Biological Conservation 198 (2016) 210–219

Contents lists available at ScienceDirect

Biological Conservation

journal homepage: www.elsevier.com/locate/bioc

Corncrake conservation genetics at a European scale: The impact of biogeographical and anthropological processes

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ARTICLE INFO

Article history:
Received 19 September 2015
Received in revised form 5 April 2016
Accepted 13 April 2016
Available online xxxx

Keywords:
Central-marginal hypothesis
Conservation genetics
Genetic diversity
Demography
Genetic structure
Approximate Bayesian computation

ABSTRACT

Understanding patterns of genetic structure, gene flow and diversity across a species range is required to determine the genetic status and viability of small peripheral populations. This is especially crucial in species distributed across a large range where spatial heterogeneity makes it difficult to predict the distribution of genetic diversity. Although biogeographical models provide expectations of how spatially structured genetic variation may be at the range scale, human disturbance may cause strong deviations from these theoretical predictions. In this study, we investigated genetic structure and demography at a pan-European scale in the corncrake *Crex crex*, a grassland bird species strongly affected by agricultural changes. We assessed population structure and genetic diversity, as well as demographic trends and direction of gene flow, in and among 15 contemporary populations of this species. Analyses revealed low genetic structure across the entire range with high levels of genetic diversity in all sites. However, we found some evidence that the westernmost populations were, to a very limited extent, differentiated from the rest of the European population. Demographic trends showed that population numbers have decreased in western Europe and remained constant across eastern Europe. Results may also indicate asymmetric gene flow from eastern to western populations. In conclusion, we suggest that the most likely scenario is that contrasting demographic regimes between eastern and western populations, driven by heterogeneous human activity, has caused not only asymmetric gene flow that has buffered small peripheral populations against genetic diversity loss, but also erased any genetic structure that may have existed. Our study not only highlights the need for coordinated action at the European scale to preserve source populations of the corncrake, but also to ensure persistence of the most threatened sites. Only by doing so will we avoid losing adaptive potential and prevent over-reliance on eastern source populations whose future may be uncertain.

1. Introduction

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

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http://dx.doi.org/10.1016/j.biocon.2016.04.018
0006-3207/

of range dynamics may need to be developed for such species. Information on gene flow and demographic trends across a range are key to identifying Evolutionarily Significant Units (ESU, Ryder, 1986) and evaluating the threats associated with changes in connectivity, i.e. inbreeding depression or the loss of adaptive potential (Hedrick and Kalinowski, 2000). Therefore such knowledge is critical in the design of informed conservation action plans.

Biogeographic models of range dynamics provide predictions regarding patterns of genetic variation across a species' distribution. Under the central-marginal model, focal species abundance is expected to be higher at the range core (i.e. the area of ecological optimum), and less abundant and more isolated at the periphery as environmental conditions gradually depart from the ecological optimum (Hengeveld and Haeck, 1982; Brussard, 1984; Brown, 1984). This has implications for the distribution of genetic variation at the range-scale (Eckert et al., 2008) and for the evolution of species' range (Hoffmann and Blows, 1994; Kirkpatrick and Barton, 1997). Although the central-marginal model is widely accepted, the hypothesis has been challenged by empirical and theoretical studies (Sagarin and Gaines, 2002; Sagarin et al., 2006; Samis and Eckert, 2007) and the model itself can generate opposite patterns. A first hypothesis implies that populations at the core have higher effective population sizes and produce more dispersing migrants than do the smaller, peripheral populations. Under this model, genetic drift in the peripheral populations is only partially compensated by limited gene flow from the core area, and therefore results in lower genetic diversity in, and higher differentiation among, these peripheral populations (Hoffmann and Blows, 1994; Lesica and Allendorf, 1995; Eckert et al., 2008). Consequently, these marginal populations are expected to be more sensitive to environmental changes – either stochastic or directional – and more prone to extinction (Lesica and Allendorf, 1995; Channell and Lomolino, 2000). In contrast, a second hypothesis suggests that if core populations are large and peripheral populations are small, there could be asymmetric gene flow from core to periphery (Kirkpatrick and Barton, 1997) analogous to that expected in a source–sink (Pulliam, 1988), or island–continent model (Slatkin, 1987). Homogenisation of genetic diversity and weak structure at the range scale is expected if the effect of the asymmetric gene flow is greater than the combined effects of drift and selection at the range margins.

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

We used the corncrake (*Crex crex*) as a model species to study genetic structure and gene flow at a continental scale. As is the case for many grassland bird species (Donald et al., 2006), agriculture intensification has severely affected the number and distribution of the corncrake (Green et al., 1997). This situation has motivated numerous conservation plans, especially in western Europe. Interestingly, because land use change and agriculture intensification are variable across Europe, the corncrake has been affected by human activity at various intensities in different parts of its range. To date, knowledge regarding genetic structure in this species is very limited and incomplete (Wettstein, 2003) and other methods (e.g. monitoring returning individuals) do not provide adequate amounts of data to determine dispersal patterns, connectivity between sites, or identify distinct evolutionary significant units in this species (Ryder, 1986). Interestingly the extensive population monitoring of the corncrake undertaken in many European countries allows survey-based demographic trends to be compared against the historical demography inferred using genetic data. The availability of such fine-scale demographic data provides an exciting opportunity to determine if apparent local trends, which usually drive conservation actions, concur with the continental-scale demographic landscape. Specifically, we tested two competing hypotheses arising from the central-marginal model: 1) peripheral populations are isolated from the core populations and are thus genetically differentiated and show a reduction of genetic diversity, 2) demographic imbalance between core and peripheral populations generates net gene flow towards the periphery that homogenises populations across the range. We used a suite of microsatellite markers to assess genetic diversity and structure across the European range of the corncrake. Approximate Bayesian computation (ABC) (Beaumont et al., 2002) was used to estimate corncrake historical demography at the population scale in order to assess fine-scale spatial variation in demographic trends across Europe. In order to assess the dynamics generating the observed pattern of genetic structuring, an ABC framework was also used to determine the direction of gene flow between western and eastern populations.

2. Methods

2.1. Study species and sample collection

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

With the collaboration of local ringers we collected 496 corncrake samples from 15 locations across Europe (Fig. 1) in 2011–2012. Samples were collected from May to July to avoid the capture of migrating birds. Individuals were attracted using playback of conspecific male calls at night during the peak of calling activity and captured using a mist net or large dipnet. Because of the playback-assisted capture method only males were sampled (Green, 1999). Depending on the local legislation and experience of the fieldworkers, different sources of DNA were collected. The different tissues sampled did not affect the quality of DNA extracted or the accuracy of the genotyping. In France, Germany, Italy, Hungary, Poland (all sites), Czech Republic, Latvia, Belarus and Russia (20 samples out of 32), ca. 50 μL of blood was collected from the brachial

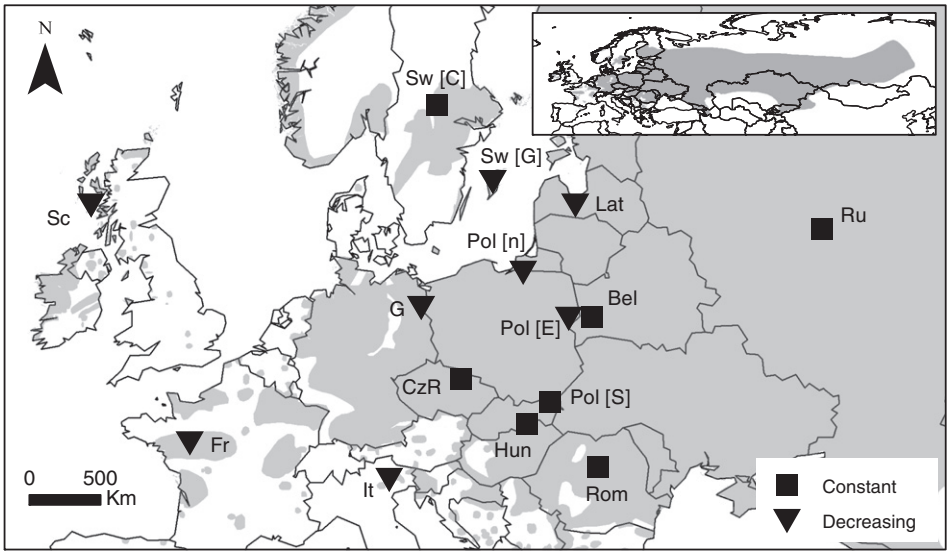

Fig. 1. Sampling locations of corncrakes across Europe, with the most probable demographic scenarios inferred by Approximate Bayesian Computation shown (squares: constant population size, down-pointing triangles: decreasing population size). The grey shading represents the distribution of the corncrake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

vein and stored in absolute ethanol. In Scotland buccal swabs served as a source of DNA, whereas feathers were collected from Romania, Sweden (all sites), and Russia (12 samples out of 32). All birds were released unharmed immediately after sampling, with the exception of a Russian individual which died from its collision with the landing net. Population sample sizes ranged from 7 to 66 (Fig. 1 and Table 1).

2.2. Microsatellite genotyping

We extracted genomic DNA using a method of salt extraction following Richardson et al. (2001). All individuals were genotyped at 15 microsatellite markers of which eight had been designed for the corncrake: *Crex1*, *Crex2*, *Crex6*, *Crex7*, *Crex8*, *Crex9*, *Crex11*, *Crex12* (Gautschi et al., 2002). The seven other markers are conserved across a large range of bird families: *CAM18* (Dawson et al., 2013), *TG02-120*, *TG04-12*, *TG04-12a*, *TG04-41*, *TG05-30* and *TG012-15* (Dawson et al., 2010). We amplified markers by Polymerase Chain Reaction (PCR) in three multiplexes (Appendix A, Table A1), using 1 μL of Qiagen Multiplex MasterMix, 1 μL of DNA (dried in the tube, ca. 15 ng) and 1 μL of 5 μM primer mix (Kenta et al., 2008) in a final PCR volume of 2 μL. The PCRs were run under the following conditions: an initial step at 95 °C for 15 min, followed by 40 cycles of 94 °C during 30 s (denaturation), 56.6 °C during 90 s (annealing) and 72 °C during 60 s (elongation). The final stage consisted of 30 min at 60 °C. Amplified fragments were mixed with a solution of formamide and GeneScan 500 ROX Size Standard (Applied Biosystems) and separated by micro-capillary electrophoresis. Alleles were subsequently scored using GeneMapper v3.7 software (Applied Biosystems).

Deviations from Hardy–Weinberg and linkage equilibria were estimated at each locus for each population using the package "adegenet" (Jombart, 2008) for R 3.0.2 (R Development Core Team, 2013) and the GENEPOP software (Rousset, 2008) respectively. Significance levels were adjusted using the Bonferroni correction for multiple comparisons. The proportion of null alleles in the dataset and its influence on the genetic differentiation between populations, as estimated by G_{ST} (Nei, 1973), was assessed using the FreeNA package (Chapuis and Estoup, 2007). Error rate was estimated using PEDANT software (Johnson and Haydon, 2007) with 10,000 simulations on 10 re-genotyped individuals. To test for the potential effect of any null alleles in the dataset, we ran a STRUCTURE analysis after exclusion of the marker which exhibited the highest rate of null alleles, using the same parameters as for the main analysis (see Section 3.2 below "Population structure").

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

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

2.3. Genetic diversity

We computed standard genetic diversity statistics for each population. Observed (H_o), expected heterozygosity (H_e), and rarefied allelic richness (A_R) were calculated using the R package "Hierfstat" (Goudet, 2005). As rarefaction depends on sample size, A_R is highly influenced by the low number of samples in Italy (9) and Hungary (7). Therefore, we also reported the rarefied allelic richness after exclusion of these two sites (A_R*). Single-locus observed and expected heterozygosity measures were also computed at each locus for each population (Appendix A, Table A3). We assessed the effect of geography on genetic diversity by testing the correlation between the genetic indices and distance-based eigenvector maps (dbMEM). dbMEM are orthogonal variables that describe the spatial structure of sampling points and are constructed from the principal coordinates of a neighbourhood matrix (Borcard and Legendre, 2002; Dray et al., 2006). dbMEM were computed using the "vegan" R package (Oksanen et al., 2015), using as truncation distance the largest distance in the minimum spanning tree

connecting all sites. All significant positive eigenvectors (Moran's I coefficients larger than the expected values) were used in a linear regression against measures of genetic diversity. We also computed the same regression analyses using longitude, latitude and their interaction instead of dbMEM as predictors of genetic diversity.

2.4. Population structure

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

Isolation by distance was tested first using Mantel tests to assess the correlation between pairwise geographic distance and pairwise genetic distance (Diniz-Filho et al., 2013). In addition to using the total geographic distances, we also ran Mantel tests using only longitudinal or latitudinal distances. However, the ability of Mantel test to detect spatial patterns has been questioned, especially when original data, not in the form of distance matrices, are used, as is the case for geographic coordinates (Legendre and Fortin, 2010; Legendre et al., 2015). Therefore, we also investigated how spatial features correlate with genetic structure using distance-based redundancy analyses (dbRDA), a method that ordinates the genetic distance matrix and uses the positive axes in a multivariate regression (Kierepka and Latch, 2015). As for the regression analyses against genetic diversity, we used as spatial predictors either the dbMEM variables or alternatively longitude, latitude and their interaction. Our individual samples are not evenly distributed in space but clustered in 15 sites, which could result in confounding isolation-by-distance and population structure (Meirmans, 2012). In order to assess the effect of the spatial configuration of sampling, we also computed partial individual analyses accounting for the identity of sampling sites. Mantel tests and dbRDA were computed using the "vegan" R package and their significance was tested with 10,000 permutations. Both types of analyses were performed both at the population and individual levels, using as the measure of genetic distance: (i) linearised pairwise population differentiation $G_{ST}/1\text{-}G_{ST}$ or (ii) pairwise individual genetic distance $\hat{a}$ (Rousset, 2000) computed using the software SPAGeDi (Hardy and Vekemans, 2002).

We estimated the contribution of within individual and within and among population variance on global genetic variation using an analysis of molecular variance (AMOVA, Excoffier et al., 1992) computed using Arlequin 3.5 (Excoffier and Lischer, 2010), with significance based on 10,000 permutations. We also tested for the presence of genetic structure using the software STRUCTURE 2.3.4 (Pritchard et al., 2000) which uses a Bayesian approach to assign individuals to genetic clusters based on allele frequencies (full detail in Appendix A, Methods A1). We used sampling locations as prior information (LOCPRIOR option) to help in the detection of weak structure (Hubisz et al., 2009) but we also reported the results of the same analysis without this option activated. We varied the number of K clusters from 1 to 15 (the number of populations sampled). The most probable number of clusters was subsequently determined using both the likelihood of K and the second order rate of change of likelihood between two consecutive values of K (ΔK) following Evanno et al. (2005). We also estimated genetic clustering of our samples using the method of Discriminant Analysis of Principal Components (DAPC, Jombart et al., 2010) implemented in the "adegenet" R package (Jombart, 2008). This approach does not assume any migration model or prior based on sampling location, but aims to identify synthetic variables that distinguish between groups while minimising within-group variation. We assessed the most likely number of clusters using Bayesian Information Criterion (BIC).

2.5. Demographic trends and gene flow

We used Approximate Bayesian Computation (ABC) to assess demographic trends in the sampling sites and to determine the direction of gene flow between western and eastern populations (Beaumont et al., 2002). The ABC approach estimates parameters in the absence of computable likelihood functions by comparing empirical observations to simulated data. It first generates a large set of simulated data using parameters randomly drawn from prior distributions. Observed and simulated data are then reduced to a set of summary statistics. The posterior probability of models and parameters are estimated using the fraction of simulated models whose summary statistics are the closest to those of observed data (Beaumont, 2010; Csilléry et al., 2010).

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

The direction of longitudinal gene flow was assessed using another set of simulations in a similar ABC workflow. Estimates of gene flow may be affected by the fact that genetic structuring across all sampled populations is unclear and that some populations showed evidence of declining size over time (see Section 3 and Figs. 1 and 2). Therefore, to simplify our model, we focused on gene flow between pairs of eastern (core and abundant) and western (small and more peripheral) populations on a France–Russia axis. We selected the three westernmost (Italy was excluded owing to its small sample size) – Scotland, France and Germany – and the three easternmost populations – Russia, Latvia, Belarus – resulting in nine pairs of populations. We determined the posterior probabilities of three models: a reference model in which the two populations exchange migrants symmetrically and two models with a unidirectional gene flow (from west to east and from east to west respectively). In order to speed-up computation and to assess uncertainty related to sample size, a single set of simulations was run, simulating only 20 individuals in each of the two populations. For each pair of populations, 20 individuals were randomly sampled in each population and this subsample was used to compute the posterior probability of each model of gene flow. This process was repeated ten times so that we reported the mean and standard-deviation of posterior probabilities across these ten replicates. The full details of the ABC methodology are given in Appendix A, Methods A1.

3. Results

3.1. Genetic diversity

The 15 microsatellites genotyped had between 9 and 34 alleles per locus (Table 1), with the corncrake specific markers being more variable

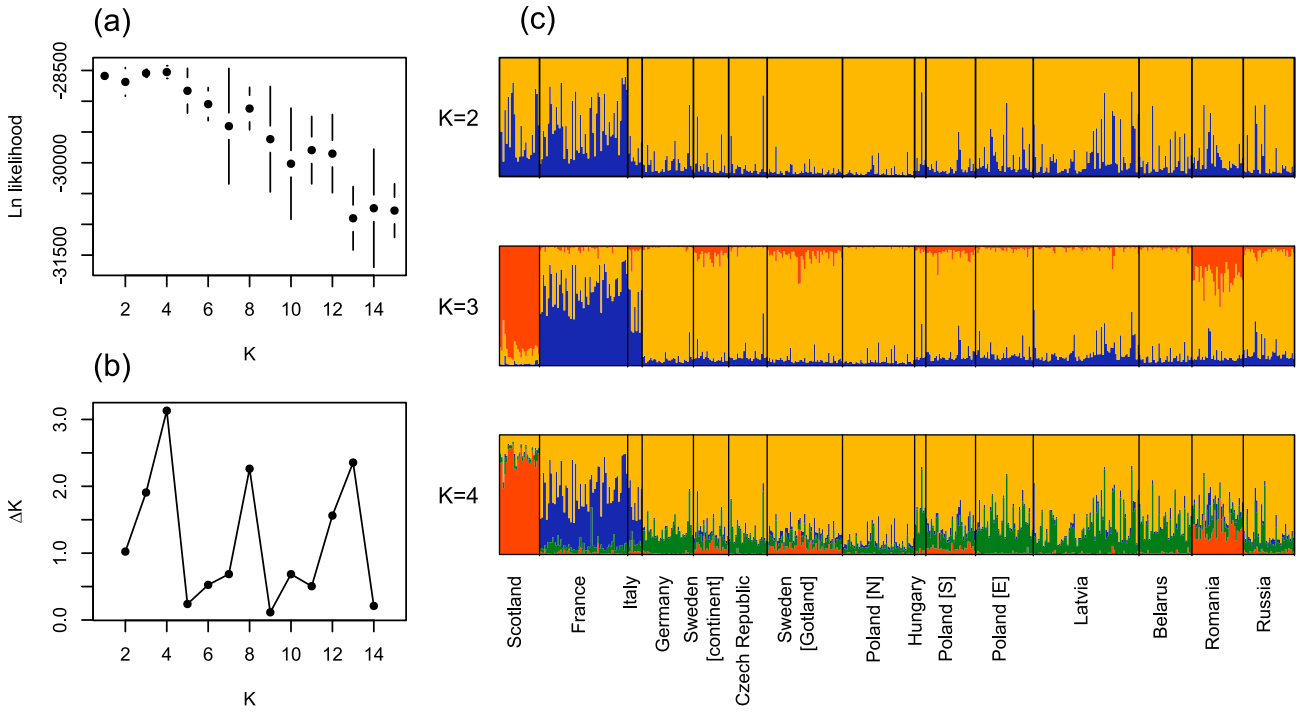

Fig. 2. Genetic structure among European corncrake populations based on the Bayesian clustering algorithm STRUCTURE, using the LOCPRIOR option (sampling locations used as priors). (a) Ln likelihood with confidence intervals of the ten replicates (b) ΔK for each value of K. The highest peak of ΔK and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

than the cross species utility markers (mean allele number: 26 vs. 12 respectively). Across 225 tests (15 populations*15 loci), 26 showed a departure from Hardy–Weinberg equilibrium, but the same loci or populations were not consistently affected (Appendix A, Table A3). Similarly, GENEPOP revealed no significant deviation from linkage disequilibrium after Bonferroni correction. The proportion of null alleles was moderate to low (mean null allele frequency over loci = 0.039, SD = 0.031), ranging from 0.011 (*Crex12*) to 0.118 (*Crex11*) and the false allele rate was estimated at 0.01. Moreover, the mean G_{ST} estimated after correction for null alleles (0.009, 95% CI = 0.006–0.013) was similar to the value calculated without taking null alleles into account (0.008, 95% CI = 0.005–0.012). Given this, and since the presence of null alleles would have little impact on Bayesian genetic clustering anyway (Carlsson, 2008), we kept all markers in further analyses.

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

3.2. Population structure

Pairwise G_{ST} and D did not show significant differentiation between populations after Bonferroni correction (P-values from 0.103 to 1.00), although 58 and 52 comparisons (out of 105) respectively were significant before Bonferroni correction, most of them involving Scotland, France, Italy or Romania (Appendix A, Table A4). Both indices exhibited very low values (mean ± SD; $G_{ST} = 0.008 \pm 0.008$; $D = 0.062 \pm 0.060$). However, the highest values constantly involved the same two populations: G_{ST} was > 0.01 in 28 (out of 105) pairwise comparisons, 14 involving Scotland and 14 involving Italy, including the G_{ST} between Scotland and Italy which was the highest in the dataset (0.033). Similarly, the highest values of D always involved Scotland and Italy. GESTE did not identify any link between population-specific F_{ST} and geographical variables, since the posterior probability of the constant model largely exceeded models that included longitude, latitude or dbMEM (Appendix A, Table A6). The AMOVA analysis revealed that the vast majority of global genetic variance was within individuals (93.4%, $P < 0.01$) while among population variation was very low (0.44% $P > 0.99$) (Appendix A, Table A7).

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

Using sampling locations as priors and following the ΔK method (Evanno et al., 2005), the Bayesian clustering analysis performed by STRUCTURE retained four genetic clusters (Fig. 2). However, the likelihoods of $K = 1$ to $K = 4$ were very close, indicating that support for $K = 4$ over K = 1, 2 or 3 was limited. Moreover, assuming $K = 4$ did not provide any useful information since the 4th cluster was split between all geographic populations. We will therefore focus on results for $K = 3$. Individual estimated memberships highlighted reasonable support for a Scottish cluster, since almost all birds sampled in Scotland had > 0.7 probability of belonging to the same cluster. French and Italian

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

	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	0.050	1.369	0.098	0.361	0.080	0.001	1.117	**0.011**	0.053	**0.020**	0.000	1.008	0.403	0.054	**0.023**
Latitude		1.075	0.338	0.100	0.210		1.138	**0.005**	0.017	0.136		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		

populations appeared to be grouped in a second cluster, suggesting the existence of a southwestern European cluster. However, membership coefficients for this group indicated a probability of belonging to several genetic clusters, thus revealing weak differentiation. This is confirmed by the fact that STRUCTURE was unable to detect any significant population structure when no spatial prior was provided (Appendix A, Fig. A2). All eastern European populations were roughly similar, with individuals mainly assigned to the 3rd cluster. Romania was the only site where more than 10% of individuals were assigned to the Scottish cluster. Details of the mean membership coefficients per sampling population are available in Appendix A, Table A8.

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

3.3. Estimation of demographic history and gene flow

A 100-fold cross-validation confirmed that our method was able to distinguish between the three different demographic scenarios (Appendix A, Table A9). The whole dataset indicated a scenario of decreasing effective population size (Table 3) with high confidence (posterior probability of the 'decreasing' model for all data pooled together = 0.98). Populations considered separately gave different results (Fig. 1, Table 3). All western European sites (Scotland, France, Italy and Germany) supported a decreasing demographic model (Table 3). In contrast, the southern and easternmost populations (Hungary, Romania, Belarus and Russia) supported a demographic scenario of constant effective population size. Among the other sites, four were assigned to the decreasing model (Sweden (Gotland), Poland (north), Poland (east) and Latvia) and three to the constant model (Czech Republic, Sweden (continent) and Poland (south)) (Table 3). In all analyses, the model of increasing population size always had a null posterior probability, indicating strong support for the rejection of this demographic scenario in all corncrake populations.

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

4. Discussion

We determined patterns of genetic variation within and among European populations of corncrake and assessed our result in comparison to theories relating to the range dynamics of a species (the central-marginal hypothesis) and to expectations from the demographic patterns observed for this species through field surveys. Our results suggest the existence of only very weak genetic structure, subtly dividing the European corncrake population into three clusters. Genetic diversity was high in all populations and showed no geographic pattern. The demographic estimates revealed different population trends, with numbers constant or decreasing depending on location. These inferred trends were mostly congruent with national field surveys.

Overall, the low G_{ST} and *D* values, the AMOVA and DAPC analyses, as well as the STRUCTURE analysis (when run with no geographic prior), revealed that there is only very limited genetic structure among corncrake populations. Analyses also failed to reveal any spatial pattern of genetic diversity or isolation by distance among populations, but

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

Population	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

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

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

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

suggested a longitudinal differentiation when analysed at the individual level. The STRUCTURE analysis, when using the sampling locations as priors for its estimation, did provide some evidence that three weakly differentiated genetic clusters may exist. One encompassed all eastern European populations and two more occurred in western European, grouping birds from France–Italy and Scotland respectively. The detection of (weakly) genetically differentiated groups in Western Europe is consistent with other data. For example, previous biometrical analyses found that French and British corncrakes were heavier than eastern European birds (Keišs et al., 2004; Schäffer and Koffijberg, 2004). Furthermore, a recent study detected not only geographic variation in male calls across Europe, but also high inter-annual variation (Budka et al., 2014). These patterns could plausibly be the result of limited genetic structuring with dispersal among distant breeding sites. Finally, as part of an ongoing study of corncrake migration, some Scottish birds have been tagged with geolocators (Green, 2013) and all recaptured individuals were found to have wintered in western Africa. Previously all corncrakes were believed to winter in eastern and southern Africa, where overwinter densities of corncrake are much higher (Schäffer and Koffijberg, 2004). Although further evidence is needed to confirm that populations are segregated in wintering grounds, these findings may indicate that western and eastern European populations have distinct wintering areas. Similarly, it has been observed that French corncrakes are infected by a distinct set of haemosporidian (avian malaria) lineages genetically different than those found in all eastern European populations (Fourcade et al., 2014). Unfortunately, due to the use of buccal samples from Scotland we could not test whether this population also had differing pathogen lineages. Taken altogether, this evidence supports the genetic evidence and suggests some limited differentiation between the western populations (France and, especially, Scotland) and the more continental populations.

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

Our ABC analyses of demography supported a model of decreasing size of the European corncrake population when all populations were included as one (the global analysis), but revealed a more complex pattern at a smaller scale. A model of demographic decline was supported for all western European populations (Scotland, France, Germany and Italy) which corroborates the trends identified from the national surveys which tend to indicate a decrease since the late 19th century (Green and Gibbons, 2000). A scenario of constant population size was supported for some of the most southern or eastern sampling sites (Czech Republic, Hungary, Romania, South Poland, Belarus and Russia) where recent population surveys also suggest that corncrake populations have remained roughly stable, or even increased (Bürger et al., 1998; Keišs, 2003; Sukhanova and Mischenko, 2003; Schäffer and Koffijberg, 2004). More surprising is that a scenario of decreasing population size was identified in Latvia and two Polish populations, despite no survey-based evidence of declining corncrake numbers in these populations. On the contrary, recent agricultural decline in former communist countries appears to have favoured population expansion (Keišs, 2003, 2005). However, human activities may have negatively affected these populations during the Soviet period (Tucker et al., 1994), leaving a genetic signature that is still detectable in the current corncrake populations. Importantly, these results indicate that the ABC method is able to identify population trends that are not detected by classical surveys, such as historical declines or trends masked by fluctuations in local population numbers. Although the time period reflected by ABC analyses remains uncertain, such methods may be particularly useful for species whose behaviour makes the accurate detection of population trends through surveys difficult. For example, species with high dispersal ability, such as the corncrake (Mikkelsen et al., 2013), may undertake long-distance movements during the breeding season to avoid unsuitable conditions, therefore causing large annual fluctuations in population sizes recorded at specific sites.

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

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

Whatever the actual drivers of the observed genetic variation, the evidence from our study suggests that all European corncrake populations are (or were recently) interconnected. Although there are also some evidence of a certain degree of site fidelity in this species (Green, 1999), recurrent long-distance dispersal events likely contribute to maintain genetic diversity within and among populations across the corncrake's European range. Despite some uncertainties regarding its exact underlying mechanisms, this high intra-European connectivity should motivate the implementation of large-scale conservation schemes. A European action plan has been published as early as 1996 (Crockford et al., 1997) but since then most management actions are restricted to small areas and European coordination remains limited. Similarly, the results of successful management experiences, such as the spectacular recoveries in some areas such as the Scottish islands (O'Brien et al., 2006), could perhaps be better shared among managers. If our hypothesis of asymmetric gene flow is confirmed, it would also suggest that the threatened populations of western Europe are sustained by birds from core eastern populations. Incoming gene flow should reduce the extinction probability of corncrake populations in western Europe, where their fate is uncertain, as long as suitable habitat is maintained and friendly agricultural practices are used. We emphasise here the need for efficient conservation management in both areas. If western European sites act, at least partly, as population sinks, the preservation of the core eastern European sites is decisive for the conservation of the species. The current favourable status of eastern populations is likely due to agriculture abandonment over the past 20 years. However, this trend is now being reversed and the renewed intensification of agriculture already impacts grassland birds in the Eurasian steppes (Kamp et al., 2015). In the long term, western sites may not be sustained by eastern source populations. Furthermore, although the observed gene flow may help avoid the negative effects of inbreeding depression and loss of adaptive potential (Frankham, 2005) in the smallest populations, the gradual replacement of western birds through a source–sink dynamics may lead to a loss of local adaptation (Kawecki and Ebert, 2004). This could be detrimental to the long-term survival of those populations. Thus conservation efforts should also focus on the isolated and declining western sites to limit the loss of any local adaption so that the survival of these populations does not rely solely on immigration from sources with an uncertain future. Finally it must be noted that their long-term persistence may also depend on their response to climate change, especially on the south-western margins of the species range. Furthermore, the existence of a slight differentiation between the western European sites – where the decline has been particularly strong – and the more eastern sites, raises the possibility of different migration routes or wintering sites for these populations, and conservation action focused on these differing sites or flyways may be necessary.

Generally, we see that the genetic approach developed here was not sufficient on its own to conclusively determine the direction of gene flow among European sites. Alternative methods to monitoring individual movements may be necessary to combine with the genetic data and facilitate a better understanding of intra-European migrations. The rate of ring recoveries being very low in this species (Green, 2004), tracking devices with GSM or satellite transmitters (Bridge et al., 2011) may soon provide an opportunity to resolve as yet unknown dispersal patterns between European populations. Likewise, the method of capture which allowed the sampling of males only may prevent from inferring sex specific dispersal patterns in the corncrake. Although there is currently no evidence for sex-biased dispersal in this species, such a bias may have demographic consequences. For example, female biased dispersal could lead to male-biased sex ratios in isolated peripheral populations and further increase extinction risk (Dale, 2001). Unfortunately, to our knowledge no unbiased capture methods is currently available. A better understanding of the processes driving the observed genetic variation may have been gained from analysing other types of markers as well as the microsatellite loci. Mitochondrial markers, being maternally inherited (Harrison, 1989), and having shorter coalescent time than nuclear loci (Zink and Barrowclough, 2008), may potentially show other patterns than the microsatellites and thus reveal more about the system. For example, a spatial structuring of mitochondrial haplotypes is expected if the almost complete panmixia we observed is caused by male dispersal only (Prugnolle and de Meeus, 2002). Furthermore, a low diversity of mitochondrial haplotypes across Europe may indicate that the lack of population structure is the result of recent population expansion across Europe (perhaps from a refugium outside of the sampled range), whereas high mitochondrial diversity may indicate that the species has long been distributed across Europe with high levels of gene flow (Provan and Bennett, 2008). Similarly, as microsatellites are often characterised by a high level of homoplasy, some subtle patterns may be only identifiable by mitochondrial markers. Such an approach may thus be a relevant axis for future research that would help to resolve some remaining uncertainties of our study.

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

Ackowledgements

The authors wish to thank all the people who helped in corncrake DNA sampling: Attila Sandor, Sandor Boldogh, Peter Olsson, Peter Sverev, Paolo Vasca, Patrik Rhönnstad, Susanne Arbeiter, Jochen Bellebaum, Joachim Sadlik and especially the people from LPO Anjou: Gilles Mourgaud, Emmanuel Séchet and Edouard Beslot. We also thank Christophe Lemaire for his useful suggestions on ABC analyses and Karl Phillips for his help in genotyping. This study was funded by a grant from Plan Loire Grandeur Nature and European Regional Development Fund to JS and DSR.

Appendix A. Supplementary data

Supplementary data to this article can be found online at http://dx.doi.org/10.1016/j.biocon.2016.04.018.

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