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1 **Corncrake conservation genetics at a European scale: the impact of biogeographical**
2 **and anthropological processes**

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29 **ABSTRACT**

30 Understanding patterns of genetic structure, gene flow and diversity across a species range
31 is required if we are to determine the genetic status and viability of small peripheral
32 populations. This is especially crucial in species distributed across a large range where
33 spatial heterogeneity makes it difficult to predict the distribution of genetic diversity.
34 Although biogeographical models provide expectations of how spatially structured genetic
35 variation may be at the range scale, human disturbance may cause strong deviations from
36 these theoretical predictions. In this study, we investigated genetic structure and demography
37 at a pan-European scale, in the corncrake *Crex crex*, a grassland bird species strongly
38 affected by agricultural changes. We assessed population structure and genetic diversity, as
39 well as demographic trends and direction of gene flow, in and among 15 contemporary
40 populations of this species. Analyses revealed low genetic structure across the entire range
41 with high levels of genetic diversity in all sites. However, we found some evidence that the
42 westernmost populations were, to a very limited extent, differentiated from the rest of the
43 European population. Demographic trends showed that population numbers have decreased
44 in western Europe and remained constant across eastern Europe. Results may also suggest
45 asymmetric gene flow from eastern to western populations. In conclusion, we suggest that
46 the most likely scenario is that contrasting demographic regimes between eastern and
47 western populations, driven by heterogeneous human activity, has caused asymmetric gene
48 flow that has buffered small peripheral populations against genetic diversity loss, but also
49 erased any genetic structure that may have existed. Our study highlight the need of
50 coordinated actions at the European scale to preserve source populations and ensure the
51 maintenance of reproductive productivity in the most threatened sites, in order to avoid

52 losing any adaptive potential and too strongly relying on sink source populations whose
53 future is uncertain.

54

55 **KEYWORDS**

56 Central-marginal hypothesis, conservation genetics, genetic diversity, demography, genetic
57 structure, Approximate Bayesian Computation

58

## 59 1. INTRODUCTION

60 Spatial heterogeneity in the environment is an important factor affecting widely distributed
61 species (Pickett & Cadenasso 1995). The distribution of factors such as ecogeographic
62 regions, natural barriers to dispersion, migration routes, or other organisms such as
63 competitors, predators or pathogens, may vary over spatial scales and affect overall
64 connectivity and local adaptation in any focal species. Similarly, when a species' range
65 overlaps several countries, it may be affected by the ecological impact of different levels of
66 economic development and environmental awareness (Dallimer & Strange 2015). Therefore,
67 the distribution of genetic variation across a species' range often emerges from a complex
68 interaction between natural biogeographic and anthropogenic processes. However the
69 pattern of the biological component may not match the pattern of the socio-economic
70 component (Moilanen & Arponen 2011). If the relative contribution of the latter is strong
71 enough it may be difficult to use classical biogeographical models to predict the range
72 dynamics of a focal species, and thus to make and implement international conservation
73 plans. Ad-hoc models of range dynamics may need to be developed for such species.
74 Information on gene flow and demographic trends across a range are key to identifying

75 Evolutionarily Significant Units (ESU, Ryder 1986) and evaluating the threats associated
76 with changes in connectivity, i.e. inbreeding depression or the loss of adaptive potential
77 (Hedrick & Kalinowski 2000). Therefore such knowledge is critical in the design of
78 informed conservation action plans.

79 Biogeographic models of range dynamics provide predictions regarding patterns of
80 genetic variation across a species' distribution. Under the central-marginal model, focal
81 species abundance is expected to be higher at the range core (*i.e.* the area of ecological
82 optimum), and less abundant and more isolated at the periphery as environmental conditions
83 gradually depart from the ecological optimum (Hengeveld & Haeck 1982; Brussard 1984;
84 Brown 1984). This has implications for the distribution of genetic variation at the range-
85 scale (Eckert *et al.* 2008) and for the evolution of species' range (Hoffmann & Blows 1994;
86 Kirkpatrick & Barton 1997). Although the central-marginal model is widely accepted, the
87 hypothesis has been challenged by empirical and theoretical studies (Sagarin & Gaines 2002;
88 Sagarin *et al.* 2006; Samis & Eckert 2007) and the model itself can generate opposite
89 patterns. A first hypothesis implies that populations at the core have higher effective
90 population sizes and produce more dispersing migrants than do the smaller, peripheral
91 populations. Under this model, genetic drift in the peripheral populations is only partially
92 compensated by limited gene flow from the core area, and therefore results in lower genetic
93 diversity in, and higher differentiation among, these peripheral populations (Hoffmann &
94 Blows 1994; Lesica & Allendorf 1995; Eckert *et al.* 2008). Consequently, these marginal
95 populations are expected to be more sensitive to environmental changes – either stochastic
96 or directional – and more prone to extinction (Lesica & Allendorf 1995; Channell &
97 Lomolino 2000). In contrast, a second hypothesis suggest that if core populations are large
98 and peripheral populations are small, there could be asymmetric gene flow from core to

99 periphery (Kirkpatrick & Barton 1997) analogous to that expected in a source-sink (Pulliam
100 1988), or island-continent model (Slatkin 1987). Homogenisation of genetic diversity and
101 weak structure at the range scale is expected if the effect of the asymmetric gene flow is
102 greater than the combined effects of drift and selection at the range margins.

103 Importantly, human disturbance, by disrupting natural dynamics, may counteract the
104 theoretical assumptions outlined above. Indeed, anthropic activity can result in barriers to
105 gene flow, fragmenting species ranges and increasing genetic isolation between populations
106 (Keller & Largiadèr 2003). On the contrary, human-assisted dispersal, or the creation of
107 corridors through changes of landscape structure, can favour genetic mixing between
108 previously isolated populations (Hale *et al.* 2001). Human activity frequently affects the
109 growth of wild populations, either positively (Garrott *et al.* 1993), or negatively (Butchart *et*
110 *al.* 2010), altering natural demographic trends and thus influencing the genetic
111 characteristics of these populations. Moreover, climate change, by driving a rapid shift in
112 species distributions, may further blur previously existing biogeographical patterns.
113 Therefore, a combination of natural and anthropogenic dynamics is responsible for the
114 observed patterns of genetic variation at large-scale. Thus it is important to consider both
115 processes in interpreting the levels of population differentiation, or differences in genetic
116 diversity, that are observed across the range of a species.

117 We used the corncrake (*Crex crex*) as a model species to study genetic structure and
118 gene flow at a continental scale. As is the case for many grassland bird species (Donald *et*
119 *al.* 2006), agriculture intensification has severely affected the number and distribution of the
120 corncrake (Green *et al.* 1997). This situation has motivated numerous conservation plans,
121 especially in western Europe. Interestingly, because land use change and agriculture
122 intensification is variable across Europe, the corncrake has been affected by human activity

123 at various intensities in different parts of its range. To date, knowledge regarding genetic
124 structure in this species is very limited and incomplete (Wettstein 2003) and other methods
125 (*e.g.* monitoring returning individuals) do not provide adequate amounts of data to determine
126 dispersal patterns, connectivity between sites, or identify distinct evolutionary significant
127 units in this species (Ryder 1986). Interestingly the extensive population monitoring of the
128 corncrake undertaken in many European countries allows survey-based demographic trends
129 to be compared against the historical demography inferred using genetic data. The
130 availability of such fine-scale demographic data provides an exciting opportunity to
131 determine if apparent local trends, which usually drive conservation actions, concur with the
132 continental-scale demographic landscape. Specifically, we tested two competing hypotheses
133 arising from the central-marginal model: 1) peripheral populations are isolated from the core
134 populations and are thus genetically differentiated and show a reduction of genetic diversity,
135 2) demographic imbalance between core and peripheral populations generates net gene flow
136 towards the periphery that homogenises populations across the range. We used a suite of
137 microsatellite markers to assess genetic diversity and structure across the European range of
138 the corncrake. Approximate Bayesian computation (ABC) (Beaumont *et al.* 2002) was used
139 to estimate corncrake historical demography at the population scale in order to assess fine-
140 scale spatial variation in demographic trends across Europe. In order to assess the dynamics
141 generating the observed pattern of genetic structuring, an ABC framework was also used to
142 determine the direction of gene flow between western and eastern populations.

143

144 **2. METHODS**

145 **2.1. Study species and sample collection**

146 The corncrake is a migratory bird that breeds on grasslands across the Palearctic (Schäffer
147 & Koffijberg 2004). Ecological niche modelling (Fourcade *et al.* 2013) and expert field
148 knowledge (Schäffer & Koffijberg 2004) suggest the species' range core is located in Russia
149 and eastern Europe, while favourable habitats are scarcer and more fragmented in western
150 Europe. Changes in anthropogenic activities, *e.g.* the intensification of agricultural practices,
151 have contributed to creating large demographic differences across the species range. In
152 western Europe, numbers have declined severely (Green & Gibbons 2000; Deceuninck *et al.*
153 2011) but the situation in eastern Europe/Asia, which includes 90% of the world's corncrake
154 population (Schäffer & Koffijberg 2004) is fundamentally different. In the east the impact
155 of agriculture intensification during the 20$^{th}$ century is difficult to assess, but was probably
156 less important than in western Europe. Indeed recent surveys highlight the positive effect of
157 agricultural abandonment after the demise of the USSR on corncrake populations (Keišs
158 2005; Mischenko 2008). Although dispersal patterns are unclear in this species due to a very
159 low recovery rate of ringed birds (< 5%, Green 1999), there is some evidence of long-
160 distance movement (> 500 Km) within the breeding season (Schäffer & Koffijberg 2004;
161 Mikkelsen *et al.* 2013). We focus on the European part of the corncrake's range. This
162 includes a core area (eastern Europe) in which corncrakes are relatively abundant and evenly
163 distributed, surrounded by several smaller populations in the north (Sweden), west
164 (Scotland, France) and south (Romania, Italy) of the range.

165 With the collaboration of local ringers we collected 496 corncrake samples from 15
166 locations across Europe (Figure 1) in 2011–2012. Samples were collected from May to July
167 to avoid the capture of migrating birds. Individuals were attracted using playback of

168 conspecific male calls at night during the peak of calling activity and captured using a mist
169 net or large dipnet. Because of the playback-assisted capture method only males were
170 sampled (Green 1999). Depending on the local legislation and experience of the
171 fieldworkers, different sources of DNA were collected. The different tissues sampled did not
172 affect the quality of DNA extracted or the accuracy of the genotyping. In France, Germany,
173 Italy, Hungary, Poland (all sites), Czech Republic, Latvia, Belarus and Russia (20 samples
174 out of 32), ca. 50 µl of blood was collected from the brachial vein and stored in absolute
175 ethanol. In Scotland buccal swabs served as a source of DNA, whereas feathers were
176 collected from Romania, Sweden (all sites), and Russia (12 samples out of 32). All birds
177 were released unharmed immediately after sampling, with the exception of a Russian
178 individual which died from its collision with the landing net. Population sample sizes ranged
179 from 7 to 66 (Figure 1 and Table 1).

180

181 **2.2. Microsatellite genotyping**

182 We extracted genomic DNA using a method of salt extraction following Richardson *et al.*
183 (2001). All individuals were genotyped at 15 microsatellite markers of which eight had been
184 designed for the corncrake: *Crex1*, *Crex2*, *Crex6*, *Crex7*, *Crex8*, *Crex9*, *Crex11*, *Crex12*
185 (Gautschi *et al.* 2002). The seven other markers are conserved across a large range of bird
186 families: *CAM18* (Dawson *et al.* 2013), *TG02-120*, *TG04-12*, *TG04-12a*, *TG04-41*, *TG05-*
187 *30* and *TG012-15* (Dawson *et al.* 2010). We amplified markers by Polymerase Chain
188 Reaction (PCR) in three multiplexes (Appendix A, Table A1), using 1 µL of Qiagen
189 Multiplex MasterMix, 1 µL of DNA (dried in the tube, ca. 15 ng) and 1 µL of 5 µM primer
190 mix (Kenta *et al.* 2008) in a final PCR volume of 2 µL. The PCRs were run under the
191 following conditions: an initial step at 95°C for 15 min, followed by 40 cycles of 94°C during

192 30 s (denaturation), 56.6°C during 90 s (annealing) and 72°C during 60 s (elongation). The
193 final stage consisted of 30 min at 60°C. Amplified fragments were mixed with a solution of
194 formamide and GeneScan 500 ROX Size Standard (Applied Biosystems) and separated by
195 micro-capillary electrophoresis. Alleles were subsequently scored using GeneMapper v3.7
196 software (Applied Biosystems).

197 Deviations from Hardy-Weinberg and linkage equilibria were estimated at each locus
198 for each population using the package "adegenet" (Jombart 2008) for R 3.0.2 (R
199 Development Core Team 2013) and the GENEPOP software (Rousset 2008) respectively.
200 Significance levels were adjusted using the Bonferroni correction for multiple comparisons.
201 The proportion of null alleles in the dataset and its influence on the genetic differentiation
202 between populations, as estimated by $G_{ST}$ (Nei 1973), was assessed using the FreeNA
203 package (Chapuis & Estoup 2007). Error rate was estimated using PEDANT software
204 (Johnson & Haydon 2007) with 10000 simulations on 10 re-genotyped individuals. To test
205 for the potential effect of any null alleles in the dataset, we ran a STRUCTURE analysis
206 after exclusion of the marker which exhibited the highest rate of null alleles, using the same
207 parameters as for the main analysis (see section below "Population structure").

208

209 **2.3. Genetic diversity**

210 We computed standard genetic diversity statistics for each population. Observed ($H_o$),
211 expected heterozygosity ($H_e$), and rarefied allelic richness ($A_R$) were calculated using the R
212 package "Hierfstat" (Goudet 2005). As rarefaction depends on sample size, $A_R$ is highly
213 influenced by the low number of samples in Italy (9) and Hungary (7). Therefore, we also
214 reported the rarefied allelic richness after exclusion of these two sites ($A_R$*). Single-locus
215 observed and expected heterozygosity measures were also computed at each locus for each

216 population (Appendix A, Table A3). We assessed the effect of geography on genetic
217 diversity by testing the correlation between the genetic indices and distance-based
218 eigenvector maps (dbMEM). dbMEM are orthogonal variables that describe the spatial
219 structure of sampling points and are constructed from the principal coordinates of a
220 neighbourhood matrix (Borcard & Legendre 2002; Dray *et al.* 2006). dbMEM were
221 computed using the "vegan" R package (Oksanen *et al.* 2015), using as truncation distance
222 the largest distance in the minimum spanning tree connecting all sites. All significant
223 positive eigenvectors (Moran's I coefficients larger than the expected values) were used in
224 a linear regression against measures of genetic diversity. We also computed the same
225 regression analyses using longitude, latitude and their interaction instead of dbMEM as
226 predictors of genetic diversity.

227

228 **2.4. Population structure**

229 Population structure was first examined using two measures of pairwise genetic
230 differentiation: Nei's $G_{ST}$ (Nei 1973), the extension of $F_{ST}$ for multi-allelic loci, and Jost's *D*
231 (Jost 2008), both using the "DEMEtics" R package (Gerlach *et al.* 2010) and corrected for
232 sample size following Nei & Chesser (1983). Significance was estimated based on 1000
233 permutations. Differentiation was considered as significant for *p*-values $<0.05$ after
234 Bonferroni correction. We tested the effect of geography on population structure using the
235 method implemented in GESTE 2.0 (Foll & Gaggiotti 2006) with its default settings. This
236 approach estimates population-specific $F_{ST}$ within a Bayesian framework and links it to
237 environmental factors. We included longitude, latitude and the dbMEM variables selected
238 in previous analysis as environmental predictors. GESTE runs all combinations of variables
239 and estimates the posterior probability of each models (including a constant model

240 incorporating only genetic drift), which allowed to test whether spatial factors influenced
241 population structure.

242 Isolation by distance was tested first using Mantel tests to assess the correlation
243 between pairwise geographic distance and pairwise genetic distance (Diniz-Filho *et al.*
244 2013). In addition to using the total geographic distances, we also ran Mantel tests using
245 only longitudinal or latitudinal distances. However, the ability of Mantel test to detect spatial
246 patterns has been questioned, especially when original data, not in the form of distance
247 matrices, are used, as is the case for geographic coordinates (Legendre & Fortin 2010;
248 Legendre *et al.* 2015). Therefore, we also investigated how spatial features correlate with
249 genetic structure using distance-based redundancy analyses (dbRDA), a method that
250 ordinates the genetic distance matrix and uses the positive axes in a multivariate regression
251 (Kierepka & Latch 2015). As for the regression analyses against genetic diversity, we used
252 as spatial predictors either the dbMEM variables or alternatively longitude, latitude and their
253 interaction. Our individual samples are not evenly distributed in space but clustered in 15
254 sites, which could result in confounding isolation-by-distance and population structure
255 (Meirmans 2012). In order to assess the effect of the spatial configuration of sampling, we
256 also computed partial individual analyses accounting for the identity of sampling sites.
257 Mantel tests and dbRDA were computed using the "vegan" R package and their significance
258 was tested with 10000 permutations. Both types of analyses were performed both at the
259 population and individual levels, using as the measure of genetic distance: (i) linearized
260 pairwise population differentiation $G_{ST}/1\text{-}G_{ST}$ or (ii) pairwise individual genetic distance $\hat{a}$
261 (Rousset 2000) computed using the software SPAGeDi (Hardy & Vekemans 2002).

262 We estimated the contribution of within individual and within and among population
263 variance on global genetic variation using an analysis of molecular variance (AMOVA,

264 Excoffier *et al.* 1992) computed using Arlequin 3.5 (Excoffier & Lischer 2010), with
265 significance based on 10000 permutations. We also tested for the presence of genetic
266 structure using the software STRUCTURE 2.3.4 (Pritchard *et al.* 2000) which uses a
267 Bayesian approach to assign individuals to genetic clusters based on allele frequencies (full
268 detail in Appendix A, Methods A1). We used sampling locations as prior information
269 (LOCPRIOR option) to help in the detection of weak structure (Hubisz *et al.* 2009) but we also
270 reported the results of the same analysis without this option activated. We varied the number
271 of *K* clusters from 1 to 15 (the number of populations sampled). The most probable number
272 of clusters was subsequently determined using both the likelihood of *K* and the second order
273 rate of change of likelihood between two consecutive values of *K* ($\Delta K$) following Evanno *et*
274 *al.* (2005). We also estimated genetic clustering of our samples using the method of
275 Discriminant Analysis of Principal Components (DAPC, Jombart *et al.* 2010) implemented
276 in the "adegenet" R package (Jombart 2008). This approach does not assume any migration
277 model or prior based on sampling location, but aims to identify synthetic variables that
278 distinguish between groups while minimizing within-group variation. We assessed the most
279 likely number of clusters using Bayesian Information Criterion (BIC).

280

281 **2.5. Demographic trends and gene flow**

282 We used Approximate Bayesian Computation (ABC) to assess demographic trends in the
283 sampling sites and to determine the direction of gene flow between western and eastern
284 populations (Beaumont *et al.* 2002). The ABC approach estimates parameters in absence of
285 computable likelihood functions by comparing empirical observations to simulated data. It
286 first generates a large set of simulated data using parameters randomly drawn from prior
287 distributions. Observed and simulated data are then reduced to a set of summary statistics.

288 The posterior probability of models and parameters are estimated using the fraction of
289 simulated models whose summary statistics are closest to those of observed data (Beaumont
290 2010; Csilléry *et al.* 2010).

291 We tested whether the changes in census size reported by national surveys (Green *et*
292 *al.* 1997; Koffijberg & Schäffer 2006) were reflected in the genetic data. Note that only
293 national-level trends were available (constant, fluctuating, declining or increasing), which
294 prevented us from being able to compare rates among the three Polish sites. For each
295 population three scenarios of demographic change over time (constant, decreasing and
296 increasing effective population size) were tested. We used ABC Toolbox (Wegmann *et al.*
297 2010) to sample parameters in our prior distributions and coalescent simulations were
298 computed using Fastsimcoal (Excoffier & Foll 2011) under the three demographic models.
299 The posterior probabilities of models were then evaluated using the 'abc' R package
300 (Csilléry *et al.* 2012) under the neural network approach, which has been shown to be less
301 sensitive to tolerance rate and correlations between summary statistics than regression-based
302 methods (Blum & François 2010).

303 The direction of longitudinal gene flow was assessed using another set of simulations
304 in a similar ABC workflow. Estimates of gene flow may be affected by the fact that genetic
305 structuring across all sampled populations is unclear and that some populations showed
306 evidence of declining size over time (see Results section and Figures 1 and 2). Therefore, to
307 simplify our model, we focused on gene flow between pairs of eastern (core and abundant)
308 and western (small and more peripheral) populations on a France – Russia axis. We selected
309 the three westernmost (Italy was excluded owing to its small sample size) – Scotland, France
310 and Germany – and the three easternmost populations – Russia, Latvia, Belarus –, resulting
311 in nine pairs of populations. We determined the posterior probabilities of three models: a

312 reference model in which the two populations exchange migrants symmetrically and two
313 models with a unidirectional gene flow (from west to east and from east to west respectively).
314 In order to speed-up computation and to assess uncertainty related to sample size, a single
315 set of simulations was run, simulating only 20 individuals in each of the two populations.
316 For each pair of populations, 20 individuals were randomly sampled in each population and
317 this subsample was used to compute the posterior probability of each model of gene flow.
318 This process was repeated ten times so that we reported the mean and standard-deviation of
319 posterior probabilities across these ten replicates. The full details of the ABC methodology
320 are given in Appendix A, Methods A1.

321

322 **3. RESULTS**

323 **3.1. Genetic diversity**

324 The 15 microsatellites genotyped had between 9 and 34 alleles per locus (Table 1), with the
325 corncrake specific markers being more variable than the cross species utility markers (mean
326 allele number: 26 vs. 12 respectively). Across 225 tests (15 populations*15 loci), 26 showed
327 a departure from Hardy-Weinberg equilibrium, but the same loci or populations were not
328 consistently affected (Appendix A, Table A3). Similarly, GENEPOP revealed no significant
329 deviation from linkage disequilibrium after Bonferroni correction. The proportion of null
330 alleles was moderate to low (mean null allele frequency over loci = 0.039, SD = 0.031),
331 ranging from 0.011 (*Crex12*) to 0.118 (*Crex11*) and the false allele rate was estimated at
332 0.01. Moreover, the mean $G_{ST}$ estimated after correction for null alleles (0.009, 95%
333 CI = 0.006-0.013) was similar to the value calculated without taking null alleles into account
334 (0.008, 95% CI = 0.005-0.012). Given this, and since the presence of null alleles would have

335 little impact on Bayesian genetic clustering anyway (Carlsson 2008), we kept all markers in
336 further analyses.

337 All populations were similar in terms of genetic diversity (Table 1); allelic richness
338 ranged from 3.86–4.68, observed heterozygosity ($H_o$), from 0.63 to 0.75, and expected
339 heterozygosity ($H_e$), from 0.70 to 0.77. Mean rarefied allelic richness was 4.42 (3.86–4.42).
340 When excluding the Italy and Hungary populations for which sample size was very small, it
341 was noted that the Scottish population had a lower allelic richness (7.27) than all the other
342 populations (8.42–9.12). None of these components of genetic diversity showed a significant
343 relationship with longitude and latitude. Similarly, the regressions against dbMEMs (3
344 variables had significant positive Moran's I and were thus used in these analyses) revealed
345 no significant relationship (Appendix A, Table A5).

346

347 **3.2. Population structure**

348 Pairwise $G_{ST}$ and $D$ did not show significant differentiation between populations after
349 Bonferroni correction ($P$-values from 0.103 to 1.00), although 58 and 52 comparisons (out
350 of 105) respectively were significant before Bonferroni correction, most of them involving
351 Scotland, France, Italy or Romania (Appendix A, Table A4). Both indices exhibited very
352 low values (mean ± SD; $G_{ST} = 0.008 \pm 0.008$; $D = 0.062 \pm 0.060$). However, the highest
353 values constantly involved the same two populations: $G_{ST}$ was > 0.01 in 28 (out of 105)
354 pairwise comparisons, 14 involving Scotland and 14 involving Italy, including the $G_{ST}$
355 between Scotland and Italy which was the highest in the dataset (0.033). Similarly, the
356 highest values of $D$ always involved Scotland and Italy. GESTE did not identify any link
357 between population-specific $F_{ST}$ and geographical variables, since the posterior probability
358 of the constant model largely exceeded models that included longitude, latitude or dbMEM

359 (Appendix A, Table A6). The AMOVA analysis revealed that the vast majority of global
360 genetic variance was within individuals (93.4%, $P < 0.01$) while among population variation
361 was very low (0.44% $P > 0.99$) (Appendix A, Table A7).

362 At the population level, no significant pattern of isolation by distance was detected
363 by Mantel tests or dbRDA analyses (Table 2 and Appendix A, Figure A1), even if there is a
364 marginal non-significant increase of genetic differentiation with geographic distance
365 ($r = 0.330$, $P = 0.064$), although probably not linear (Appendix A, Figure A1). Conversely,
366 at the individual level, the dbRDA analysis revealed a significant link between genetic
367 distance and all spatial factors tested (dbMEM – 8 variables had a significant positive
368 Moran's I and were retained, and coordinates, Table 2). Similarly, Mantel test highlighted a
369 significant relationship with longitudinal distance. However, dbRDA with sampling site as
370 conditional variable revealed no significant pattern (Table 2), suggesting that significant
371 results were mostly a result of the spatial aggregation of samples.

372 Using sampling locations as priors and following the $\Delta K$ method (Evanno *et al.*
373 2005), the Bayesian clustering analysis performed by STRUCTURE retained four genetic
374 clusters (Figure 2). However, the likelihoods of $K = 1$ to $K = 4$ were very close, indicating
375 that support for $K = 4$ over K = 1, 2 or 3 was limited. Moreover, assuming $K = 4$ did not
376 provide any useful information since the 4$^{th}$ cluster was split between all geographic
377 populations. We will therefore focus on results for $K = 3$. Individual estimated memberships
378 highlighted reasonable support for a Scottish cluster, since almost all birds sampled in
379 Scotland had > 0.7 probability of belonging to the same cluster. French and Italian
380 populations appeared to be grouped in a second cluster, suggesting the existence of a
381 southwestern European cluster. However, membership coefficients for this group indicated
382 a probability of belonging to several genetic clusters, thus revealing weak differentiation.

383 This is confirmed by the fact that STRUCTURE was unable to detect any significant
384 population structure when no spatial prior was provided (Appendix A, Figure A2). All
385 eastern European populations were roughly similar, with individuals mainly assigned to the
386 3rd cluster. Romania was the only site where more than 10% of individuals were assigned to
387 the Scottish cluster. Details of the mean membership coefficients per sampling population
388 are available in Appendix A, Table A8.

389 Eighty principal components (PC) had to be kept in the discriminant analysis of
390 principal components (DAPC) to retain more than 80% of the total variation, indicating that
391 there was no clear or simple partitioning of genetic variation. Following Bayesian
392 information criterion, the optimal number of genetic clusters was five (Appendix A, Figure
393 A3). However, these five clusters did not match the geographic distribution of samples and
394 were mixed between individuals from multiple origins. DAPC was thus unable to identify a
395 reliable population structure in our dataset.

396

397 **3.3. Estimation of demographic history and gene flow**

398 Using a 100-fold cross-validation confirmed that our method was able to distinguish between
399 the three different demographic scenarios (Appendix A, Table A9). The whole dataset
400 indicated a scenario of decreasing effective population size (Table 3) with high confidence
401 (posterior probability of the 'decreasing' model for all data pooled together = 0.98).
402 Populations considered separately gave different results (Figure 1, Table 3). All western
403 European sites (Scotland, France, Italy and Germany) supported a decreasing demographic
404 model (Table 3). In contrast, the southern and easternmost populations (Hungary, Romania,
405 Belarus and Russia) supported a demographic scenario of constant effective population size.
406 Among the other sites, four were assigned to the decreasing model (Sweden (Gotland),

407 Poland (north), Poland (east) and Latvia) and three to the constant model (Czech Republic,
408 Sweden (continent) and Poland (south)) (Table 3). In all analyses, the model of increasing
409 population size always had a null posterior probability, indicating strong support for the
410 rejection of this demographic scenario in all corncrake populations.

411 The cross-validation procedure highlighted that our model selection analysis was
412 unable to perfectly discriminate between models of gene flow, especially between the east-
413 to-west and symmetric models, although the pseudo-observed datasets were assigned to the
414 model they belonged to in most cases (Appendix A, Table A9). The simulations resulted in
415 the strong rejection of the west-to-east gene flow model for all populations pairs (posterior
416 probability $< 0.002$ in all cases) (Table 4). However, the analyses failed to clearly distinguish
417 which of the unidirectional east-to-west or symmetric gene flow models was the most likely.
418 Although mean posterior probabilities attributed three population pairs to the east-to-west
419 model and six to the symmetric model, standard-deviations across the 10 replicates
420 overlapped values of east-to-west and symmetric models in all cases, indicating that the two
421 hypotheses are statistically equally probable (Table 4).

422

423 **4. DISCUSSION**

424 We determined patterns of genetic variation within and among European populations of
425 corncrake and assessed our result in comparison to theories relating to the range dynamics
426 of a species (the central-marginal hypothesis) and to expectations from the demographic
427 patterns observed for this species through field surveys. Our results suggest the existence of
428 only very weak genetic structure, subtly dividing the European corncrake population into
429 three clusters. Genetic diversity was high in all populations and showed no geographic
430 pattern. The demographic estimates revealed different population trends, with numbers

431 constant or decreasing depending on location. These inferred trends were mostly congruent
432 with national field surveys.

433 Overall, the low $G_{ST}$ and $D$ values, the AMOVA and DAPC analyses, as well as the
434 STRUCTURE analysis (when run with no geographic prior), revealed that there is only very
435 limited genetic structure among corncrake populations. Analyses also failed to reveal any
436 spatial pattern of genetic diversity or isolation by distance among populations, but suggested
437 a longitudinal differentiation when analysed at the individual level. The STRUCTURE
438 analysis, when using the sampling locations as priors for its estimation, did provide some
439 evidence that three weakly differentiated genetic clusters may exist. One encompassed all
440 eastern European populations and two more occurred in western European, grouping birds
441 from France-Italy and Scotland respectively. The detection of (weakly) genetically
442 differentiated groups in Western Europe is consistent with other data. For example, previous
443 biometrical analyses found that French and British corncrakes were heavier than eastern
444 European birds (Keišs *et al.* 2004; Schäffer & Koffijberg 2004). Furthermore, a recent study
445 detected geographic variation in male calls across Europe, but also high inter-annual
446 variation (Budka *et al.* 2014). These patterns could plausibly be the result of limited genetic
447 structuring with dispersal among distant breeding sites. Finally, as part of an ongoing study
448 of corncrake migration, some Scottish birds have been tagged with geolocators (Green 2013)
449 and all recaptured individuals were found to have wintered in western Africa. Previously all
450 corncrakes were believed to winter in eastern and southern Africa, where overwinter
451 densities of corncrake are much higher (Schäffer & Koffijberg 2004). Although further
452 evidence is needed to confirm that populations are segregated in wintering grounds, these
453 findings may indicate that western and eastern European populations have distinct wintering
454 areas. Similarly, it has been observed that French corncrakes are infected by a distinct set of

455 haemosporidian (avian malaria) lineages genetically different than those found in all eastern
456 European populations (Fourcade *et al.* 2014). Unfortunately, due to the use of buccal
457 samples from Scotland we could not test whether this population also had differing pathogen
458 lineages. Taken altogether, this evidence supports the genetic evidence and suggest some
459 limited differentiation between the western populations (France and, especially, Scotland)
460 and the more continental populations.

461 If we are to believe the evidence of a subtle amount of genetic structure, then two
462 different scenarios may be responsible for the low differentiation observed. First, the pattern
463 may result from the existence of shared ancestral polymorphism with recent, or ongoing,
464 isolation of the western populations from an originally panmictic European population. We
465 know from historical data that the corncrake was still common and widespread in Europe in
466 the early 20$^{th}$ century (Green *et al.* 1997) and we can assume that favourable habitats were
467 highly connected. However, habitat fragmentation (Donald *et al.* 2001; Tockner & Stanford
468 2002) may have since caused the limited population differentiation. The second possibility
469 is that it results from contemporary gene flow among corncrake populations that were
470 structured in the past. Assuming a constant migration rate, the more recent asymmetry in
471 sizes between eastern and western populations would result in a higher number of effective
472 migrants leaving the core populations for the smaller peripheral populations than vice versa.
473 Thus, differences in demographic regimes may have gradually erased most, if not all, of any
474 initial difference in allele frequencies between Scotland, France, and Eastern Europe,
475 resulting in an observed pattern of high gene flow with low, but still just apparent, genetic
476 structure. We performed ABC analyses to test for this hypothesis of asymmetric gene flow
477 between eastern and western populations. Unfortunately, we could not conclusively
478 distinguish between a simple model of symmetric gene flow and a scenario of east-to-west

479 asymmetric gene flow, possibly because gene flow is not strictly unidirectional or
480 symmetric. However, all models showed a very strong rejection of the west-to-east model,
481 which tends to support the opposite hypothesis of asymmetric gene flow towards western
482 populations.

483 Our Approximate Bayesian Computation analyses of demography supported a model
484 of decreasing size of the European corncrake population when all populations were included
485 as one (the global analysis), but revealed a more complex pattern at a smaller scale. A model
486 of demographic decline was supported for all western European populations (Scotland,
487 France, Germany and Italy) which corroborates the trends identified from the national
488 surveys which tend to indicate a decrease since the late 19$^{th}$ century (Green & Gibbons
489 2000). A scenario of constant population size was supported for some of the most southern
490 or eastern sampling sites (Czech Republic, Hungary, Romania, South Poland, Belarus and
491 Russia) where recent population surveys also suggest that corncrake populations have
492 remained roughly stable, or even increased (Bürger *et al.* 1998; Keišs 2003; Sukhanova &
493 Mischenko 2003; Schäffer & Koffijberg 2004). More surprising is that a scenario of
494 decreasing population size was identified in Latvia and two Polish populations, despite no
495 survey-based evidence of declining corncrake numbers in these populations. On the contrary,
496 recent agricultural decline in former communist countries appears to have favoured
497 population expansion (Keišs 2003, 2005). However, human activities may have negatively
498 affected these populations during the Soviet period (Tucker *et al.* 1994), leaving a genetic
499 signature that is still detectable in the current corncrake populations. Importantly, these
500 results indicate that the ABC method is able to identify population trends that are not
501 detected by classical surveys, such as historical declines or trends masked by fluctuations in
502 local population numbers. Although the time period reflected by ABC analyses remains

503 uncertain, such methods may be particularly useful for species whose behaviour makes the
504 accurate detection of population trends through surveys difficult. For example, species with
505 high dispersal ability, such as the corncrake (Mikkelsen *et al.* 2013), may undertake long-
506 distance movements during the breeding season to avoid unsuitable conditions, therefore
507 causing large annual fluctuations in population sizes recorded at specific sites.

508 Although patterns of genetic variation at the range scale and related hypotheses, have
509 been studied for many years, there are still various unresolved issues regarding theoretical
510 expectations (Sagarin *et al.* 2006; Eckert *et al.* 2008). The central-marginal hypothesis may
511 result either in the differentiation of peripheral populations with a reduction of their genetic
512 diversity, or on the contrary in a source-sink dynamics which homogenise populations
513 (Sagarin & Gaines 2002; Eckert *et al.* 2008). In our study, levels of genetic differentiation
514 indicated that considerable gene flow occurs, or at least has occurred, between corncrake
515 populations. We also did not detect a clear reduction in genetic diversity in peripheral
516 populations. Indeed, all measures of genetic diversity remained notably high across the entire
517 European range (Table 1), although measures of allelic richness suggest a slight reduction
518 of diversity in Scotland, the most north-western site. The apparent homogeneity of genetic
519 variation would therefore be more congruent with a source-sink model. However, analyses
520 also show a weak signature of a longitudinal differentiation between western and eastern
521 populations of the corncrake. This pattern does not involve all marginal populations – no
522 differentiation of the northernmost or southernmost populations was observed – and thus
523 does not seem to match the classical hypotheses of the central-marginal model either. In
524 conclusion, we show that a classical biogeographical model seems unable to predict the
525 pattern of genetic structure – very weak and longitudinal – and the maintenance of high
526 genetic diversity observed across the European range of the corncrake. However, most

527 results could be explained by the spatial heterogeneity of human activity which drove
528 demographic differences and may subsequently be responsible for either an asymmetric east
529 to west gene flow, or a recent divergence of the westernmost populations. Finally our vision
530 of the global pattern of genetic variation in the corncrake remains limited by our sampling
531 which covered its European range only. A larger view may reveal a different pattern: in their
532 Asian breeding area, where habitat is still relatively undisturbed, corncrake populations may
533 show more “natural” dynamics closer to the expectations of the central-marginal hypothesis.
534 It is also possible that, at this larger scale, Asian and European populations are more
535 significantly structured. In this regard, analysing samples from Asia, including peripheral
536 sites, as well as more western European sites such as Ireland would provide clues to the
537 determination of the actual differentiation between the western and eastern sites and between
538 core and marginal populations.

539 Whatever the actual drivers of the observed genetic variation, the evidence from our
540 study suggests that all European corncrake populations are (or were recently) interconnected.
541 Although there are also some evidence of a certain degree of site fidelity in this species
542 (Green 1999), recurrent long-distance dispersal events likely contribute to maintain genetic
543 diversity within and among populations across the corncrake’s European range. Despite
544 some uncertainties regarding its exact underlying mechanisms, this high intra-European
545 connectivity should motivate the implementation of large-scale conservation schemes. A
546 European action plan has been published as early as 1996 (Crockford *et al.* 1997) but since
547 then most management actions are restricted to small areas and European coordination
548 remains limited. Similarly, the results of successful management experiences, such as the
549 spectacular recoveries in some areas such as the Scottish islands (O’Brien *et al.* 2006), could
550 perhaps be better shared among managers. If our hypothesis of asymmetric gene flow is

551 confirmed, it would also suggest that the threatened populations of western Europe are
552 sustained by birds from core eastern populations. Incoming gene flow should reduce the
553 extinction probability of corncrake populations in western Europe, where their fate is more
554 uncertain, as long as the suitable habitat is maintained and friendly agricultural practices are
555 used. We emphasize here the need for efficient conservation management in both areas. On
556 the one hand, if western European sites act, at least partly, as population sinks, the
557 preservation of the core eastern European sites is decisive for the conservation of the species.
558 The current favourable status of eastern populations is likely due to a recent event of
559 agriculture abandonment. However, this trend is reversed and the renewed intensification of
560 agriculture through the reconversion of abandoned croplands already impacts grassland birds
561 in Eurasian steppes (Kamp *et al.* 2015). In the long term, western sites may not be sustained
562 by eastern source populations. On the other hand, although the observed gene flow may help
563 avoiding the negative effects of inbreeding depression and loss of adaptive potential
564 (Frankham 2005) in the smallest populations, the gradual replacement of western European
565 birds through a source-sink dynamics may also, potentially, lead to a loss of local adaptation
566 (Kawecki & Ebert 2004) which could be detrimental to long-term survival of populations.
567 Thus, even if western populations are sustained by eastern birds, conservation efforts should
568 aim at preserving isolated and declining western sites to enhance local recruitment and limit
569 the loss of local adaptive characteristics so that their survival would not rely on immigration
570 from sources with uncertain future. However, it must be noted that their long-term
571 persistence may also depend on their response to global warming, especially on the southern
572 margins of the species range.

573 Furthermore, the existence of a slight differentiation between the western European
574 sites – where the decline has been particularly strong – and the more eastern sites, raises the

575 possibility of different migration routes or wintering sites for these populations, and
576 conservation action focused on these differing sits or flyways may be necessary. Generally,
577 we see that the genetic approach developed here was not sufficient on its own to conclusively
578 determine the direction of gene flow among European sites. Alternative methods to
579 monitoring individual movements may be necessary to combine with the genetic data and
580 facilitate a better understanding of intra-European migrations. The rate of ring recoveries
581 being very low in this species (Green 2004), tracking devices with GSM or satellite
582 transmitters (Bridge *et al.* 2011) may soon provide an opportunity to resolve as yet unknown
583 dispersal patterns between European populations. Likewise, the method of capture which
584 allowed the sampling of males only may prevent from inferring sex specific dispersal
585 patterns in the corncrake. Although there is currently no evidence for sex-biased dispersal in
586 this species, such a bias may have demographic consequences. For example, female biased
587 dispersal could lead to male-biased sex ratios in isolated peripheral populations and further
588 increase extinction risk (Dale 2001). Unfortunately, to our knowledge no unbiased capture
589 methods is currently available. A better understanding of the processes driving the observed
590 genetic variation may be gained from analysing different types of markers. Mitochondrial
591 markers, being maternally inherited (Harrison 1989), and having shorter coalescent time that
592 nuclear loci (Zink & Barrowclough 2008), may potentially show other patterns than those
593 revealed by microsatellites. For example, a spatial structuring of mitochondrial haplotypes
594 is expected if the almost complete panmixia we observed is caused by male dispersal only
595 (Prugnolle & de Meeus 2002). Furthermore, a low diversity of mitochondrial haplotypes
596 across Europe may indicate the lack of population structure is the result of recent population
597 expansion across Europe (perhaps from a refugium outside of the sampled range), whereas
598 high mitochondrial diversity may indicate the species has long been distributed across
599 Europe with high levels of gene flow (Provan & Bennett 2008). Similarly, as microsatellites

600 are often characterised by a high level of homoplasy, some subtle patterns may be only
601 identifiable by mitochondrial markers. Such an approach may thus be a relevant axis for
602 future research that would help to resolve some remaining uncertainties of our study.

603 Approximate Bayesian computation (ABC) (Beaumont *et al.* 2002) provides an
604 innovative simulation-based tool which is now widely used to distinguish between
605 demographic scenarios (Bertorelle *et al.* 2010). Here, an ABC approach was used to identify
606 discrepancies between population trends observed in surveys and variation in effective
607 population size. It provides opportunities to better quantify the relative importance of natural
608 and human pressures on the contemporary dynamics of ranges in the face of the current
609 changes (Mace *et al.* 2010). This method, associated with more classical clustering and
610 spatial analyses, helped us to unravel the consequences of different levels of anthropogenic
611 pressure across a large species range on the resulting spatial genetic structure of that species.
612 Developing and improving similar approaches in other species would certainly provide
613 insights into range dynamics of species across large continental landmasses like the
614 Palearctic. It is notably a strong help to improve the spatial scaling of conservation actions
615 by highlighting the current levels of range-wide connectivity. It may also raise the awareness
616 of practitioners to some aspects of human disturbance such as the loss of adaptive potential
617 that could remain neglected by more local studies. In general, large-scale genetic approaches
618 have the potential to take an important part in the guidance of conservation actions
619 (Frankham 2010), for example by revealing historical demographic processes in species that
620 have experienced contrasting effects of human activity across their range.

621

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630

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865 **Appendix A:** Supplementary material

866 **Methods A1:** Detailed methods of genetic analyses
867 **Fig. A1:** Isolation by distance (IBD) plots, at the population and individual levels.
868 **Fig. A2:** STRUCTURE results, computed without using the LOCPRIOR option
869 **Fig. A3:** Inference of genetic clusters following Discriminant Analysis of Principal
870 Components (DAPC).
871 **Table A1:** Genetic diversity statistics calculated for each microsatellite locus and
872 details on genotyping.
873 **Table A2:** Prior distributions of parameters and summary statistics used for ABC
874 simulations
875 **Table A3:** Genetic diversity for each of the 15 loci and 15 populations
876 **Table A4:** Pairwise differentiation between populations**,** $G_{ST}$ and Jost's *D*
877 **Table A5:** Regressions between within–population genetic diversity and spatial
878 factors
879 **Table A6:** Posterior probabilities of models inferred by GESTE
880 **Table A7:** Analysis of molecular variance (AMOVA)
881 **Table A8:** Individual membership coefficients averaged by sampling population, *i.e.*
882 mean probability of belonging to one of the 4 clusters inferred by STRUCTURE
883 **Table A9:** Confusion matrix of demographic and gene flow models

884

885 **Highlights**

886 • Understanding range-scale genetic patterns is essential in conservation biology
887 • Predictions of biogeographical models can be disturbed by human activity
888 • In the Corncrake *Crex crex*, we observed a low genetic structure at the European
889 scale
890 • Approximate Bayesian Computation informed about gene flow and demographic
891 trends
892 • European-scale coordination is required for efficient conservation of the corncrake

893

894 **TABLES**

895

896 **Table 1:** Genetic diversity statistics calculated for each sampled location (population). $n$:
897 number of individuals genotyped, $H_o$: observed heterozygosity, $H_e$: expected heterozygosity
898 or gene diversity, $A_R$: rarefied allelic richness, $A_R$*: rarefied allelic richness calculated
899 excluding the two populations with a low sample size, $N_A$: total number of alleles.

900

| Population | $n$ | $H_o$ | $H_e$ | $A_R$ | $A_R$* | $N_A$ |
|---|---|---|---|---|---|---|
| Scotland | 25 | 0.64 | 0.70 | 3.99 | 7.27 | 120 |
| France | 55 | 0.66 | 0.75 | 4.49 | 8.52 | 179 |
| Italy | 9 | 0.64 | 0.69 | 3.86 | | 85 |
| Germany | 32 | 0.63 | 0.74 | 4.54 | 8.88 | 168 |
| Sweden (continent) | 22 | 0.64 | 0.72 | 4.33 | 8.42 | 144 |
| Czech Republic | 24 | 0.75 | 0.76 | 4.56 | 8.43 | 146 |
| Sweden (Gotland) | 47 | 0.65 | 0.73 | 4.43 | 8.56 | 181 |
| Poland (north) | 45 | 0.68 | 0.72 | 4.43 | 8.57 | 178 |
| Hungary | 7 | 0.73 | 0.77 | 4.68 | | 94 |
| Poland (south) | 31 | 0.70 | 0.73 | 4.48 | 8.64 | 164 |
| Poland (east) | 36 | 0.68 | 0.73 | 4.47 | 8.97 | 183 |
| Latvia | 66 | 0.70 | 0.77 | 4.67 | 9.12 | 208 |
| Belarus | 33 | 0.63 | 0.74 | 4.50 | 8.68 | 164 |
| Romania | 32 | 0.71 | 0.73 | 4.43 | 8.50 | 160 |
| Russia | 32 | 0.71 | 0.74 | 4.48 | 8.56 | 164 |

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906 **Table 2:** Isolation-by-distance analysis results based on mantel tests and distance-based redundancy analyses (dbRDA). Mantel tests compared
907 pairwise genetic distances to 1) geographic distances (log transformed) and 2) longitudinal and latitudinal distances. dbRDA tested the effect of
908 1) longitude and latitude, and 2) distance-based Moran's eigenvector maps (dbMEM, 8 factors) on genetic distances. Analyses were computed
909 at the population level – genetic distances among populations being inferred by $G_{ST}/1\text{-}G_{ST}$ – and at the individual level with inter-individual
910 genetic distances corresponding to Rousset's *â*. Individual-level analyses were also computed after partialling out by sampling site identity.
911 Statistically significant results are indicated in bold font.

912

| | Population level | | | | | Individual level | | | | | Individual level (partialled out by sampling site) | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | dbRDA | | | Mantel test | | dbRDA | | | Mantel test | | dbRDA | | | Mantel test | |
| | *adj* $R^2$ | *F* | *P* | *r* | *P* | *adj* $R^2$ | *F* | *P* | *r* | *P* | *adj* $R^2$ | *F* | *P* | *r* | *P* |
| *log* Geographic distance | | | | 0.330 | 0.064 | | 0.012 | 0.087 | | | | | | 0.011 | 0.103 |
| Longitude | | 1.369 | 0.098 | 0.361 | 0.080 | | 1.117 | **0.011** | 0.053 | **0.020** | | 1.008 | 0.403 | 0.054 | **0.023** |
| Latitude | 0.050 | 1.075 | 0.338 | 0.100 | 0.210 | 0.001 | 1.138 | **0.005** | 0.017 | 0.136 | 0.000 | 0.957 | 0.823 | 0.016 | 0.152 |
| Longitude × Latitude | | 1.290 | 0.146 | | | | 1.439 | **<0.001** | | | | 0.942 | 0.904 | | |
| dbMEM | 0.008 | 1.036 | 0.399 | | | 0.002 | 1.105 | **<0.001** | | | 0.000 | 0.979 | 0.895 | | |

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916 **Table 3:** Posterior probability of the three demographic models – for each population and
917 for all data pooled together – inferred from the neural network method. The highest posterior
918 probability is highlighted in bold. The last column shows local demographic trends inferred
919 from population surveys. Data come from Schäffer & Koffijberg (2004) unless stated
920 otherwise.

921

| | Decreasing | Constant | Increasing | Local survey trend |
|---|---|---|---|---|
| All data | **0.98** | 0.02 | 0.00 | |
| Scotland | **1.00** | 0.00 | 0.00 | Decreasing / increasing[a] |
| France | **0.85** | 0.15 | 0.00 | Decreasing |
| Italy | **1.00** | 0.00 | 0.00 | Decreasing |
| Germany | **0.98** | 0.02 | 0.00 | Increasing ?[b] |
| Sweden (continent) | 0.36 | **0.64** | 0.00 | Increasing[c] |
| Czech Republic | 0.12 | **0.88** | 0.00 | Increasing |
| Sweden (Gotland) | **0.91** | 0.09 | 0.00 | Decreasing[d] |
| Hungary | 0.24 | **0.76** | 0.00 | Fluctuating |
| Poland (north) | **0.73** | 0.27 | 0.00 | |
| Poland (south) | 0.46 | **0.54** | 0.00 | Increasing |
| Poland (east) | **1.00** | 0.00 | 0.00 | |
| Latvia | **0.91** | 0.09 | 0.00 | Increasing |
| Belarus | 0.26 | **0.74** | 0.00 | Constant |
| Romania | 0.11 | **0.89** | 0.00 | Increasing |
| Russia | 0.40 | **0.60** | 0.00 | Fluctuating |

939
940 [a] Long-term decrease (Green 1995) followed by recent recovery (O'Brien *et al.* 2006)
941 [b] Schäffer & Koffijberg (2004) indicate an increasing population but Busche (1994) indicates a declining population in
942 Northern Germany
943 [c] Berg & Gustafson (2007)
944 [d] Green *et al.* (1997a)
945

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947 **Table 4:** Posterior probability (mean ± SD) of the three models of gene flow for nine pairs
948 of populations (three western sites: France, Scotland, Germany vs. three eastern sites:
949 Belarus, Latvia, Russia). For each pair of population, posterior probabilities were inferred
950 from 10 random samples of 20 individuals per site (the highest posterior probability for each
951 comparison is underlined and highlighted in bold).

952

| Population pairs | West ← East | | West ↔ East | | West → East | |
|---|---|---|---|---|---|---|
| France-Belarus | 0.493 | ±0.018 | **<u>0.507</u>** | ±0.018 | 0.000 | ±0.000 |
| France-Latvia | 0.486 | ±0.020 | **<u>0.514</u>** | ±0.020 | 0.000 | ±0.000 |
| France-Russia | **<u>0.510</u>** | ±0.023 | 0.490 | ±0.023 | 0.000 | ±0.000 |
| Scotland-Belarus | **<u>0.503</u>** | ±0.017 | 0.496 | ±0.017 | 0.001 | ±0.000 |
| Scotland-Latvia | 0.484 | ±0.009 | **<u>0.514</u>** | ±0.009 | 0.002 | ±0.001 |
| Scotland-Russia | **<u>0.500</u>** | ±0.024 | 0.499 | ±0.024 | 0.000 | ±0.000 |
| Germany-Belarus | 0.488 | ±0.023 | **<u>0.511</u>** | ±0.023 | 0.001 | ±0.003 |
| Germany-Latvia | 0.498 | ±0.020 | **<u>0.501</u>** | ±0.020 | 0.000 | ±0.001 |
| Germany-Russia | 0.485 | ±0.020 | **<u>0.515</u>** | ±0.020 | 0.000 | ±0.000 |

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954

955 **FIGURES LEGENDS**

956

957 **Figure 1:** Sampling locations of corncrakes across Europe, with the most probable
958 demographic scenarios inferred by Approximate Bayesian Computation shown (squares:
959 constant population size, down-pointing triangles: decreasing population size). The grey
960 shading represents the distribution of the corncrake according to the IUCN. Sampling sites
961 names are abbreviated: Sc: Scotland, Fr: France, It: Italy, G: Germany, Sw[C]: Sweden
962 (continent), CzR: Czech Republic, Sw[G]: Sweden (Gotland), Pol[n]: Poland (north), Hun:
963 Hungary, Pol[S]: Poland (south), Pol[E]: Poland (east), Lat: Latvia, Bel: Belarus, Rom:
964 Romania, Ru: Russia. Sample sizes and posterior probabilities of ABC models are given in
965 Tables 1 and 3 respectively.

966

967 **Figure 2:** Genetic structure among European corncrake populations based on the Bayesian
968 clustering algorithm STRUCTURE, using the LOCPRIOR option (sampling locations used as
969 priors). (a) Ln likelihood with confidence intervals of the ten replicates (b) $\Delta K$ for each value
970 of $K$. The highest peak of $\Delta K$ and Ln likelihood at $K = 4$ indicates most support for four
971 genetic clusters. (c) Bar plots of individual membership to each cluster where $K = 2$, $K = 3$
972 and $K = 4$. Sampling sites are separated by vertical bars and plotted according to their
973 longitude. Visual inspection of plots revealed that no further information can be gained by
974 considering $K = 4$ over $K = 3$.

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