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Extreme genetic structure in a social bird species despite high dispersal capacity

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Abstract

Social barriers have been shown to reduce gene flow and contribute to genetic structure among populations in species with high cognitive capacity and complex societies, such as cetaceans, apes and humans. In birds, high dispersal capacity is thought to prevent population divergence unless major geographic or habitat barriers induce isolation patterns by dispersal, colonization or adaptation limitation. We report that Iberian populations of the red-billed chough, a social, gregarious corvid with high dispersal capacity, show a striking degree of genetic structure composed of at least 15 distinct genetic units. Monitoring of marked individuals over 30 years revealed that long-distance movements over hundreds of kilometres are common, yet recruitment into breeding populations is infrequent and highly philopatric. Genetic differentiation is weakly related to geographic distance and habitat types used are overall qualitatively similar among regions and regularly shared by individuals of different populations, so that genetic structure is unlikely to be due solely to isolation by distance or isolation by adaptation. Moreover, most population nuclei showed relatively high levels of genetic diversity, suggesting a limited role for genetic drift in significantly differentiating populations. We propose that social mechanisms may underlie this unprecedented level of genetic structure in birds through a pattern of isolation by social barriers not yet described, which may have driven this remarkable population divergence in the absence of geographic and environmental barriers.

Introduction

Genetic differentiation among populations at large geographic scales is caused primarily by geographic barriers to gene flow and isolation by distance (e.g. Wright 1943; Slatkin 1987). However, complex social patterns are also known to play a role in restricting gene flow among populations (Ross 2001). The evolutionary history of humans is the clearest example of complex gene-culture interactions (Laland 2008; Gintis 2011), where diverse socio-cultural traits such as ethnolinguistic boundaries, social organization, and traditional lifestyles, act as barriers to gene flow and thereby promote genetic differentiation among populations (Ségurel *et al*. 2008; Novembre *et al*. 2009; Tishkoff *et al*. 2009; Ross *et al*. 2013). This mechanism of isolation, mediated by social barriers, has been rarely reported in animals other than social mammals, where patterns of neutral genetic structure are typically consistent with patterns of isolation by limitation to dispersal (i.e., geographic distance), isolation by colonization (geographic barriers), or isolation by adaptation (ecological barriers) (Orsini *et al*. 2013; Andrews 2014).

There is evidence that complex social processes, similar to those in humans, occur in several non-human primate and cetacean species with variable dispersal capacity and an associated ability to cross geographical barriers (Pilot *et al*. 2010; Kopp *et al*. 2014; Kopps *et al*. 2014; Foote *et al*. 2016). In terrestrial birds, however, fine-scale genetic structure is relatively rare, and generally arises from major geographical barriers, low dispersal capacity, or a combination of both (Francisco *et al*. 2007; Naka *et al*. 2012; Bertrand *et al*. 2014). Among birds with high cognitive capacity, corvids have developed complex patterns of social behaviour (Marzluff & Angell 2005; Clayton & Emery 2007; Loretto *et al*. 2012), and there is some evidence that socio-cultural factors may have played a role in driving genetic structure at local scales when combined with low dispersal and small population size

(Abdelkrim *et al*. 2012). However, this has not been the case in most studies at large geographic scales (Omland *et al*. 2000; McCormack *et al*. 2008; van Els *et al*. 2012; Zhang *et al*. 2012). Demonstrating the role of social factors in driving population divergence at large scales requires ruling out alternative factors such as limited dispersal capacity, geographic barriers to gene flow, and small-scale habitat specialization through local adaptation.

Here, we examine patterns of population genetic structure, individual dispersal and breeding recruitment within and among Iberian populations of the red-billed chough (*Pyrrhocorax pyrrhocorax*), a highly social corvid with high dispersal capacity. We aimed to explore the relative roles of extrinsic factors (e.g. geographic barriers, distance, or habitat) versus potential intrinsic factors (e.g. natal dispersal, social behaviour) in driving population differentiation. We inferred population structure from individual genotypes at 11 microsatellite loci and sequences from two mitochondrial DNA regions. We also used data from a region-wide long-term monitoring program of marked red-billed choughs over the last 30 years to assess dispersal capacity and recruitment probability into breeding populations.

Material and methods

Long-term population monitoring and genetic sampling

Red-billed choughs (choughs hereafter) are highly social, medium-sized, long-lived corvids, showing strict mate and nesting-site fidelity (Banda & Blanco 2014). They are widespread at continental scales, but rare at regional scales, in Europe, Africa, and Asia (Cramp 1988). They forage mostly in open landscapes (Blanco *et al*. 1996 and 1998) and nest in caves, crevices and human-made structures (Blanco *et al*. 1996; Banda & Blanco 2009). The study area comprises the Iberian Peninsula, the European stronghold for this species, where numerous fragmented population nuclei are distributed across open, montane and rocky inland and coastal areas (Fig. 1) (Blanco 2003; Cuevas & Blanco 2009).

Biological samples for molecular analysis were collected at 25 population nuclei throughout the Iberian distribution range (Table 1; Fig. 1). In twelve of these areas, we also individually marked nestlings and full-grown individuals from 1985 to 2016 in order to monitor dispersal movements and breeding recruitment (Table 2). Throughout the entire study period we regularly searched for ringed breeders by surveying nesting sites and capturing breeding pairs. We recorded if they were ringed as nestlings to determine their natal origin (Blanco & Tella 1999; Banda & Blanco 2014). Communal roosts and foraging flocks were also regularly monitored to record the movements of ringed individuals identified with spotting scopes. Multiple captures of communally roosting birds were also conducted to ring and record the presence of previously marked individuals (Table 2).

Microsatellite genotyping

Genomic DNA was extracted from blood and feather samples. A total of 642 individuals were genotyped at 11 polymorphic microsatellite loci specifically developed for choughs (Dávila *et al*. 2015) (see Supplementary Methods for details on DNA extraction, amplification and genotyping). Individuals with missing data at three or more loci were excluded from the dataset. All loci within each population were screened for the presence of genotyping errors, null alleles, large allele dropout and stuttering with MICRO-CHECKER v.2.2.3 (Van Oosterhout *et al*. 2004) using Bonferroni (Dunn-Sidak) adjusted confidence intervals (95%) derived from 10,000 Monte Carlo simulations. The probability of null alleles was negligible for all loci except PpyA1.9, which was excluded from all subsequent analyses. To minimize potential bias due to family sampling, individuals from each sampling site were screened for close genetic relatedness (presence of full-sibs) using COLONY v.2.0.5.8 (Jones & Wang 2010), and applying the full-likelihood method, long length of run, medium likelihood precision, and no sibship prior, assuming a monogamous mating system and an

inbreeding model for isolated populations (Keller & Waller 2002). After four long runs, only one member of each full-sib pair detected with a probability >0.90 was included in the final genotype data set used in the study. LOSITAN (Antao *et al*. 2008) was used to detect evidence of selection at individual loci.

Linkage disequilibrium (LD) between pairs of loci and deviations from Hardy-Weinberg equilibrium per sampling location were calculated with GENEPOP v.4.4.2 (Rousset 2008) using the Markov chain method (10,000 dememorisation steps, 1000 batches and 5000 iterations/batch), and a sequential Bonferroni correction. Microsatellite genetic diversity was characterised by the number of alleles per locus (N_A), observed and expected heterozygosities (H_O and H_E) calculated with GENAIEX v.6.5 (Peakall & Smouse 2012), allelic richness (A_R) obtained using FSTAT v.2.9.3.2, and fixation index (F_{IS}) values estimated using GENEPOP v.4.4.2 (Rousset 2008). Polymorphism information content (PIC) for all loci per population was obtained using CERVUS v.3.0.3 (Kalinowski *et al*. 2007).

Mitochondrial DNA sequencing and analysis

Partial sequences of the control region (CR) and NADH dehydrogenase gene subunit 2 (*ND2*) were used to assess mitochondrial genetic diversity and structure (see Supplementary Methods for amplification details). The sequences of all haplotypes identified were submitted to GenBank under accessions KX024396-KX024431 (CR sequences) and KX024343-KX024378 (*ND2* sequences). MtDNA genetic diversity indices (*H*, number of haplotypes; *h*, haplotype diversity; *K*, average number of differences; and π, nucleotide diversity) were obtained with DNASP v.5.10.01 (Librado & Rozas 2009). A median-joining statistical parsimony network was constructed using the program NETWORK v.4.6.1.3 (Bandelt *et al*. 1999).

Bayesian clustering analysis and population genetic differentiation

Red-billed chough population structure across Iberia was inferred using Bayesian clustering algorithms, which use multilocus genotype data to assign individuals to clusters under the assumption of Hardy–Weinberg and linkage equilibrium within each population (Pearse & Crandall 2004; Manel *et al*. 2005). The available Bayesian clustering software packages differ slightly in the model assumptions and methodological principles, which may lead to incongruent results among different methods (François & Durand 2010). To support the reliability of the population genetic structure, we used three different strategies implemented in GENELAND v.4.0.5 (Guillot *et al*. 2005a), BAPS v.6.0 (Corander *et al*. 2008) and STRUCTURE v.2.3.4 (Pritchard *et al*. 2000), respectively.

GENELAND uses a Reversible Jump Markov Chain Monte Carlo (RJMCMC) algorithm for the inference of number of populations, which can be implemented using a spatially explicit model that assumes the geographical location of the samples. In this study, the inferences were carried out using 20 independent runs with *K* varying from 1 to 10 under the following conditions: 1 000 000 MCMC interactions with a thinning of 100, a maximum rate of Poisson process of 590 (equal to the number of individuals in the dataset), a maximum number of nuclei of 1770 (3 x maximum rate of Poisson process), an uncorrelated allele frequency model, a true spatial model and a false null allele model. The uncorrelated allele model was chosen since there is evidence that the correlated model may overestimate the number of populations (Guillot *et al*. 2005b; Cullingham & Moehrenschlager 2013). The uncertainty of the coordinates was set to zero assuming that individuals have the same geographic provenance within each sampling region.

In BAPS, the Bayesian clustering model uses a stochastic optimization algorithm to infer the posterior mode of the genetic structure (Corander *et al*. 2006), allowing the non-spatial and spatial clustering of "individuals" or "groups of individuals". In this approach, the spatial clustering of groups was implemented with 10 replicates for each *K* (from 2 to 10). To estimate admixture coefficients among clusters, the program was run with the following parameters: 1000 interactions, 2000 reference individuals and 10 interactions for reference individuals.

The program STRUCTURE infers the optimal number of populations (*K*) using a Markov chain Monte Carlo (MCMC) algorithm. Considering the complexity of our dataset, we followed a strategy similar to an approach previously reported for highly structured data to select the best *K* (Tishkoff *et al.* 2009). Thus, the analysis consisted of 20 independent runs for all *K* values (varying from 1 to 10), each with 1,000,000 MCMC iterations after a burn-in of 100,000 iterations, under a model of admixture and correlated allele frequencies. The simulations were performed using sampling localities as priors (locprior option) (Hubisz *et al*. 2009). The CLUMPAK server (Kopelman *et al*. 2015) was used to process the structure outputs to obtain assignment probabilities of individuals (*q*) to each cluster (using a MCL threshold for similarity scores of 0.90) and to display the results in a graphical interface. The likelihood distributions were analysed with STRUCTURE HARVESTER (Earl & von Holdt 2012). The optimal *K* value was selected considering the following assumptions: (1) the likelihood distribution reached a maximum and began to plateau or decrease; (2) high stability of clustering patterns between runs (at least 60% of the 20 runs were similar in the primary mode); and (3) *K*max + 1 no longer identify new clusters (i.e., the genetic structure at *K*max + 1 is equal to *K*max). STRUCTURE analyses were also performed using a hierarchical approach to detect fine-scale population structure (Evanno *et al*. 2005). Thus, population

clusters differentiated at the first level of the STRUCTURE analysis (q>0.5) were analysed separately in a second level analysis. The best K for each cluster was selected following the procedures described above for the first level, after running the programme n+2 times, considering n the number of sampling localities included in the clusters.

The level of genetic differentiation among populations was estimated based on data from 10 microsatellite loci and two mtDNA markers (CR and *ND2*). For microsatellite markers, global and pairwise genetic differentiation was characterised by F_{ST} (Weir & Cockerham 1984) and Jost's D values (Jost 2008). Computations for F_{ST} were performed in GENALEX v.6.5 using the analysis of molecular variance (AMOVA) with 9,999 permutations. The calculation of D_{est} values was also carried out in GENALEX v.6.5 using 99 permutations (maximum number of permutations allowed by the software due to missing data in some loci) to test significance at a 0.05 level. The mitochondrial genetic differentiation among the chough populations was calculated in ARLEQUIN v.3.1 (Excoffier *et al*. 2005) with 9,999 permutations to assess the significance of pairwise F_{ST} estimates.

Testing for departures from mutation/drift and migration/drift equilibria

The analysis of departures from neutral equilibrium associated with selection (mutation-drift disequilibrium) or population processes (migration-drift disequilibrium) provides important data to identify demographic events (e.g. bottlenecks or restriction in gene flow) which may have influenced populations in the past. The program BOTTLENECK v.1.2.02 (Piry *et al*. 1999) was used to detect evidence of "recent" bottleneck events. Additionally, the evidence for genetic bottlenecks in our data was also tested through the *M*-ratio model implemented in the software M_P_Val (Garza & Williamson 2001). See the Supplementary Methods for a detailed explanation of these program settings.

The test for deviations from migration–drift equilibrium was based on the estimations and comparison of the relative likelihoods of "gene flow/drift" and "drift alone" models obtained with the program 2MOD (Ciofi *et al*. 1999). In the estimation of relative probabilities, this method assumes that the effect of microsatellite mutations is negligible after population divergence (Ciofi *et al*. 1999). Five independent runs of 1,000,000 MCMC iterations were executed, with the first 100,000 steps discarded as burn-in.

Estimation of gene flow

The magnitude and direction of contemporary gene flow was assessed using a non-equilibrium Bayesian method implemented in BAYESASS v.3.0.4 (Wilson and Rannala, 2003). The analysis consisted of five independent runs of 2×10^8 iterations (with a burn-in of 2×10^7 iterations) and a sampling frequency of 1000. The mixing parameters were adjusted ($\Delta_M = 0.1$; $\Delta_F = 0.5$; $\Delta_A = 0.3$) and replicate runs were performed with different starting-seed values to verify the consistency of the results. Convergence of MCMC chains was monitored and analysed with TRACER v.1.6 (Rambaut *et al.* 2014).

Correlation of genetic structure with geography

To identify potential drivers of population genetic structure, we used Mantel tests to estimate the correlation between matrices of genetic and geographic distances. Under a scenario of isolation by dispersal limitation or distance (IBD), we expect a linear increase of genetic distance with geographic distance. Isolation by distance was analysed using a Mantel test implemented in the ISOLDE extension in GENEPOP (Rousset 1997), with 10,000 permutations, to compare matrices of genetic distance expressed as $F_{ST}/(1\text{-}F_{ST})$ and geographical distance (ln[km]) among the populations previous characterised. Matrices of different genetic distances (F_{ST} and D_{est} for microsatellites, and F_{ST} for mitochondrial genes)

were compared using PASSAGE v.2.0.11.6 (Rosenberg & Anderson 2011) and the significance of the Mantel tests was assessed using 100,000 permutations.

Results

Genetic structure, demographic events and gene flow estimated from microsatellite loci

The microsatellite dataset revealed marked levels of genetic structure among chough populations across the Iberian Peninsula. The three clustering methods based on microsatellite markers revealed a consistent and marked degree of structure among chough populations. The programs differed slightly in their estimates of the optimal number of populations (*K*), but the main partitions were very consistent (Fig. 1). The genetic clusters obtained with GENELAND (*K*=7), BAPS (*K*=9) and the first level of STRUCTURE (*K*=8) clearly differentiated two population clusters in Portugal (L1 and L2-L3) and three clusters in Spain (L13, L18 and L20-L25) (Fig. 1). The remaining genetic clusters are slightly different between the three Bayesian approaches (see Supplementary Results), yet the number of clusters remains similar.

All pairwise F_{ST} and D_{est} estimates were highly significant (P<0.001) and highly correlated (r=0.772; P<0.001), with the highest values among the three Portuguese population groups (P1, P2 and P3) and moderate to high differentiation among Spanish populations (pairwise estimates for P4 to P15) (Table S1).

The results of the analysis with BOTTLENECK suggested historical population contraction events in some populations (P1, P6-P9 and P12-P15) only when assuming an infinite allele model (IAM) (Table S2). No evidence of bottlenecks was found using the two-phase model (TPM) or the stepwise mutation model (SMM), although significant values for

heterozygote deficiency were obtained for one population (P1) using TPM, and three populations (P2, P12, P15) using SMM (Table S2). The mode-shift test did not support any historical bottleneck event (Table S2). The third approach, based on an M-ratio test, was the most consistent, indicating probable population contractions (values of M<Mc) in all sites when pre-bottleneck N_e values of 50 and 500 were tested (Table S2). For higher N_e values (5000) the results did not support a recent bottleneck event in six populations (P4-P6, P8, P10 and P13) (Table S2). The test for migration-drift equilibrium implemented in the 2MOD indicated that all Iberian chough populations are in migration-drift equilibrium (100% supported).

Migration rates (*m*) among chough genetic clusters calculated with BAYESASS were generally low, with a global mean value of 0.017 (ranging from 0.003 to 0.240) for the proportion of individuals of migrant origin in each population (Table S3). The mean values of migration rates among Iberian chough populations never exceed 0.03 (Fig. S1), indicating very low levels of immigration and revealing very local breeding recruitment over recent generations.

Genetic structure and coalescence analysis using mtDNA markers

Genetic structure among populations based on mtDNA markers was expectedly less marked than that based on microsatellites, but also revealed a considerable degree of differentiation, particularly in central Iberia. Concatenated sequences of *ND2* (680 bp) and the control region (512 bp) amplified in 577 chough samples, yielded 36 haplotypes defined by 25 polymorphic sites (Table S4). The two highest-frequency haplotypes (H1 and H2) were shared by most populations and formed starlike phylogenies in the haplotype network (Fig. 2), thus representing shared ancestral polymorphism. Haplotype H12 is restricted to central Spain and Portugal, and haplotype H13 is restricted to central Spain (Fig. 2). Pairwise F_{ST}

values estimated from mtDNA data ranged from 0 to 0.945, with 79% of the estimates being significant at the 0.05 level (Table S5). A detailed characterization of mitochondrial haplotype distribution among Iberian populations is available in Supplementary Results.

Genetic diversity

All microsatellite loci were polymorphic in all populations, with a total of 176 different alleles amplified. The number of alleles per locus ranged from 9 (Ppy2P16) to 30 (Ppy2P7), with an average of 17.3 alleles. Average values of allelic richness ranged between 3.908 and 5.892 (Table S6). Mean observed heterozygosity ranged between 0.611 (Ppy51) and 0.817 (Ppy104), and the mean expected heterozygosity ranged between 0.644 (Ppy51) and 0.797 (Ppy104) (Table S6). PIC values support the high genetic diversity and informativeness of the markers with mean values ranging between 0.597 (Ppy51) and 0.755 (PpyA1.12) (Table S6). Average F_{IS} values were close to zero (Table S6) as expected in randomly mating populations.

The 37 mitochondrial haplotypes identified were defined by 26 polymorphic sites, consisting of 19 parsimony informative sites, five singleton variable sites and two insertions/deletions (Table S4). Overall mitochondrial haplotype diversity was moderate (global Hd=0.589), low in population P1 (Hd=0.083) and absent in P3 and P5 (Table S7). The average values for the number of nucleotide differences ranged from zero in P3 to 2.648 in P14 (average K=1.498), and nucleotide diversity ranged from zero in P3 to 0.0023 in P14 (average π=0.0014) (Table S7).

Dispersal movements of marked individuals

Monitoring of spatial movements of individuals revealed a very high dispersal capacity of choughs among population nuclei (Fig. 3). About nine thousand choughs were ringed in twelve population nuclei over the last 30 years (Table 2), and regular monitoring allowed the multiple re-sighting of many ringed individuals (Table 2). Choughs were frequently recorded outside their natal areas, performing long-distance movements of up to several hundred kilometres (Figs. 3 and S2, Table 2). Between 1985 and 2016, 1957 individual movements longer than 50 km were recorded, averaging 198 km. Of these, 210 movements were longer than 300 km and involved 135 individuals from seven different population nuclei (Table 2). Movement data from marked individuals is consistent with our data showing that birds from different Iberian regions form mixed feeding flocks on variable foraging substrates in agricultural and pasture land, and congregate in communal roosts, during variable time periods throughout the year (GB, unpublished data). Even in populations where sampling effort was relatively low (Table 2), we were able to detect both short and long distance movements (Figs. 3 and S2, Table 2). We have accumulated more data on movements in central Spain simply because the recording effort was much greater in this area (Fig. S2). Breeding recruitment events of individuals banded as nestlings were rare, but all of them (n =124) occurred in their natal population nuclei (Table 2).

Drivers of population genetic structure

The correlation between geographical distances and genetic differentiation (F_{ST}) measures was weak and marginally significant (r=0.266, P=0.045), and the RMA regression line for F_{ST} explained only 6.81% of the variation (Fig. S3). When the peripheral, highly differentiated and isolated populations P1 and P2 (Fig. 1) were excluded from the analysis, no significant correlation was found between geographical distance and F_{ST} (r=0.207, P=0.117).

Discussion

Our molecular data revealed marked genetic structure and highly restricted gene flow among Iberian populations of red-billed choughs despite evidence of high dispersal capacity. These results are striking given the absence of major geographic barriers and the weak correlation between genetic differentiation and geographic distances, and suggest that gene flow is not strongly influenced by the drivers typically acting in wild animal populations.

Genetic structure consisted of at least seven major genetic clusters clearly supported by three different Bayesian clustering approaches. The most geographically isolated populations were in general clearly differentiated from the remaining nuclei, as expected from their small population size (Farinha 1991; Blanco 2004). Thus, they exhibit high susceptibility to factors like genetic drift, inbreeding, demographic stochasticity and/or reduced gene flow (Fischer & Lindenmayer 2007). In spite of our extensive but still partial sampling of the extant population nuclei found across the Iberian Peninsula, the analysis of genetic structure identified as many as 15 different genetic units in an area under 580,000 km^2, revealing an unusual degree of genetic differentiation at local and regional scales. Even though choughs are frequently described as sedentary and engaging in mostly local, altitudinal, and occasionally long-distance movements (Bullock *et al*. 1983; Cramp 1988; Moore 2006), our long-term field study clearly shows that long distance movements are frequent, and that individuals from different nuclei often shared common foraging areas and roosting sites across Iberia. Indeed, non-breeding individuals seem to show a nomadic existence, regularly returning to their natal areas, but never permanently dispersing to regions other than their natal areas, engaging in wandering movements that can occur multiple times during an individual's lifetime (GB, unpublished data).

Local adaptation may have fitness costs for individuals dispersing away from their natal areas (Bonte *et al.* 2012), as individuals may have evolved phenotypic adaptations to specific local conditions that may reduce their performance elsewhere, and this may contribute to the genetic structure found. In-depth ecomorphological analyses will be needed to determine if small differences between populations exist. Moreover, given that our assessment of habitat type is qualitative, more detailed quantitative analysis of environmental variables at local scales will be needed to definitively rule out the influence of habitat differences in restricting gene flow among populations. However, fitness costs of dispersal appear to be low in choughs. Short and long distance dispersal events show no clear seasonal or altitudinal patterns and involve both non-breeding individuals (juvenile and older floaters), and breeding individuals outside the breeding season. Multiple movements of individual choughs were recorded between the regions considered for the genetic analysis, even on a daily basis, including round trips of several hundred kilometres within a week, and involving the crossing of major geographical barriers such as high mountains. The strong genetic structure is not consistent with the long-distance movements and population connectivity observed. The correlation between geographic distance and genetic differentiation was weak when all Iberian populations were considered, and non-significant after excluding the two most peripheral and isolated populations from Portugal (localities L1-L3). Geographical barriers and landscape/ecological features can be major barriers to gene flow between populations (Storfer *et al*. 2007; Sexton *et al*. 2014). However, these constraints seemed to be negligible for choughs considering their high dispersal capacity, and long-movement frequency and distance throughout Iberia during the last 30 years. To the best of our knowledge, cases of such extreme genetic structure in the absence of geographic or ecological barriers have not been previously reported in birds.

The most peripheral and isolated population nuclei showed reduced genetic diversity, as expected (Frankham 1996; Willi *et al*. 2007), yet most Iberian populations show high levels of genetic diversity. This suggests that the differentiation and structure patterns between population nuclei were caused by restricted gene flow among populations, and not by demographic events such as population bottlenecks. This is in sharp contrast with the low diversity reported for chough populations in the British Isles, which are the result of postglacial colonization from southern latitudes accentuated by the isolation of small populations (Wenzel *et al*. 2012). Overall nuclear genetic diversity of Iberian choughs is also higher than values reported for corvid species with low genetic differentiation patterns, the Clark's Nutcracker (*Nucifraga columbiana*), and fine-scale genetic structure, the New Caledonian crows (*Corvus moneduloides*) (Abdelkrim *et al.* 2012; Dohms *et al.* 2013; Rutz *et al.* 2012). The recent coalescence of Iberian haplotypes and the widespread distribution of ancestral haplotypes, indicates a young postglacial origin for the current genetic variation, coinciding with the concomitant role of the Iberian Peninsula as a major refugium for Palearctic vertebrates (Gómez & Lunt 2007; Ferrero *et al*. 2011; Abellán & Svenning 2014).

The extreme level of genetic structure in the red-billed chough suggests a strongly structured social organization with different social and behavioural identities. The most similar case of genetic differentiation was reported for humans (Rosenberg *et al*. 2002; Tishkoff *et al.* 2009), and was attributed to socio-cultural factors (Ségurel *et al*. 2008; Tishkoff *et al*. 2009; Ross *et al*. 2013). Socio-cultural features in non-human animal species are more difficult to document, but there is evidence that the influence of cultural inheritance on gene flow between primates and cetacean populations can lead to marked population differentiation and complex patterns of genetic structure (Kopp *et al*. 2014; Kopps *et al*. 2014; Foote *et al*. 2016). Geographical and ecological barriers to dispersal capacity, such as rivers,

mountains and anthropogenic habitat fragmentation for primates, or continental masses for cetaceans, can influence gene flow and genetic differentiation patterns between populations. However, in most cases, the complex genetic structure cannot be explained simply by geographical barriers (reviewed in Andrews 2014).

Corvids are known for their high cognitive capacity and several studies on social learning have been conducted (Clayton & Emery 2007). Previous studies have reported a likely association of tool use in Caledonian Crows with fine-scale genetic structure across a few kilometres (Abdelkrim *et al*. 2012; Rutz *et al*. 2012). Choughs also show remarkably complex social and familiar interactions (Bignal *et al*. 1997; Blanco & Tella 1999), including a versatile repertoire of foraging habits, requiring learning from conspecifics (Bignal *et al*. 1997; variable nesting systems and communal roosting patterns involving complex hierarchical, social and ritualized interactions among group members (Still *et al*. 1987; Blanco *et al*. 1997; Blanco & Tella 1999); high mate and nest-site fidelity (Banda & Blanco, 2014); and complex vocalizations (Laiolo *et al*. 2000, 2001), all of which may potentially influence genetic differentiation of the different population nuclei by cultural evolution.

Dispersal patterns of highly social species are frequently influenced by socio-cultural features (Andrews 2014). Restricted dispersal due to social barriers has been reported to influence fine-scale spatial genetic structure in the unrelated white-winged chough, *Corcorax melanorhamphos* (Beck *et al*. 2008). In contrast, while Iberian red-billed choughs show high dispersal capacity, recruitment appears to happen only at natal areas. This level of strict natal philopatry can contribute to genetic differentiation (Nyholm 1986; Weatherhead & Forbes 1994), but it fails to explain the lack of isolation by distance within the continuous distribution range of the Iberian chough. Genetic structure at large scales in bird species with

moderate-to-high dispersal capacity is often significantly associated with a pattern of isolation by distance (Agudo *et al*. 2011; Mira *et al*. 2013; Graciá *et al*. 2015; Pellegrino *et al*. 2015), and a lack of isolation by distance is generally due to highly fragmented and isolated small populations (Agudo *et al*. 2011). The extreme level of genetic structure of Iberian choughs is unlike any case reported for birds that are unconstrained by geographical or ecological barriers at regional scales. Natal-range philopatry or philopatry to particular habitat types and/or social structure features, such as strong group stability and sex-biased dispersal, can explain the pronounced genetic structure observed in several species (Andrews 2014). Furthermore, cultural factors like ethnolinguistic boundaries and traditional social practises are also important factors regulating gene flow in human populations (Tishkoff *et al*. 2009; Ross *et al*. 2013). There is also increasing evidence that a combination of genetically and culturally inherited evolutionary changes can drive genetic differentiation in wild species with pronounced social features (Langergraber *et al*. 2011; Rutz *et al*. 2012; Kopps *et al*. 2014; Foote *et al*. 2016). In light of this evidence, we propose a mechanism of "isolation by social barriers" to explain evolutionary divergence, partly induced by non-random patterns of pairing and recruitment, deserving of future research.

Conclusion

Our long-term study demonstrates that choughs engage in regular long-distance movements and interact regularly with individuals of different population nuclei across Iberia. Given this high dispersal capacity, the marked genetic structure observed is unprecedented, and unlikely to be due solely to patterns of isolation by distance, colonization or adaptation. To our knowledge, the complex genetic structure, described here for the first time for the Iberian choughs, is unique among birds, and only comparable to that reported for humans, some wild non-human primates, and cetaceans. Our findings suggest that complex patterns of social

interactions may be responsible for genetic differentiation. Forthcoming approaches aimed at identifying the proximate mechanisms driving this strong genetic structure are needed, including the potential existence of social group identity and within-group reproductive skew. There is also a need to expand our knowledge on other highly complex vertebrate societies, reviewing the main hypotheses on genetic structuring of wild populations, where phenomena of isolation by social barriers must also be considered.

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Data Accessibility

The field monitoring data and microsatellite allele datasets have been archived in the Dryad repository (doi: 10.5061/dryad.684v0). Mitochondrial DNA sequences are available from GenBank (accessions KX024343-KX024378 and KX024396-KX024431).

Author contributions

F.M., J.A.D., B.M. and G.B. designed the study; J.A.C., E.B. and G.B. coordinated the study; F.M., J.A.C., O.F., J.L.G., P.T., D.C. and G.B. collected biological samples; O.F., J.L.G., and G.B. conducted the bulk of the long-term population banding and monitoring; F.M. and J.A.D. performed the laboratory analyses; F.M., J.A.D., B.M. and G.B. analysed the data and contributed to the interpretation of the results; F.M., B.M. and G.B. wrote the manuscript. All authors read and approved the submitted version.

Conflicts of interest

The authors declare no conflicts of interest.

Tables and Figures

Table 1 Sampling localities, habitat variables, sample sizes, and genetic diversity indices based on individual genotypes from microsatellite loci. N_A – mean allele number per locus, H_O - observed heterozygosity, H_E - expected heterozygosity, with standard deviation (SD) shown in parentheses.

Country	Area	Locality	Dominat habitat[a]	Nest sites[b]	Altitude range[c]	Genetic cluster	Sample size (mtDNA)[d]	Sample size (microsat)[d]	N_A	H_O	H_E
Portugal	Sagres	L1	A,B,C	A	0-200	P1	24	24	4.50 (0.48)	0.62 (0.05)	0.60 (0.04)
Portugal	Porto de Mós	L2	A,C	B	200-600	P2	25	25	5.10 (0.38)	0.63 (0.06)	0.62 (0.04)
Portugal	Serra da Estrela	L3	A,C	B	500-2000	P2	13	13	5.10 (0.57)	0.54 (0.07)	0.59 (0.06)
Portugal	Barroso	L4	A,C	B	700-1300	P3	6	6	3.60 (0.40)	0.55 (0.09)	0.57 (0.05)
Spain	Enciña da Lastra	L5	A,C	B	500-900	P4	10	10	6.00 (0.52)	0.76 (0.07)	0.72 (0.03)
Spain	Vilarín	L6	A,C	B	600-900	P4	19	19	7.00 (0.54)	0.74 (0.05)	0.74 (0.03)
Spain	Riodeporcos	L7	A,C	B	200-600	P4	16	16	7.30 (0.65)	0.71 (0.03)	0.73 (0.04)
Spain	Costa da Morte	L8	A	A	0-200	P5	14	14	5.10 (0.55)	0.65 (0.04)	0.63 (0.04)
Spain	Asturias	L9	A	B	500-2000	P6	40	38	9.30 (0.99)	0.74 (0.03)	0.79 (0.02)
Spain	Urbasa	L10	A	B	600-1000	P7	37	39	9.70 (0.98)	0.75 (0.02)	0.79 (0.03)
Spain	Los Monegros	L11	B,C	C	200-500	P8	35	38	10.30 (0.76)	0.74 (0.05)	0.79 (0.04)
Spain	Teruel	L12	B,C	C	1000-1200	?	24	24	9.80 (0.79)	0.73 (0.04)	0.79 (0.02)
Spain	Albacete	L13	B,C	B	500-700	P9	9	14	5.80 (0.55)	0.68 (0.04)	0.70 (0.02)
Spain	Murcia	L14	B,C	B,C	300-1200	P10	24	24	9.80 (0.80)	0.80 (0.03)	0.81 (0.02)
Spain	Granada	L15	A,B,C	B,C	1000-2000	P10	23	23	9.00 (0.68)	0.85 (0.04)	0.77 (0.03)
Spain	Málaga	L16	B,C	B	500-800	P11	35	42	10.30 (1.22)	0.72 (0.04)	0.75 (0.04)
Spain	Ciudad Real	L17	B,C	B,C	500-700	?	15	9	5.60 (0.48)	0.81 (0.05)	0.72 (0.04)
Spain	Hornachos	L18	A,B,C	B	400-900	P12	17	17	6.20 (0.51)	0.72 (0.04)	0.70 (0.03)
Spain	Castuera	L19	A,B,C	B,C	300-600	?	9	9	6.90 (0.64)	0.81 (0.04)	0.79 (0.02)
Spain	Guadalajara	L20	B,C	B	900-1200	P13	14	17	8.40 (0.50)	0.85 (0.03)	0.80 (0.02)
Spain	Río Lobos	L21	B,C	B	1000-1200	P14	15	15	7.20 (0.49)	0.80 (0.02)	0.75 (0.02)
Spain	Azálvaro	L22	A,C	C	1200-1600	P14	23	23	8.30 (0.60)	0.80 (0.04)	0.79 (0.02)
Spain	Segovia	L23	A,B,C	B,C	1000-1400	P14	17	18	7.70 (0.65)	0.82 (0.02)	0.78 (0.02)
Spain	Madrid west	L24	B,C	B,C	500-700	P15	57	56	9.80 (1.15)	0.74 (0.05)	0.77 (0.03)
Spain	Madrid east	L25	B,C	B,C	600-800	P15	56	57	10.60 (0.81)	0.80 (0.04)	0.78 (0.03)
	TOTAL						577	590			

[a] **(**A**)** Grassland ; (B) agricultural areas (cereal crops); (C) short scrubland.
[b] (A) Crevices and caves (sea cliff); (B) crevice and caves (inland); (C) human-made structures.
[c] Aproximate minimum and maximum altitudes of the areas used by each population.
[d] Number of samples with successful PCR amplification for mitochondrial DNA and microsatellites without full-sibs.

Table 2 Results of the mark-recapture and field monitoring of 12 chough population nuclei in the Iberian Peninsula.

Location	Marked individuals		Recaptured/resighted		Nestlings recruited[b]	Number of movement records[c] 50-300/>300 km (mean distance, km)	Monitoring period
	nestlings	fully-grown adults	individuals	records			
Porto de mós (L2)	29	0	3	7	-	0/6 (424)	2012-2015
Barroso (L4)	3	0	3	3	-	1/0 (84)	2012-2014
Los Monegros (L11)	3322	3133	1715	4242	93	443/188 (176)	1985-2016
Teruel (L12)	21	45	27	30	-	10/0 (183)	2011-2015
Murcia (L14)	9	1	1	2	-	2/0 (126)	2014-2015
Granada (L15)	19	0	3	4	-	0/4 (410)	2014-2015
Ciudad Real (L17)	100	16	39	98	-	21/1 (177)	2011-2015
Azálvaro (L22)	326	49	143	799	6	49/1 (123)	1993-2016
Segovia (L23)	154	1	76	480	2	179/8 (108)	2011-2016
Madrid (L24 and L25)	915	602	734	5269	23	1041/2 (99)	1988-2016
Navarra[a]	127	117	9	55	-	1/0 (270)	2014-2016
TOTAL	5025	3964	2753	10253	124	1747/210 (198)	1985-2016

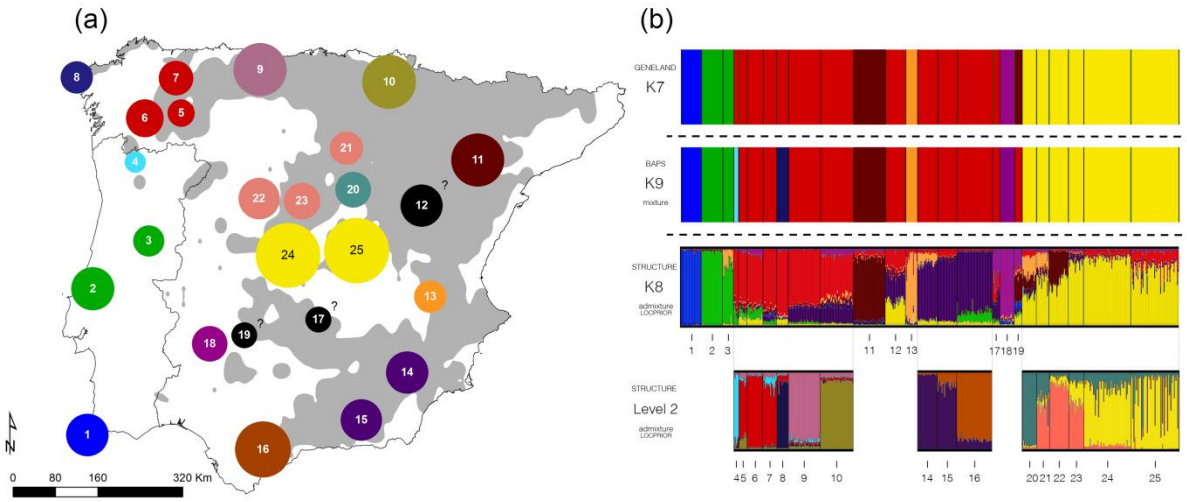
[a] Population recently monitored and not included in the genetic analysis.
[b] Nesting recruited as breeders in their natal areas; no individual was found recruited outside its natal area.
[c] Frequencies of movements longer than 50 km from the natal areas to recapture/resighting localities from a subsample of the records for which the distance of movements was calculated (n = 9862).

Figure legends

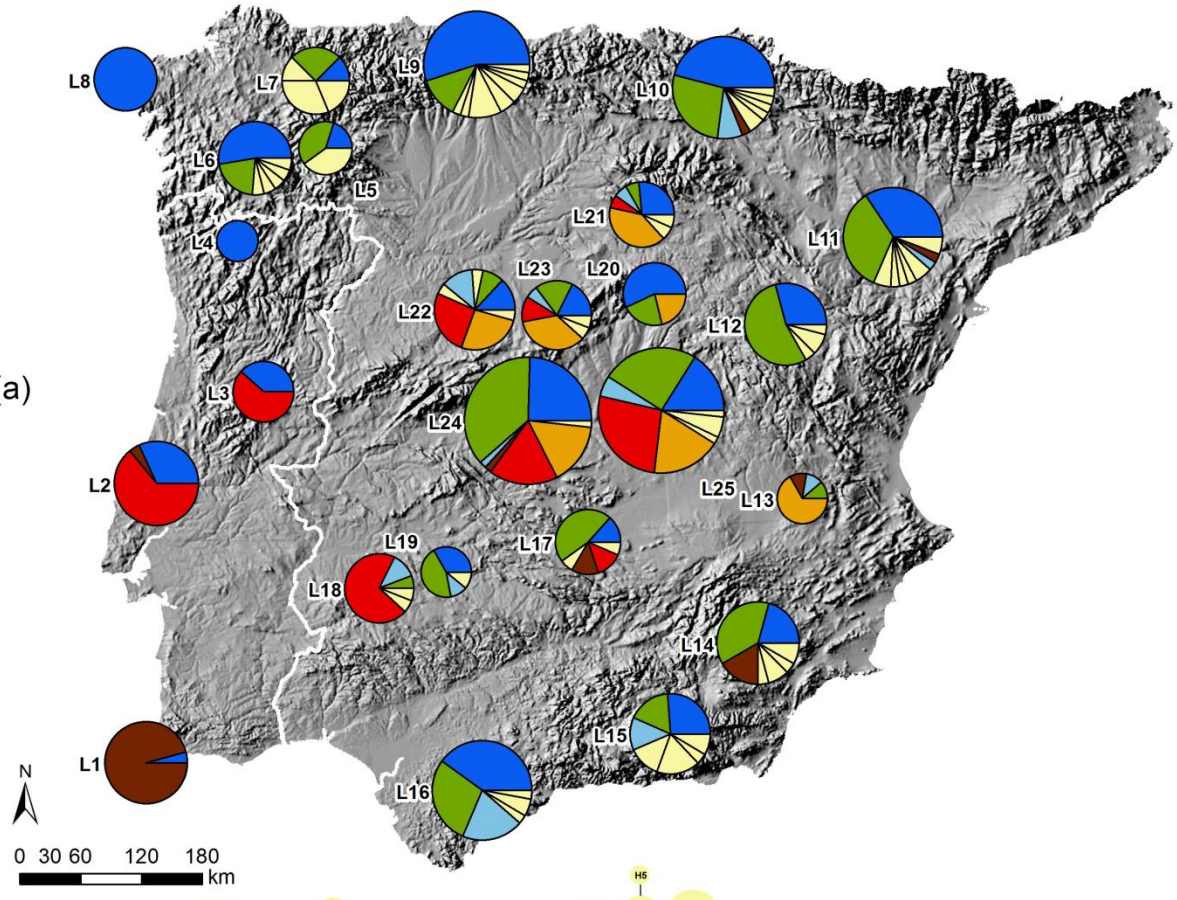
Fig. 1 Genetic structure among Iberian populations of the red-billed chough. (*a*) Map of the red-billed chough distribution range (grey area) and sampling localities (numbered circles, with circle size proportional to sample size, see Table 1). Colours correspond to genetic clusters in (*b*). (*b*) Results of the analysis of genetic structure inferred from microsatellite data using GENELAND, BAPS and STRUCTURE. Vertical bars correspond to individual choughs and colours represent the posterior probability of assignment to each of an optimal number of clusters estimated by each program (K=7 for GENELAND, K=9 for BAPS, and K=8 for STRUCTURE).

Fig. 2 Geographical distribution (*a*) and median-joining network (*b*) of the concatenated mtDNA sequences. Pie charts in the map indicate the frequency of each haplotype, with colours corresponding to those in the haplotype network. The size of each pie chart is proportional to sample size (see Table 1). The network includes the 37 haplotypes identified, highlighting the six most common haplotypes with different colours; all haplotypes are separated by a single nucleotide change, and circle sizes are proportional to haplotype frequencies.

Fig. 3 Dispersal capacity of Iberian red-billed choughs. (*a*) Dispersal range of 12 chough population nuclei. Lines represent individual movements from population nuclei (indicated by different colours), to the resighting/recapture site. Each line represents single or multiple movements of the same or different individuals. Only movements longer than 50 km are shown. See Fig. S2 for the exact location of individual records. (*b*) Examples of movements recorded for three individual choughs (band numbers 1N4, H1L and 6FR) from three different population nuclei.



(a)



(b)