0.0008$). All loci were used in the analyses. Expected heterozygosity and allelic richness
221 were at very similar levels in Villafranca and Pego-Oliva, while lower in Mallorca (Table 1b).

222 Program STRUCTURE attributed the highest likelihood to $K = 1$, both with and without the
223 LOCPRIOR model. Consistently, the STRUCTURE bar plots of assignment likelihoods obtained
224 for $K = 2$ (Fig 3b) showed almost no differences among individuals. It is not possible to calculate
225 ΔK for $K = 1$, because ΔK is based on the rates of change between the previous and the next K . In
226 our case, we could only calculate ΔK for $K = 2-9$, and for these values we did not observe any clear
227 peaks of this statistics. More clarity was obtained with DAPC, which~~Differently, DAPC~~ supported
228 the occurrence of two distinct genetic clusters (Fig 4b) , which and highlighted the difference
229 between the two Spanish continental sites, while the Mallorcan sample included birds from both
230 clusters in similar proportions (Fig 5b). With the AMOVA analysis, we found a significant but low
231 genetic differentiation among sampling sites ($F_{ST} = 0.052$, $p < 0.001$). Comparing the genetic
232 differentiation between the pairs of sampling sites, we found significant though low differentiation
233 in all comparisons (Mallorca – Pego-Oliva: $F_{ST} = 0.017$, $p = 0.036$; Mallorca – Villafranca: $F_{ST} =$
234 0.063 , $p = 0.018$; Pego-Oliva – Villafranca: $F_{ST} = 0.078$, $p < 0.001$), and, consistently with the
235 DAPC results, the highest differentiation was found between Pego-Oliva and Villafranca. The
236 migration rates calculated between sampling sites using MIGRATE-N were lower from Mallorca to
237 the continental sites than in the opposite direction and between the two continental sites (Table 2).
238 We also merged sampling sites based on the DAPC clusters (cluster 1: Pego-Oliva + Mallorca;
239 cluster 2: Villafranca + Larache; Fig. 5b); the migration rate was higher from cluster 2 to cluster 1
240 ($M = 4.677$; CI 95% 4.291 - 5.085) than in the opposite direction ($M = 1.758$; CI 95% 1.608 -
241 1.920).

242 We found no signs of recent population size reductions from BOTTLENECK analysis. Garza-
243 Williamson indexes were higher than or almost equal to 0.68 (Table 1b), thus there were no
244 indications for past bottlenecks.

245

246 **Discussion**

247

248 Cetti's Warbler

249 The lower expected heterozygosity and allelic richness found in Mallorca may be a consequence of
250 fewer arrivals of immigrant individuals and of the lower population numbers in this island site than
251 in the continental breeding site. Yet, the standard deviations of diversity estimates from Mallorca
252 and Larache overlapped widely (Table 1a), thus the difference in genetic diversity between the two
253 sampling sites is not strong.

254 The weak population structuring and the significant but low genetic differentiation suggest
255 that the large distance (~ 960 km) and the sea limit gene flow between the two sampling sites, but
256 do not prevent it. This suggests that Cetti's Warbler has a good dispersal ability, consistently with
257 information from a mark-recapture study of the British population (Robinson et al. 2007). The
258 recent colonization of Britain obviously shows the capability of this species to cross the sea during
259 dispersal and our results indicate that the species is able to fly over stretches of sea notably wider
260 than the English Channel. The slightly lower migration rate from Mallorca to Larache than in the
261 opposite direction may indicate a lower tendency of the insular individuals to disperse.

262 The demographic history of the Cetti's Warbler was partly similar in the two sampling areas,
263 although the bottleneck was more recent and severe in Larache than in Mallorca. The results
264 suggest that the Western Mediterranean population of this species was reduced for a relatively long
265 period (approx. 10000 years). The bottleneck period estimated for Mallorca started during the cold
266 and dry period MIS2, included the Younger Dryas (approx. 13000 – 12000 yr BP; also a dry and
267 cool period) and ended in a transition period to a wetter and less continental climate (Fletcher and
268 Sánchez Goñi 2008). A long period of severe climatic conditions in the Western Mediterranean,
269 with the dominance of semi-desertic vegetation (Fletcher and Sánchez Goñi 2008), may explain the

270 observed population reduction. In the case of the Larache population, the estimated bottleneck time
271 started shortly before the Younger Dryas, but also included wetter periods with less continental
272 climate; a possible relationship between the population size reduction and climate is therefore not
273 clearly identifiable.

274

275 Great Reed Warbler

276 As observed for the Cetti's Warbler, the lower expected heterozygosity and allelic richness found in
277 Mallorca may be explained by limited immigration or smaller population size in the Mallorca Island
278 than in the continental breeding sites. However, also in this case the standard deviations of diversity
279 estimates overlapped widely (Table 1b). Overall, our results of population differentiation indicate
280 high levels of gene flow among sampling sites that is nevertheless limited, possibly due to long
281 distances between sampling sites and high philopatry of this species (e.g., Procházka and Reif 2000;
282 Hansson et al. 2002; Mátrai et al. 2012; Koleček et al. 2015). The different results obtained through
283 STRUCTURE analysis (K=1) and DAPC (K=2) are probably due to the different assumptions of
284 the analysis methods (Jombart et al. 2010), which can lead to identification of different numbers of
285 distinct genetic clusters (Jombart et al. 2010; Kraus et al. 2013). In any case, the results of the two
286 analysis methods are compatible; in fact, likelihood values calculated by STRUCTURE often fail in
287 identifying the real number of genetic clusters (Evanno et al. 2005), and it was not possible to use
288 the more reliable *ad hoc* statistics ΔK to compare $K = 1$ and $K = 2$. The strong connection among
289 our sampling sites is consistent with the available information from mark-recapture data, which
290 have revealed cases of long distance dispersal for up to more than 100 km (Hansson et al. 2002;
291 Mátrai et al. 2012). Our results suggest that, at least in the case of our study population, the sea does
292 not represent any more effective dispersal barrier than a similarly wide extension of land. In fact,
293 Pego-Oliva is approximately equally distant from Mallorca and from Villafranca (~ 290 km), but
294 the differentiation is even slightly higher between the two continental sites than between the island

295 and mainland sites. The lower migration rates from the insular to the continental sites than in the
296 opposite direction suggest a lower tendency to long-distance dispersal in the Mallorcan breeding
297 population. The demographic history was similar in Mallorca and in the continental breeding areas,
298 i.e. no population bottlenecks were detected.

299

300 Interspecific comparison

301 In both species, we observed slightly lower genetic diversity in the insular than in the continental
302 breeding areas. A further pattern shared by both warblers is the similarity between the demographic
303 history in the Mallorcan and the continental samples, although in the Cetti's Warbler the estimated
304 bottleneck time was partly different between populations. The main interspecific difference is
305 represented by the evidence for past bottlenecks found in the Cetti's Warbler but not in the Great
306 Reed Warbler. This suggests that, in the case of the Great Reed Warbler, higher dispersal capability
307 may have allowed constant arrival of individuals from other areas. Such capability may also have
308 been advantageous for the Great Reed Warbler in facing climatic and environmental changes, e.g.,
309 by finding new breeding areas, and/or by migrating over long distances to find suitable wintering
310 areas. The higher dispersal capability of the Great Reed Warbler is also supported by the migration
311 rates among sampling sites, which were generally higher in this species than in the Cetti's Warbler.

312 The interspecific comparison of the genetic differentiation and migration rates among sampling
313 sites needs to be taken cautiously, given that most of Great Reed Warblers were sampled at sites
314 separated by shorter distances than in the case of Cetti's Warblers. However, according to the
315 STRUCTURE results, the two Great Reed Warblers from Larache were very similar to those
316 sampled in the Spanish mainland and in Mallorca (Fig 3b), suggesting higher gene flow than in the
317 Cetti's Warbler. A previous study carried out in the same area (including the Mallorca Island) on two
318 other passerines also reported higher gene flow in the long-distance migratory species, the Eurasian
319 Reed Warbler *Acrocephalus scirpaceus*, than in the mainly resident Moustached Warbler

320 *Acrocephalus melanopogon* (Ceresa et al. 2015). Possibly, as hypothesized in Ceresa et al. (2015,
321 2016), also in our study, the higher gene flow in the long-distance migratory species may be partly
322 explained by a higher capability of crossing the sea.

323

324 **Acknowledgements** - We acknowledge M. Rebassa, H. Rguibi-Idrissi, M. Marín, J. Gómez, Santi,
325 Luis, Kames and family and many others, who have helped us with the field work. We would also
326 like to thank the authorities of the Marjal de Pego-Oliva Natural Park, the S'Albufera de Mallorca
327 Natural Park, “Consejería de medio Ambiente y Desarrollo Rural de Castilla-La Mancha”, the
328 “Servei de Conservació de la Biodiversitat de la Generalitat Valenciana” and the “Haut
329 Commissariat aux Eaux et Forêts et à la lutte contre la désertification de Rabat, Morocco” for
330 providing the facilities to work in protected areas and for the relevant permits. We are grateful to M.
331 Serra and the researchers and Ph.D. students of the Laboratory of Evolutionary Ecology (Institute
332 Cavanilles of Biodiversity and Evolutionary Biology – University of Valencia), for providing the
333 laboratory for DNA extraction. This study has been partly financed by Projects CGL2005-
334 02041/BOS and CGL2010-21933-C02-02 of the Spanish Ministry of Science and Innovation and
335 by the University of Oulu. F. Ceresa was supported by an “Atraent talent” grant from the University
336 of Valencia.

337

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520 *Phylogenet Evol* 113:113-125

521 Table 1. Sample sizes (N), expected heterozygosity (H_e), allelic richness (A), inbreeding coefficient
 522 (F_{IS}) and Garza-Williamson index (G-W) of (a) Cetti's Warbler and (b) Great Reed Warbler,
 523 calculated from microsatellite data for each sampling site and for the total sample. Standard
 524 deviation are shown in parentheses.

a)

Sampling site	N	H_e (SD)	A (SD)	F_{IS} (SD)	G-W
Mallorca	15	0.503 (0.271)	3.164 (2.113)	-0.317 (0.447)	0.650
Larache	20	0.543 (0.272)	4.134 (2.852)	-0.071 (0.348)	0.612
Total	35	0.544 (0.266)	3.995 (2.657)	-0.110 (0.378)	0.631

b)

Sampling site	N	H_e (SD)	A (SD)	F_{IS} (SD)	G-W
Pego-Oliva	20	0.440 (0.259)	3.340 (2.202)	-0.136 (0.264)	0.739
Mallorca	10	0.390 (0.297)	2.783 (1.981)	-0.004 (0.422)	0.690
Villafranca	12	0.439 (0.251)	3.213 (2.304)	0.009 (0.320)	0.672
Total	42	0.442 (0.244)	4.358 (3.562)	-0.033 (0.234)	0.701

Table 2. Migration rates (M) among Great Reed Warbler's sampling sites, calculated from microsatellite data.

Direction	M (95% CI)
Mallorca → Pego-Oliva	8.202 (7.503 - 8.943)
Villafranca → Pego-Oliva	11.854 (11.010 - 12.739)
Pego-Oliva → Mallorca	13.298 (12.165 - 14.499)
Villafranca → Mallorca	10.758 (9.742 - 11.841)
Pego-Oliva → Villafranca	9.811 (9.077 - 10.584)
Mallorca → Villafranca	5.888 (5.326 - 6.488)

525 **Figure legends**

526

527 Figure 1. A map showing the sampling locations: (1) Larache, (2) Villafranca de los Caballeros, (3)
528 Pego-Oliva Natural Park and (4) S'Albufera de Mallorca.

529

530 Figure 2. Cetti's warbler STRUCTURE results: mean likelihood (\pm SD) obtained for each K value
531 between 1 and 10, (a) without and (b) including prior spatial information of sampling sites, and ΔK
532 statistics obtained (c) without and (d) including prior spatial information of sampling sites.

533

534 Figure 3. Bar plots of the STRUCTURE results based on microsatellite data of (a) Cetti's Warbler
535 and (b) Great Reed Warbler for $K = 2$, obtained (1) without geographic information about the
536 sampling sites and (2) by adding such information. Each column represents an individual and
537 indicates its probability to belong to one of the two clusters.

538

539 Figure 4. Number of distinct genetic clusters (K) identified through the discriminant analysis of
540 principal components (DAPC) for (a) the Cetti's Warbler and (b) the Great Reed Warbler. For both
541 species, the Bayesian information criterion (BIC) supported the occurrence of two clusters.

542

543 Figure 5. Bar plots of the discriminant analysis of principal components (DAPC) results based on
544 microsatellite data of (a) Cetti's Warbler and (b) Great Reed Warbler for $K = 2$. Each column
545 represents an individual and indicates its probability to belong to one of the two clusters.

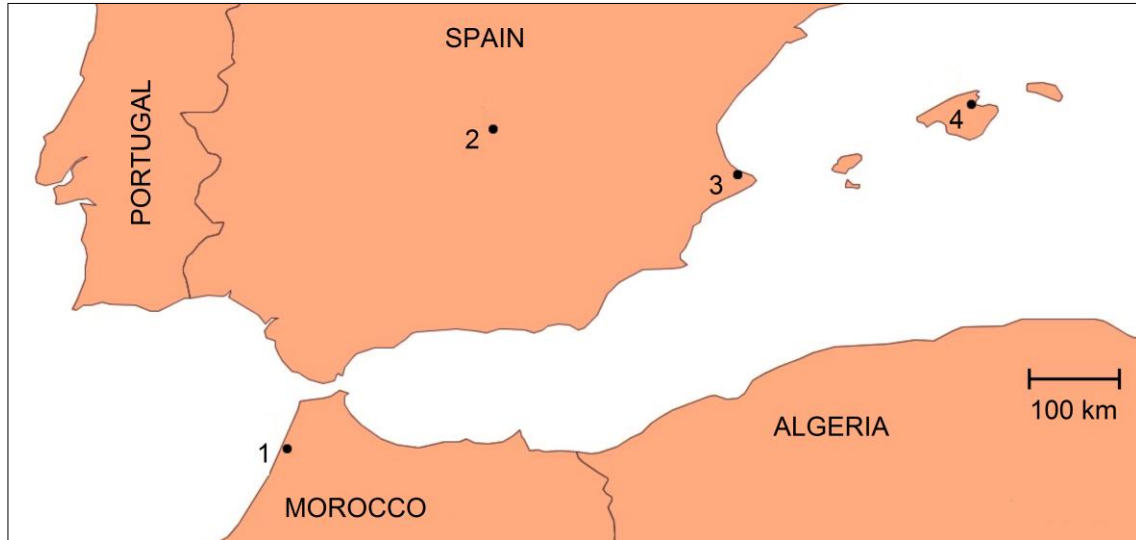
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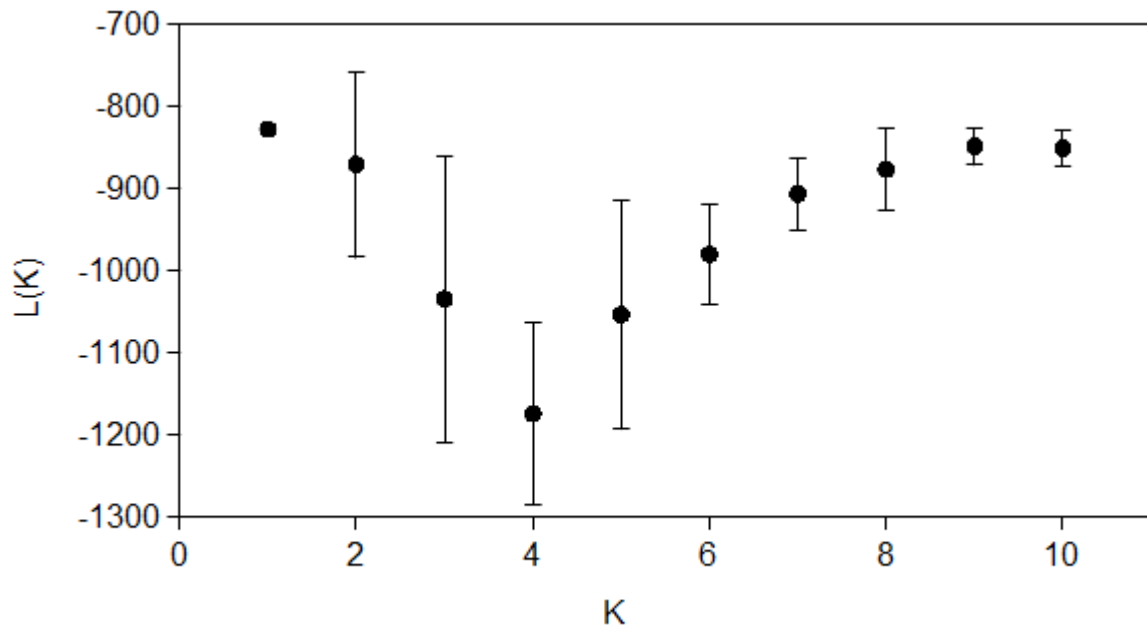
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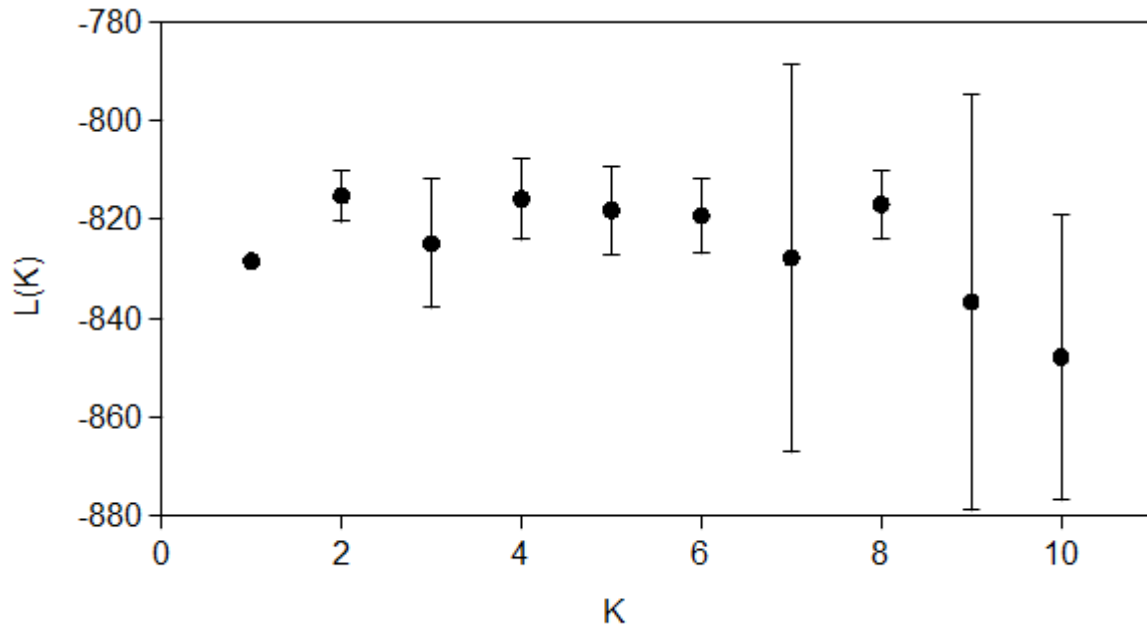
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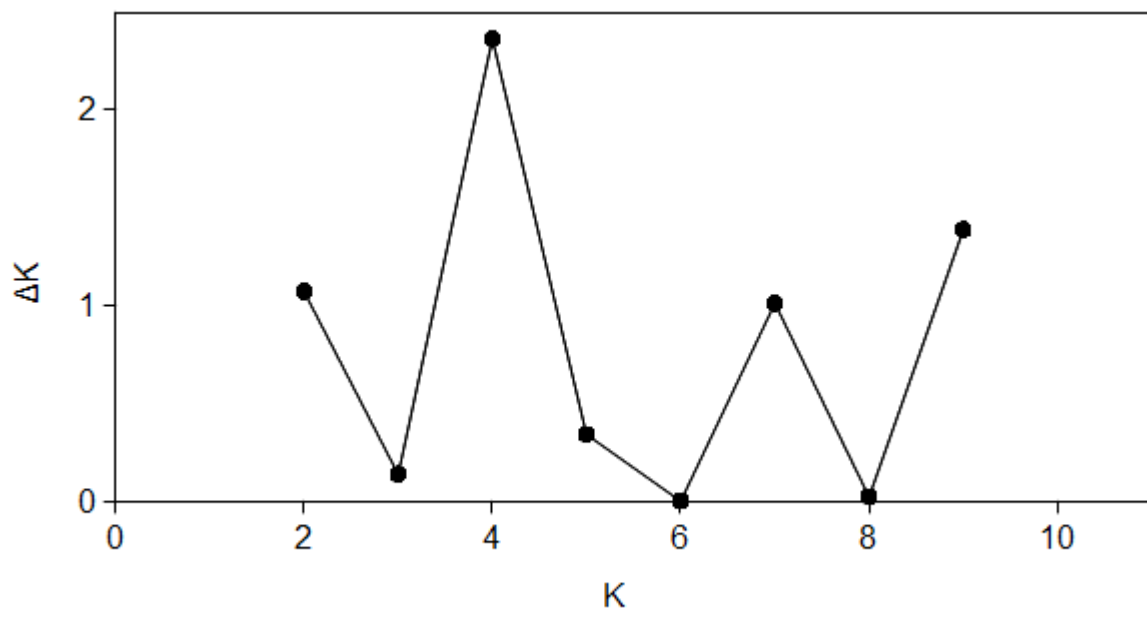




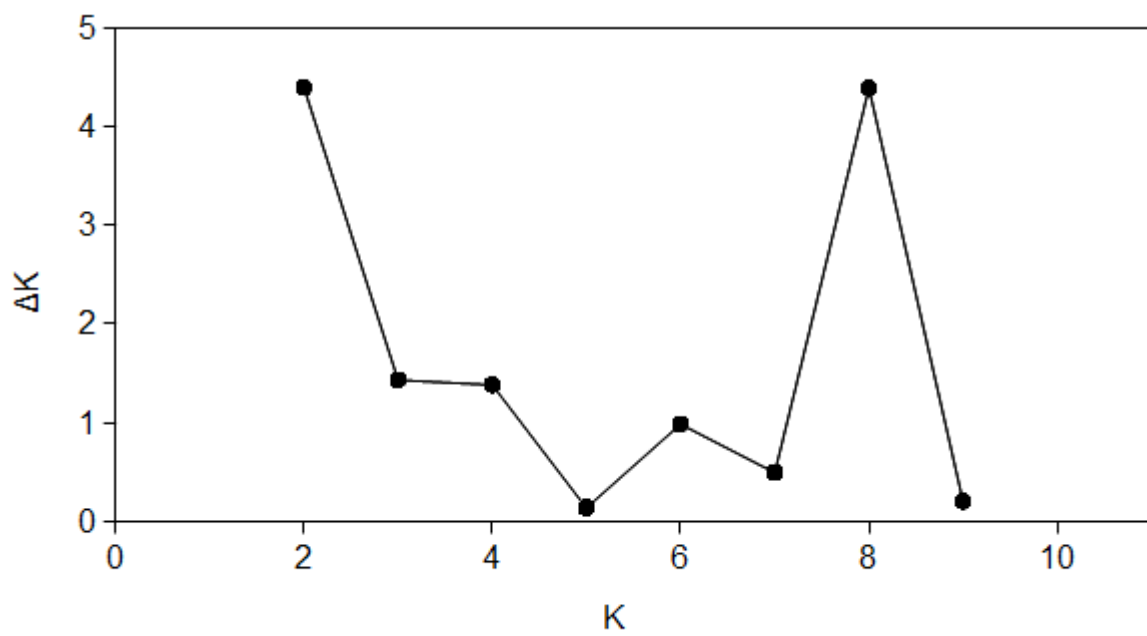
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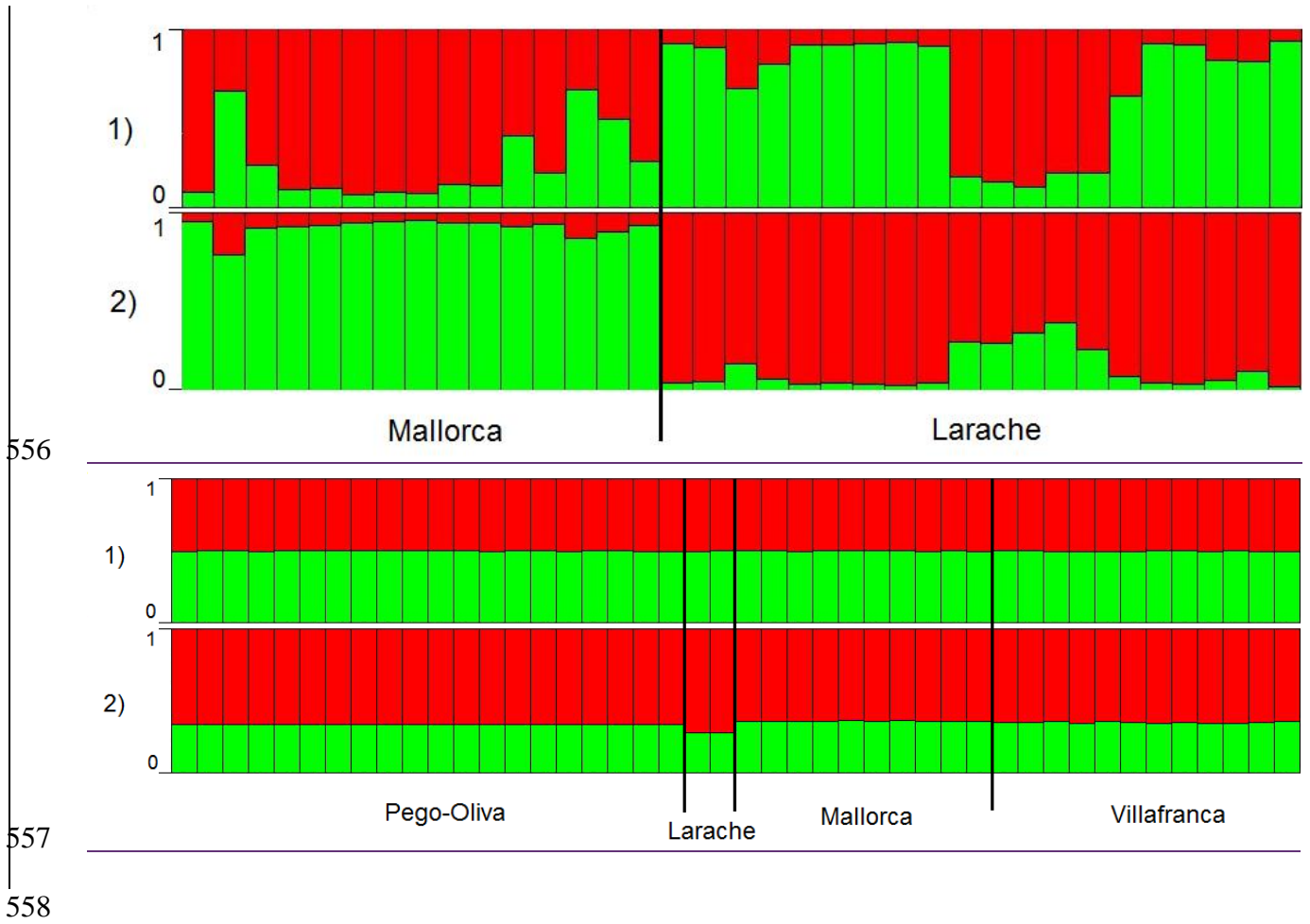


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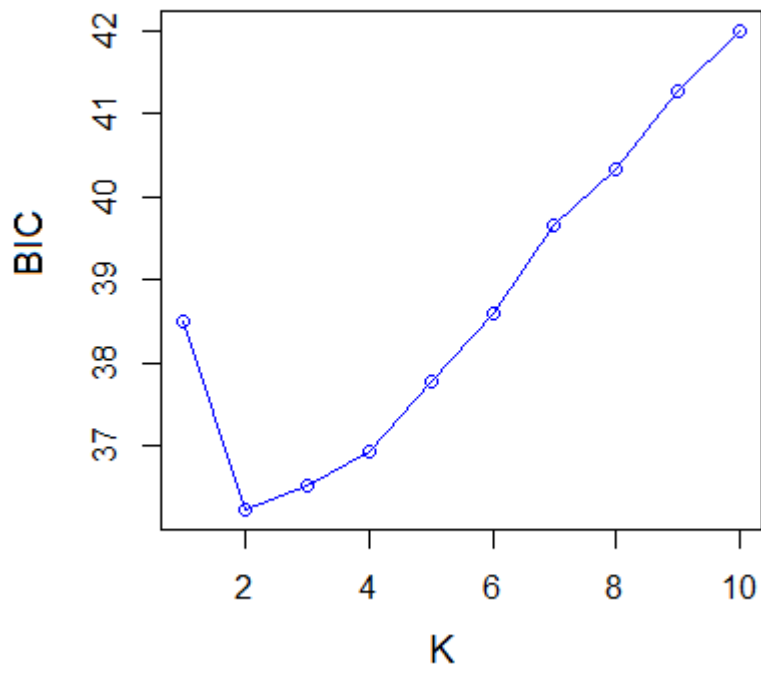


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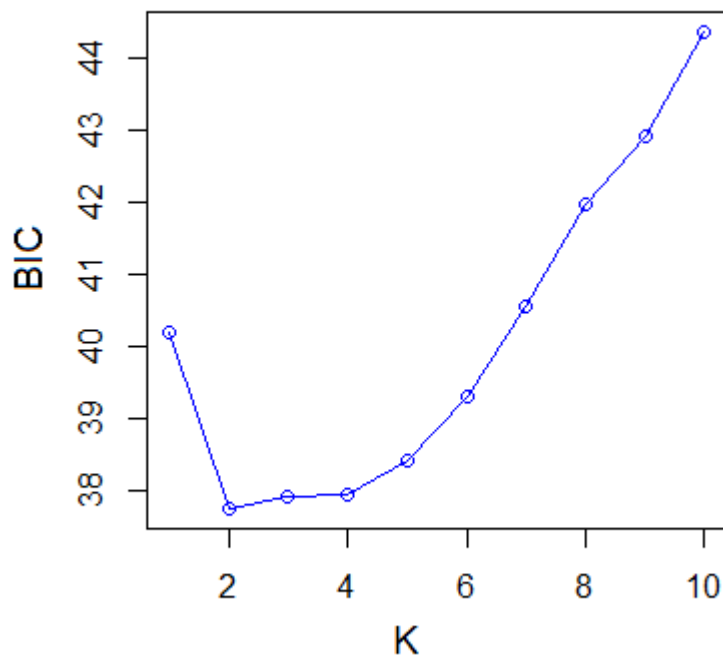


a)



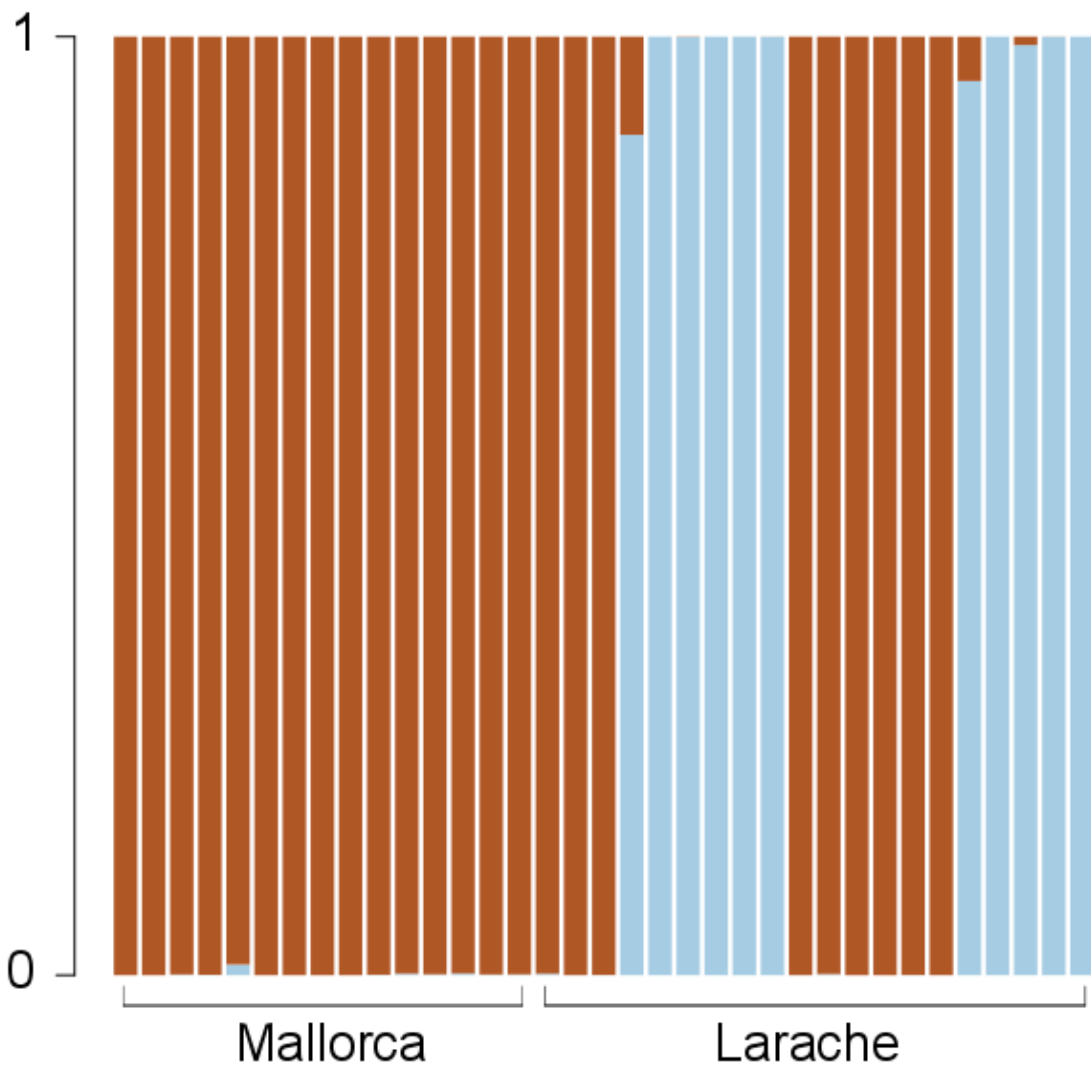
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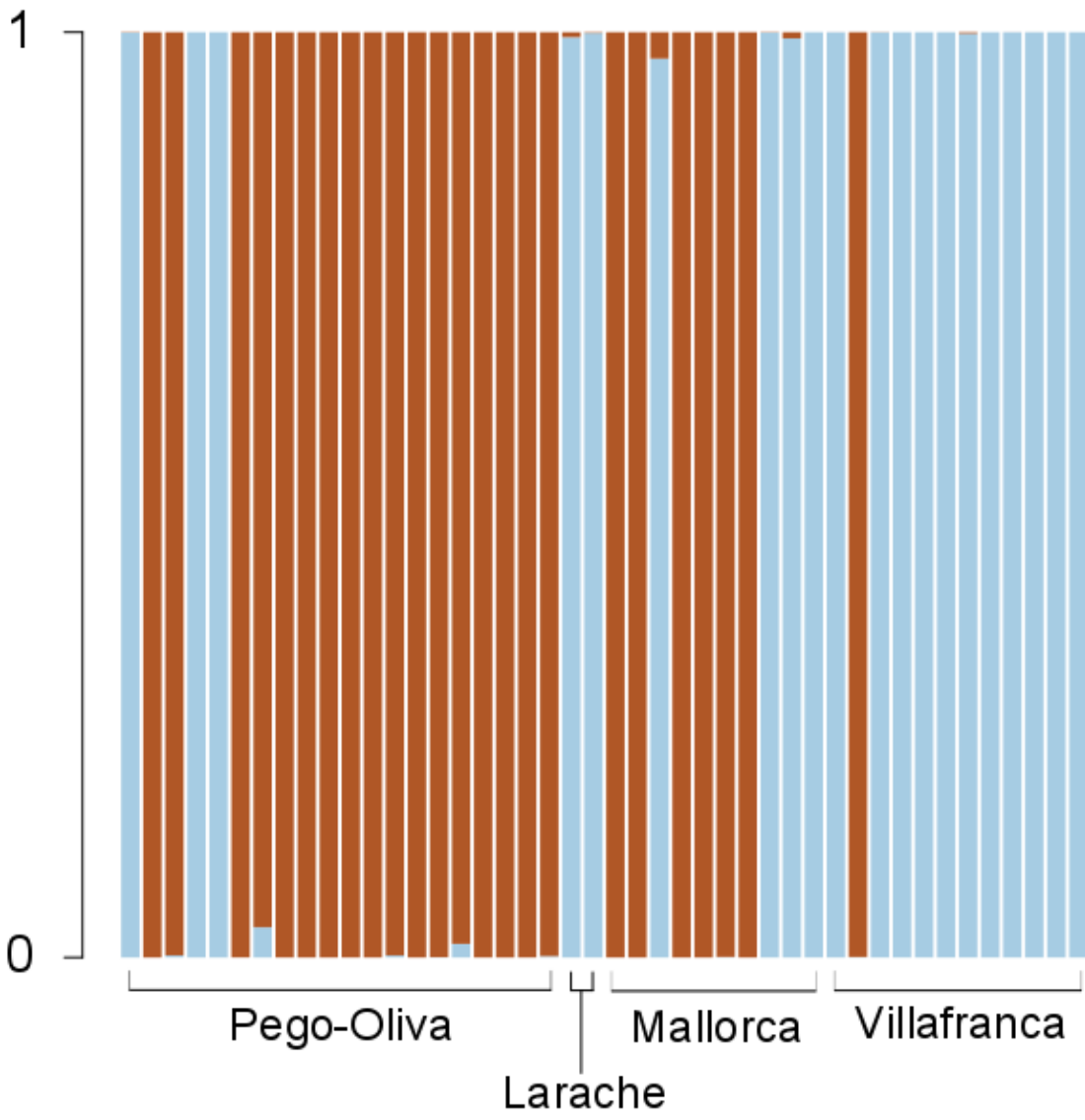


560

a)



b)



562