No evidence for a reduction of genetic diversity despite a strong population decline in the Corncrake *Crex crex*

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Abstract

The preservation of genetic diversity is an important aspect of conservation biology, since small populations frequently suffer from inbreeding and loss of genetic diversity that can increase their risk of extinction. Here, we report changes in various measures of genetic diversity over 12 years in a declining population of corncrake *Crex crex*, a grassland bird species of high conservation concern throughout Europe. Despite a twofold demographic decline during the same period, we found no evidence for a reduction of genetic diversity. The maintenance of genetic diversity is an opportunity that may help the implementation of effective conservation actions.

Introduction

The preservation of genetic diversity is recognised as an important aspect of conservation biology (Haig et al. 2016). Small populations frequently suffer from inbreeding and loss of genetic diversity. Inbreeding reduces survival and fecundity as a result of an increase in the frequency of deleterious alleles or a reduction of heterozygote advantage, thus directly increasing the extinction risk of small populations (Reed & Frankham 2003). On the long-term, maintaining sufficiently high genetic variation within species and populations is key to make them able to adapt to new environmental conditions (Crandall et al. 2000). In an era of rapid environmental changes driven by human activity, the observed decline of many populations is expected to reduce their adaptive potential and jeopardises their long-term persistence. Hence, monitoring temporal changes in the genetic diversity of wild populations can serve to prioritize management actions and as an indicator of the strength of human impact.

The corncrake *Crex crex* is a grassland bird species of high conservation concern throughout Europe (Schäffer & Koffijberg 2004). Its main threat is the intensification of agricultural practices , and especially early mowing, that strongly reduces the survival of fledglings (Tyler et al. 1998). It has resulted in a severe population decline in several western European countries (Koffijberg et al. 2016), such as France that experienced a 90% reduction of corncrake numbers in the last 30 years (Hennique et al. 2013). So far, we ignore whether this demographic decline of corncrake populations has affected their genetic diversity. Reduced population sizes and demographic bottlenecks are usually associated with a loss of allelic richness and a reduction of heterozygosity (Reed & Frankham 2003), but incoming gene flow from larger, not genetically depleted, populations may contribute to maintaining genetic diversity. In the corncrake, a recent population genetic study revealed high genetic diversity within, and large levels of gene flow among, European populations (Fourcade et al. 2016). However, temporal changes in genetic diversity have not been investigated. It is therefore unknown whether the most threatened corncrake populations gradually lose genetic diversity as a result of their demographic decline.

In this study, we examined the changes in genetic diversity that occurred over a 12-years period in a corncrake population in western France that experienced a 2-fold decline during the same period. Individuals were sampled and genotyped in 2000, 2011 and 2012, and we estimated allelic richness, heterozygosity and effective population size in each year to test for a decline in genetic diversity over this period. We also analysed temporal population structure to assess whether this population became gradually genetically different over time.

Methods

The corncrake is a migratory bird that breeds in floodplain meadows and extensive grasslands across the Palearctic. The mechanisation of mowing and the abandonment of traditional haymaking practices has led to a decline of corncrake populations throughout western Europe during the last century (Schäffer & Koffijberg 2004). In France, a national monitoring scheme of singing males implemented from the 1980s revealed that, at the national level, the number of individuals declined from ca. 2000 calling males in 1983 to ca. 200 in 2016. At the same time, the national distribution of corncrakes became highly fragmented and contracted in the floodplain meadows around the city of Angers (-0.1154°W, 47.4216°N, see Figure 1) in western France, which now host the majority of breeding corncrakes in France. In this region, the number of singing males has severely declined in the past decades, from ca. 500 in 1983 to only ca. 60 in 2016 (Figure 1).

We used 55 blood samples of male corncrakes collected respectively from 25 and 30 individuals in 2011 and 2012 around Angers, and previously used to assess the genetic structure of populations at the European scale (Fourcade et al. 2016). We also analysed 24 new blood samples collected in 2000. Birds were captured using playbacks between May and July as described in Fourcade et al. (2016). All birds were ringed which ensured that no individual was sampled twice in this study. We extracted genomic DNA from blood samples using a salt extraction protocol and genotyped all individuals at 15 microsatellite markers, including eight corncrake-specific markers (Gautschi et al. 2002) and seven markers conserved across many bird species (Dawson et al. 2010;

Dawson et al. 2013). The full genotyping procedure followed the protocol described in Fourcade et al. (2016).

We computed the observed and expected heterozygosity, as well as the rarefied allelic richness, of each locus in each sampling year, using the "hierfstat" R package (Goudet 2005). Effective population size was estimated using the linkage disequilibrium approach implemented in NeEstimator 2.1 (Do et al. 2014), excluding rare alleles with frequency < 0.05. Additionally, we tested for a temporal differentiation of the sampled population. First we computed pairwise indices of population differentiation between sampling years: G''_{ST}, an unbiased and standardized analogue of F_{ST} (Meirmans & Hedrick 2011), and D (Jost 2008), a measure of population differentiation based on the number of alleles instead of on heterozygosity. Confidence intervals around these indices were calculated based on a bootstrap approach with 10000 permutations as implemented in the "diveRsity" R package (Keenan et al. 2013). Finally, we implemented the clustering algorithm of the program STRUCTURE (Pritchard et al. 2000), which uses a Bayesian approach to assign to each individual a membership probability to an *a priori* number of genetic clusters. We ran 10 STRUCTURE replicates for K = 1, K = 2 and K = 3 clusters with the following settings: admixture model with correlated allele frequencies (Falush et al. 2003), and 100 000 burn-in steps followed by 500 000 iterations. We also turned the LOCPRIOR option on, which makes use of prior information to facilitate the detection of weak genetic structures, usually the sampling location (Hubisz et al. 2009). Here, instead, we used the years of sampling as prior genetic clusters.

Results

There was no evidence for a decline in genetic diversity over time (Figure 2). Observed heterozygosity was 0.70 (± 0.07 s.e.m) in the oldest sample (2000), then 0.62 (± 0.05 s.e.m) in 2011 and 0.68 (± 0.06 s.e.m) in 2012. Similarly, there was almost no difference between sampling years in terms of expected heterozygosity, with a mean of 0.70 (± 0.07 s.e.m) in 2000, 0.76 (± 0.04 s.e.m) in 2011 and 0.75 (± 0.05 s.e.m) in 2012. There was a weak decrease in allelic richness over time, from 9.50 (± 1.44 s.e.m) alleles per locus in 2000 on average, to 9.15 (± 0.10 s.e.m) in 2011 and 8.87 (±

1.08 s.e.m) in 2012. Finally, although the mean estimate of effective population size was highly variable between years (183.3 in 2000, 60.5 in 2011 and 643.9 in 2012), the upper confidence interval was always infinity, showing that there was no signal of linkage disequilibrium in our data that can distinguish it from being indeterminably large.

Pairwise measures of genetic differentiation between years were not significant, as bootstrap confidence intervals always included zero (Table 1). However, we note that G''_{ST} and D calculated between the samples collected in 2000 and those collected in 2011 or 2012 were considerably larger that between 2011 and 2012 (Table 1). STRUCTURE provided no evidence for a temporal genetic structure neither, as shown by a larger likelihood for one genetic cluster and an estimated membership of individuals to two or three genetic clusters that did not match the temporal structure of the data (Supplementary material, Figure S1). Nevertheless, we observed that samples collected in 2011 and 2012 were more closely related with each other than with the 2000 sampling. For instance, assuming K = 2, samples from 2000 were assigned to the red cluster of Figure S1 by 80 % on average, while the mean membership for this cluster was 62 % both for the samples collected in 2011 and in 2012.

Discussion

We demonstrated that, despite a strong and continuous demographic decline, the corncrake population of Western France did not simultaneously face a reduction of its genetic diversity. Not only did genetic diversity remained stable, it was also remarkably high for a population estimated around 180 males (in 2012). There is generally a strong correlation between microsatellite heterozygosity and population size in birds (Evans & Sheldon 2008). At first, it is therefore surprising to observe that genetic diversity did not reflect the population drop recorded in the field. Several hypotheses can explain this result, which have profound implications for the management of this endangered population.

In light of the available data, the most likely scenario is that genetic diversity was maintained by gene flow from distant populations that have not suffered from the same demographic decline.

Although the available evidence remain scarce, there are records of occasional, within-season, long-distance movements (up to 1,500 km) revealed by ring recoveries (Schäffer & Koffijberg 2004; Koffijberg et al. 2016) and song analyses (Mikkelsen et al. 2013). We also previously described large levels of gene flow among European populations of corncrakes that we attributed to the dispersal of individuals from the most productive sites of eastern Europe to the declining western European populations (Fourcade et al. 2016). (Koffijberg et al., 2016)(Koffijberg et al., 2016)In this regard, the estimates of effective population size estimates could not be distinguished from infinity, suggesting that the samples originated from a larger population than the few hundreds individuals recorded in the study area. We also observed what might be a sign of a slow temporal differentiation. It may reveal the gradual change in the genetic characteristics of the original population, in agreement with the hypothesis that the maintenance of genetic diversity was achieved by regular immigration events.

The fact that the extreme decline of corncrake numbers was not followed by a similar decrease of their genetic diversity is good news in a conservation perspective. For example, it is known that low levels of heterozygosity, when they reflect a reduced genetic diversity at immune-related loci, can result in a higher susceptibility to pathogens (Hawley et al. 2005). In this regard, we already observed that western European corncrake populations, including France, did not suffer from higher malaria prevalence than the larger populations of eastern Europe (Fourcade et al. 2014). It suggests that, as the population is not genetically depleted, it may be able to recover successfully if effective management actions are implemented.

Longer genetic monitoring may however be needed to rule out the possibility that a slow decline of genetic diversity is occurring. Indeed, it is possible that the population decline was still too recent to result in a reduction of heterozygosity and that in a clear genetic signal will be detectable later. In the absence of annual monitoring between 1998 and 2006, it is also unknown whether population decline started before or after 2000 (Figure 1). Samples from the 1980s or earlier, when corncrake numbers where high both at the local and national levels (Hennique et al. 2013), would help identifying the association between demography and genetic diversity in corncrake populations. Interestingly, here, allelic richness showed a slight trend towards decline, which might be an early

warning of a loss of genetic diversity, since allelic richness is usually more sensitive to a decrease of population size than heterozygosity (Allendorf 1986).(Reif & Vermouzek 2018) Accordingly, previous analyses using approximate Bayesian computation suggested that contemporary genetic data were compatible with a scenario of ongoing population decline (Fourcade et al. 2016). So far, the agri-environmental schemes implemented to protect the corncrake in the region have failed to halt its decline. Future environmental policies should now take advantage of the fact that genetic diversity has been maintained to implement effective conservation strategies that may reverse the unfavourable demographic trend.

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Tables

Table 1: Pairwise genetic differentiation between sampling years, expressed as G''_{ST} below diagonal and *D* above diagonal. Numbers in brackets show the 95% confidence intervals obtained through 10000 permutations.

	2000	2011	2012
2000	--	0.0145 [-0.0216 - 0.0632]	0.0054 [-0.0270 - 0.0469]
2011	0.0474 [-0.0105 - 0.1132]	--	0.0010 [-0.0285 - 0.0440]
2012	0.0297 [-0.0148 - 0.0761]	0.0115 [-0.0345 - 0.0735]	--

Figures

Figure 1: Location of sampling site in France shown as a black rectangle, with the estimated European distribution of the Corncrake represented in light blue (left); population trend in the study region based on the number of singing males recorded during annual surveys (right). For visualisation purpose, the blue line shows the estimated trend ± confidence interval according to a Poisson generalized additive model with k = 4 for the smooth term. The years of sampling (2000, 2011 and 2012) are represented as arrows.

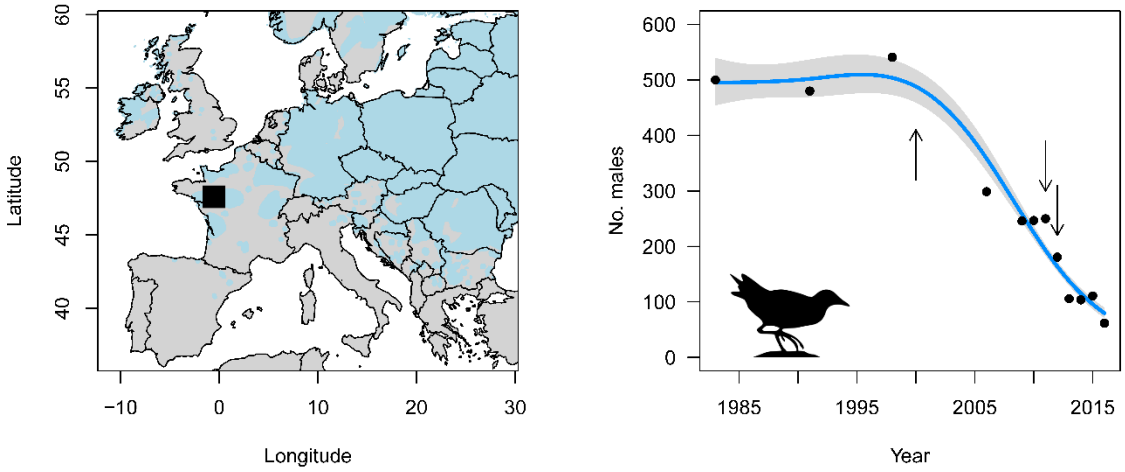
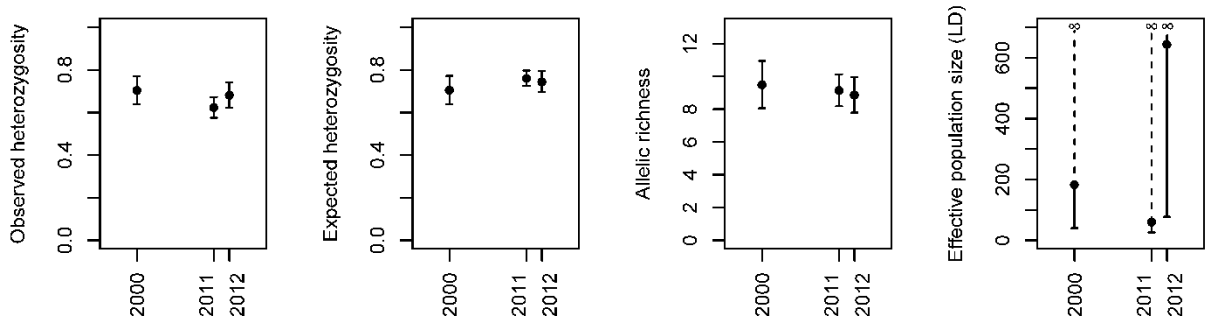


Figure 2: Change in genetic diversity between sampling years, expressed as estimates of observed heterozygosity, expected heterozygosity, allelic richness (mean across loci ± standard error), and effective population size based on linkage disequilibrium (± 95% confidence intervals).



Supplementary material

Figure S1: Results of the STRUCTURE analysis. A: Likelihood of the data to belong to one, two or three genetic clusters (mean and standard deviation across 10 replicates). B: Estimated membership of each individual (represented as vertical bars) to two (top) or three (bottom) genetic clusters.