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ORIGINAL PAPER

Genetic structure and diversity of breeding Montagu's harrier (*Circus pygargus*) in Europe

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Abstract The Montagu's harrier (*Circus pygargus*) is a long-distance migratory raptor, breeding in Europe and Western Asia and wintering in sub-Saharan Africa. The population of the species has declined in Europe during the twentieth century, and Montagu's harrier is red-listed in many European countries as declining or threatened. The main aims of the study were to evaluate the genetic diversity of European breeding populations and estimate the genetic differentiation among them, using polymorphism in the hypervariable domain of the mitochondrial control region. We analysed 158 individuals from central Spain, Germany, the Czech Republic and Poland. The results indicated high genetic diversity in the European breeding population, probably reflecting the large population size of the species. However, we found decreased genetic variability in the breeding population of Germany. Among the 18 identified haplotypes, 2 were of high frequency. There was no clear connection between the position of the haplotype in the genealogy and its geographical distribution. Genetic structure was weakly pronounced (H_{ST}=0.053, P<0.001). SAMOVA indicated the presence of three genetic groups: The first group consisted of samples from central Spain and northeastern Poland, the second from southern Poland and the Czech Republic, and the third group separated samples from Germany from the other regions. Genetic differentiation between pairs of groups was low, suggesting a low level of philopatry and a high dispersal ability of Montagu's harrier.

Keywords Montagu's harrier · *Circus pygargus* · Accipitride · Control region · mtDNA · Population genetics

Introduction

There are many factors influencing the geographic distribution of intraspecific genetic diversity, including genetic drift, selection, mutation, species-specific dispersal ability accompanied by gene flow, and the interaction of these factors with histor-

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ical processes, such as past demographic fluctuations and glaciations (Taberlet et al. 1998; Hewitt 2004; Bazin et al. 2006; Gaggiotti et al. 2009; Leffler et al. 2012). Simultaneously, the identification and understanding of processes that influence genetic diversity in wild populations and the genetic differentiation among them are extremely important, because preserving an appropriate gene pool is a fundamental objective of wildlife conservation and management (Crozier 1997; Reed and Frankham 2003; Spielman et al. 2004; Allentoft and O'Brien 2010).

Early studies of wild animals suggested that genetic differentiation among populations of birds is generally low (Avise and Aquadro 1982; Barrowclough 1983). This general trend was ascribed mainly to high mobility, assuring high levels of gene flow (Barrowclough 1983; Crochet 2000). However, consistent accumulation of genetic data has revealed more and more examples of bird species with substantial differentiation (e.g., Rhodes et al. 1993; Mundy et al. 1997; Lee et al. 2001; Caizergues et al. 2003; Rutkowski et al. 2012).

Raptors of the Accipitridae family—mainly large, highly mobile and wide-ranging birds—usually present a low level of genetic structuring. High movement capacity, especially natal dispersal (Greenwood and Harvey 1982), allows subpopulations separated by hundreds or thousands of kilometres to be genetically connected (Kretzmann et al. 2003; Martínez-Cruz et al. 2004; Sonsthagen et al. 2004; Cadahía et al. 2007). In such cases, the pattern of differentiation is sometimes shaped by isolation-by-distance, reflecting dispersal capabilities (e.g., Mira et al. 2013). Additionally, environmental variability in temperate regions might have homogenized the genetic pool through increased dispersal and the migratory behaviour of raptors (Newton 2003; Sonsthagen et al. 2004). However, historical processes, such as isolation in glacial refugia (de Volo et al. 2013), behavioural characteristics, such as philopatry (Godoy et al. 2004) or different migratory routes (Hull and Girman 2005), geographical barriers (Sonsthagen et al. 2012), isolation on islands and adaptation to island habitats (Kretzmann et al. 2003) may all have induced the appearance of significant genetic differentiation.

Montagu's harrier (*Circus pygargus*) is a long-distance migratory raptor species, wintering in sub-Saharan Africa and the Indian subcontinent, and breeding over large areas within Europe and Western Asia (Cramp and Simmons 1980; Clarke 1996). The migratory routes of the species have been thoroughly studied (Clarke 1996; Agostini and Logozzo 1997; García and Arroyo 1998; Trierweiler et al. 2014). A recent study using satellite telemetry identified three autumn migration routes for northern European harriers: via Spain, Italy and Greece (Trierweiler et al. 2014). The authors also indicated that circular migration is rather interlinked with a clockwise loop migration of birds from eastern European breeding areas, whereas western harriers tend to travel through more western routes. The level of philopatry in the species seems to be low and independent of the sex of the birds (Limiñana et al. 2012), while wintering areas and migration routes of harriers from different breeding populations could overlap (Trierweiler et al. 2014). A phylogeographical study based on mitochondrial DNA indicated no significant differentiation when birds were divided according to migratory routes, although low but significant genetic differentiation was found between groups of populations breeding in southwestern and northeastern Europe (García et al. 2011; Rutkowski et al. 2014).

For many years, Montagu's harrier has been a rare example of a bird exhibiting substantial differences in nesting habitat between eastern and western Europe—in the eastern part of the continent, the majority of breeding pairs gathered around vast marshlands, whereas in western Europe, significantly more pairs nested in crop fields (Arroyo et al. 2002). However, during the mid-1990s, the proportion of birds nesting in crops in central and eastern Europe systematically decreased, with a simultaneous reduction of breeding pairs in wetland areas (marshes and river valleys) (Mrlík et al. 2002; Krupiński et al. 2012).

In general, the population of Montagu's harrier has declined in central Europe during the twentieth century as a consequence of extensive habitat destruction and human persecution (Clarke 1996; Krogulec 1997). Since then, the species has been red-listed in many European countries as declining or threatened. The population from the Iberian Peninsula constitutes the western European stronghold of the species (García et al. 2011), whereas in central and eastern Europe, a significant decrease in the number of birds and size of populations was observed during the 1980s (Clarke 1996; Tomiałojć and Stawarczyk 2003). Since then, at least in some countries (e.g. Poland), the number of birds seems to have systematically increased (Tomiałojć and Stawarczyk 2003). However, present trends in population size are difficult to determine.

Biological, behavioural and demographic processes that have had an impact on the European population of Montagu's harrier should influence the distribution of genetic diversity within the species. Differences in migration patterns and nesting habitats could induce the appearance of genetic structure, while different demographic trends could alter the level of genetic variability within some local populations. Although previous studies suggested a rather low level of differentiation among local populations from Europe and a lack of differences in genetic variability, they were based on mitochondrial genes (García et al. 2011) and cross-amplified microsatellites (Rutkowski et al. 2014), which could have presented excessively low polymorphism to efficiently reflect the population's genetic structure. Hence, we aimed to supplement previous data by using polymorphisms in the hypervariable domain of the mitochondrial control region. Specifically, we focused on (i) evaluating the genetic diversity of breeding Montagu's harriers, (ii) estimating the genetic differentiation among some

European breeding areas and (iii) analysing whether recent changes in population sizes are reflected in the pattern of polymorphism of the mitochondrial DNA (mtDNA) control region.

Material and methods

We collected feathers of Montagu's harriers in five areas of Central Europe: Germany (Brandenburg, denoted as GER-B, n=27), the Czech Republic (Moravia, denoted as CZR-M, n=37), Lower Silesia in southern Poland (denoted as POL-S, n=15), the southern part of the Podlasie region of eastern Poland (denoted as POL-SP, n=12), Northern Mazovia and the northern part of the Podlasie region in northeastern Poland (denoted as POL-NMP, n=21), and central Spain (the area surrounding Madrid and Guadalajara Province, n=46) (Fig. 1). In Poland, molted feathers were collected around the nest during ringing activities and nest monitoring, performed as a part of several projects to conserve the species (www.pygargus.pl) in 2007–2011. From each nest or its surrounding area, only one feather was genetically analysed; hence, it can be assumed that only samples from unrelated individuals were included in the genetic studies. Samples from Spain, Germany and the Czech Republic were delivered by local ornithologists. Feathers in Spain were collected in 2010, in Germany in 2009 and 2011, and in the Czech Republic in 2010 and 2011. Detailed data about samples are presented in Appendix 1. The feathers were stored in separate vials, either dry or in 96 % alcohol. DNA extraction was performed using QIAamp DNA Mini Kit (Qiagen, Hilden, Germany) as previously described (Rutkowski et al. 2010).

A fragment of mitochondrial DNA encompassing the positions of 1103 to 1937 of the complete mitochondrial genome of *Accipiter gentilis* (accession number NC_011818) was amplified using primers CIR_CRFm (5′-CACTAACCGGAGCCCTAGAA-3′) and CIR_CRR (5′-CATATGTGAGGGCCACCTGT-3′). Primers were designed using PRIMER3 (Rozen and Skaletsky 2000), based on complete sequences of several mitochondrial genomes of the Accipitrinae subfamily available in GenBank. The forward primer was complementary to part of cytochrome b (positions 1103–1122), and the reverse primer was located within the control region. Amplification conditions were as follows: initial denaturation of 5 min at 95 °C, 35 cycles of 30 s at 95 °C, 30 s at 59 °C, 30 s at 72 °C, and the final extension step of 5 min at 72 °C. The final amplification volume of 15 μl contained 7.5 μl Bio Mix (BioLine) and 0.2 μM of each primer. The cleanup reaction of PCR products before sequencing was performed with 1 U FastAP™ (Thermo Scientific) and 10 U Exo I (Thermo Scientific). The reaction's mixture was incubated at 37 °C for 15 min and stopped by heating at 80 °C for 15 min. The sequencing of PCR products was performed by Oligo.pl (Oligo, IBB, Warsaw, Poland). DNA sequence

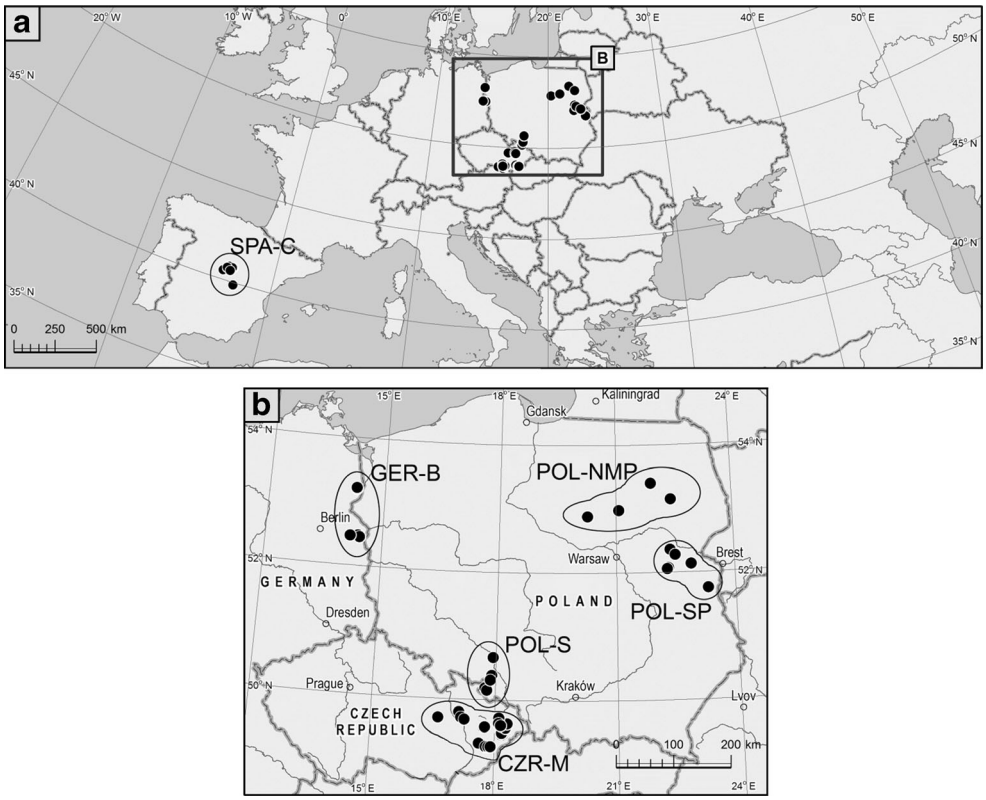

Fig. 1 Distribution of sampling sites and grouping of sampling sites into geographical regions. **a** The overall picture; **b** central Europe. *SPA-C* Spain (central Spain, Madrid surroundings and Guadalajara Province), *GER-B* Germany (Brandenburg), *CZR-M* Czech Republic (Moravia), *POL-S* southern Poland (Lower Silesia), *POL-SP* Poland, southern part of the Podlasie region, *POL-NMP* Poland, northern Mazovia and northern part of the Podlasie region

chromatograms were analysed using FinchTV v.1.4.0 (Applied Biosystems). The sequences obtained in this study were aligned in BioEdit software v.7.0.5.3 (Hall 1999), and alignments were checked manually.

Although the designed primers amplified a fragment of about 800 base pairs in length, only 534 base pairs were clearly readable. The sequences were aligned with the complete mitochondrial genome of *Accipiter gentilis* (accession number NC_011818), indicating that the analysed fragment represented the 5′ end of the mitochondrial control region and corresponds to positions 1.288–1.823.

Data analysis

The number of haplotypes, haplotype diversity, nucleotide diversity and mean number of nucleotide differences among the haplotypes in the total sample were calculated using DNAsp 5.10 (Librado and Rozas 2009). Then, we used SAMOVA 2.0 (Dupanloup et al. 2002) to perform a spatial analysis of the molecular variance in order to determine how well the geographic regions correspond to the genetic groups. This software defines groups of geographically homogenous populations and maximizes the proportion of genetic variance due to differences between populations (F_{CT}) based on the number of groups (K) defined a priori through simulated annealing procedures. We ran SAMOVA using 100 simulated annealing procedures for K values from 2 to 5. The analysis was performed with a constraint about the geographic composition of the groups. The most likely number of groups was selected based on the highest significant F_{CT} value.

Next, we estimated the number of haplotypes, haplotype diversity, nucleotide diversity and mean number of nucleotide differences among the haplotypes (k) for each genetic group, indicated by SAMOVA. ARLEQUIN v3.5.1.2 (Excoffier and Lischer 2010) was used to identify unique haplotypes. Haplotype richness—the number of haplotypes corrected for sample size—was estimated by rarefaction using the Contrib 1.02 program (Petit et al. 1998). We used a rarefaction size of 25 individuals. Differences in nucleotide and haplotype diversity between pairs of genetic groups were tested with the t test in PAST (Hammer et al. 2001), using mean and standard deviation values from DNAsp and Arlequin.

A median-joining haplotype network (Bandelt et al. 1999) was constructed in NETWORK v4.6.1.1. (Fluxus Technology Ltd.).

We calculated the pairwise F_{ST} among genetic groups using both haplotype frequencies and distance among haplotypes. We applied the Kimura two-parameter model of nucleotide substitution (Kimura 1980). The test for significance was performed with 1000 permutations. The global genetic structure based on haplotype frequencies was also estimated using H_{ST} (Hudson et al. 1992; Eq. 2) in DNAsp. Significance for global estimate was determined by permutation test, using 1000 replicates.

The demographic history of each genetic group was investigated by applying two basic neutrality tests to detect past population expansion: Fu's F_S, and Tajima's D tests. Both tests use the infinite site model without recombination to test for departures from selective neutrality and population equilibrium for intraspecific data. Fu's F_S, Tajima's D and related statistics show signals of an excess of rare mutations when the values are negative and significantly differ from zero. Fu's F_S uses information from the haplotype distribution and has greater statistical power to detect population expansion than other available tests (Fu 1997). Low F_S values indicate an excess of single substitutions usually due to expansion. Tajima's D test compares the number of nucleotide differences between sequences and the number of differences between segregating sites. Population expansion will result in a significant negative departure from zero (Tajima 1989). The significance of both values was determined by a coalescent simulation, using 1000 replicates in DNAsp. Because Fu (1997) showed that in the case of F_S, a significance of 0.02 was equivalent to a 0.05 level, the test was considered significant when $P \leq 0.02$. Additionally, Fu and Li's D^* and F^* statistics were used to test for the confounding effect of background selection. Significant values are associated with selection (Fu and Li 1993). Comparing F_S, D^* and F^* can permit population expansion to be discriminated from background selection. A significant F_S with non-significant D^* and F^* supports an interpretation of population expansion, whereas the reverse indicates selection (Fu 1997).

Additionally, we performed a Bayesian Skyline plot (BSP) reconstruction of the changes in the size of the Montagu's harrier population through time using BEAST v1.8.0 (Drummond et al. 2012). For this analysis, we pooled all 158 samples from all European locations. We used the HKY+G substitution model chosen according to the Bayesian information criterion using ModelGenerator v0.85 (Keane et al. 2006). For BEAST analysis, we used a piecewise-linear Skyline model with six groups. The per-lineage substitution rate was fixed to 7.4 % Ma^{-1} [half of the divergence rate, reported for domain I of the control region (Wenink et al. 1996) and a generation time of 4 years]. The MCMC chain was run for 100 million steps with samples taken every 10,000th step, and the first 10 % of the samples were discarded as burn-in. We checked the support for the Bayesian Skyline by rerunning the analysis using a constant population size model. Bayes factor analysis was performed by the estimation and comparison of marginal likelihoods for both models using stepping-stone and path sampling protocols (Baele et al. 2012, 2013). A separate BSP reconstruction using the same settings was also performed for each of the three groupings indicated by the SAMOVA results.

Results

We analysed a 534 base pair long fragment of the control region for 158 Montagu's harriers. We found 12 polymorphic sites within this fragment (10 parsimony-informative sites). All variable sites represented transitions, except for one transversion. The base composition of the Montagu's harrier control region was similar to that of other bird species (Baker and Marshall 1997; Ruokonen and Kvist 2002): A, 29.02 %; C, 25.26 %; T, 30.55 %; and G, 15.18 %. Among 158 obtained sequences, we identified 18 haplotypes (GenBank Acc. no: KJ934201–KJ934218). Overall haplotype diversity was 0.820±0.020 with an associated nucleotide diversity of 0.00269±0.0001. The average number of nucleotide differences among haplotypes was low (k=1.434, Table 1), indicating that in the majority of cases, pairs of haplotypes differed by a single substitution (also Fig. 2).

Only 3 out of 18 identified haplotypes had a total frequency higher than 10 % (Table 2).

There was no clear association between the position of a unique haplotype in the median-joining network and its geographic distribution (Fig. 2). For example, haplotypes originating from H2 (one of the three 'core haplotypes') were found in SPA-C, GER-B, CZR-M and POL-S.

The highest and only significant F_{CT} value in SAMOVA was observed under the K=3 model (F_{CT}=0.117; P=0.022), placing the geographic regions of SPA-C, POL-NMP and POL-SP (denoted as SPA-C&POL-NMP&POL-SP) into the first genetic group, CZR-M and POL-S (denoted as CZR-M&POL-S) into the second group and separating GER-B from the other regions. The number of haplotypes was similar in the first and second group, but haplotype diversity and nucleotide diversity were higher (although not significantly) in the CZR-M&POL-S group (Table 1).

The global genetic structure was weak but significant (H_{ST}=0.053, P<0.001). The genetic differentiation among genetic groups indicated a low but significant F_{ST} between CR-M&POL-S and SPA-C&POL-NMP&POL-SP (F_{ST}= 0.08 and 0.06 for haplotype frequencies and genetic distance respectively, P<0.05) and was clearly higher when comparing these groups with GER-B (CR-M&POL-S, F_{ST}=0.152 and 0.144 for haplotype frequencies and genetic distance respectively, P<0.05; SPA-C&POL-NMP&POL-SP, F_{ST}=0.158 and 0.083 for haplotype frequencies and genetic distance respectively, P<0.05).

Our results indicated that Tajima's D did not deviate significantly from neutrality (Table 3). Significant F_S values (Table 3) indicating a recent expansion were found in SPA-C&POL-NMP&POL-SP and CR-M&POL-S genetic groups and in the total sample. Non-significant values of F* and D* indicated that selection has not influenced the distribution of genetic diversity within the analysed mtDNA region. In GER-B, we found a relatively high and positive value of D, as well as a positive F_S, suggesting that this population may have suffered a recent bottleneck or is presently decreasing.

For the BEAST analysis of all samples pooled, Bayesian Skyline was strongly supported over the constant population size model with ln Bayes factors of 10.7 and 11.1 for path sampling and stepping-stone protocols, respectively. The Bayesian Skyline reconstruction showed no indication of population size decrease over the last almost 8000 years (Fig. 3), with a median effective population size at present estimated at around 31,600 females, although with wide credibility intervals (95 % HPD, 260–250,000 females). Separate BSP reconstructions for groupings resulting from the SAMOVA analysis also did not exhibit any significant signal of changes in population size (Fig. 3).

Discussion

In analysing single fragment of mitochondrial DNA (part of the control region), we found high genetic diversity of the Montagu's harrier in Europe. The haplotype and nucleotide diversity was slightly higher than that found previously in COI and ND1 mtDNA genes across the European breeding range of Montagu's harrier in a study comprising a broader geographic range (García et al. 2011). The authors reported

Table 1 Sample size and genetic characteristics of mtDNA CR polymorphism in Montagu's harrier in genetic groups indicated by SAMOVA and total sample

	N	h	h [25]	U	H [SD]	π [SD]	k
SPA-C& POL-NMP&POL-SP	79	11	6.631	7	0.770 [0.037]	0.00225 [0.0002]	1.200
CZR-M& POL-S	52	10	6.609	5	0.823 [0.031]	0.00249 [0.0002]	1.328
GER-B	27	4	3.000	1	0.707 [0.054]	0.00209 [0.0003]	1.664
Total	158	18		13	0.820 [0.020]	0.00269 [0.0001]	1.434

N sample size, h number of identified haplotypes, h [25] haplotype richness, calculated from a sub-sample of 25 individuals, U number of unique haplotypes, H [SD] haplotype diversity and corresponding standard deviation, π [SD] nucleotide diversity and corresponding standard deviation, k average number of pairwise nucleotide differences, *SPA-C* Spain (central Spain, Madrid surroundings and Guadalajara Province, n=46), *GER-B* Germany (Brandenburg, n=27), *CZR-M* Czech Republic (Moravia, n=37), *POL-S* southern Poland (Lower Silesia, n=15), *POL-SP* Poland, southern part of the Podlasie region (n=12), *POL-NMP* Poland, northern Mazovia and northern part of the Podlasie region (n=21)

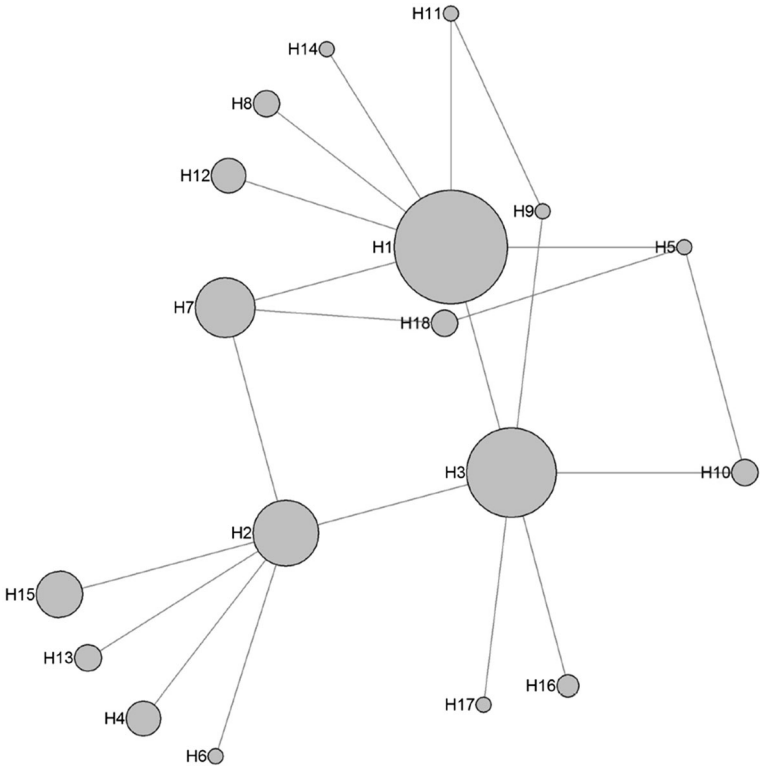

Fig. 2 Median-joining network of haplotypes. The size of a circle is proportional to the frequency of a haplotype in the total sample. *Each line* connecting *circles* represents a single mutation

haplotype diversity of 0.663 and nucleotide diversity of 0.0008, compared to the values of 0.82 and 0.0027 obtained in the present study. However, we analysed fragments of hypervariable domains I and II of the non-coding mitochondrial control region, which usually shows higher polymorphisms than other mitochondrial genes (Ruokonen and Kvist 2002).

Table 2 Distribution of Montagu's harrier haplotypes in the genetic groups, indicated by SAMOVA

	SPA-C& POL-NMP&SP	CZR-M& POL-S	GER-B	Total
H1	0.418	0.154	0.444	0.335
H2	0.051	0.192	0.148	0.114
H3	0.203	0.327		0.209
H4		0.096		0.032
H5		0.019		0.006
H6		0.019		0.006
H7	0.114	0.115		0.095
H8	0.038			0.019
H9	0.013			0.006
H10	0.038			0.019
H11	0.013			0.006
H12	0.063			0.032
H13	0.038			0.019
H14	0.013			0.006
H15		0.019	0.296	0.057
H16		0.038		0.013
H17		0.019		0.006
H18			0.111	0.019

Frequency in the group and total frequencies are reported

No polymorphisms were found in the mitochondrial ATP6 and CO3 sequences of the endemic and rare Black harrier (*Circus maurus*) of South Africa (Fuchs et al. 2014). However, these mitochondrial genes have a considerably lower mutation rate than the control region; hence, it is hard to compare the results of these studies. Haplotype diversity seems to be higher for Montagu's harrier than for some of the other European Accipitridae that had their hypervariable domains of the control region analysed: Spanish imperial eagle *Aquila adalberti* (Martínez-Cruz et al. 2004), white-tailed eagle *Haliaeetus albicilla* (Hailer et al. 2007; Honnen et al. 2010; Langguth et al. 2013; Ponnikas et al. 2013), Bonelli's eagle *Hieraaetus fasciatus* (Cadahía et al. 2007), bearded vulture *Gypaetus barbatus* (Godoy et al. 2004; García et al. 2012) and red kite *Milvus milvus* (Roques and Negro 2005). However, these studies used shorter control region fragments than our study to estimate diversity. We can assume that the large size (around 50,000 pairs; Kuczyński and Krupiński 2014) of the European breeding population of Montagu's harrier ensures the maintenance of genetic variability. Indeed, the BSP analysis suggested an effective population of females at ~30,000, although 95 % CI of our estimate was very large (260–250,000).

A significant reduction in genetic diversity (estimated based on mtDNA control region polymorphism) in bird

Table 3 Results of neutrality tests with corresponding *P* values for Montagu's harrier mtDNA haplotypes

	Tajima's test		Fu's test		Fu and Li's tests			
	D	*P*	F_S	*P*	*F**	*P*	*D**	*P*
SPA-C& POL-NMP&SP	−0.651	ns	−4.507	0.012	0.131	ns	0.497	ns
CZR-M& POL-S	−0.676	ns	−3.770	0.017	−1.513	ns	−1.578	ns
GER-M	1.055	ns	0.576	ns	1.415	ns	1.070	ns
Total	−0.972	ns	−10.645	0.001	−0.285	ns	0.096	ns

populations experiencing bottleneck seems to be evident only if the number of individuals has dropped below 500 individuals (Jackson et al. 2013). It is possible that the reduction in the numbers experienced by the central European Montagu's harrier population in the 1980s, for example in Poland (Kuczyński and Krupiński 2014), was not as severe as to significantly decrease genetic diversity. Similarly, the genetic after-effects of population decline were not detected in the case of the Japanese northern goshawk, suggesting that the past population decline has not been very serious (Asai et al. 2008). The BSP analysis suggested no significant changes in the effective population size during the last 8000 years. The neutrality test (negative and significant values of Fu's F_S) suggested a recent expansion of the European population. Hence, it is possible that the high genetic diversity of European Montagu's harrier could be interlinked with the population expansion accompanied by a generation of new polymorphisms in the mtDNA control region. The rapid postglacial colonization of northern Europe and major population expansion were also suggested as an explanation of the higher genetic diversity of the northern population of the white-tailed eagle *H. albicilla* (e.g., Estonia, Hailer et al. 2007).

The lowest genetic diversity was found in Germany. The low genetic diversity of the population from this region was also suggested by García et al. (2011), when analysing other mitochondrial genes. This suggests that in this region, genetic diversity is indeed reduced compared to other parts of Europe. Also, the neutrality tests indicated that this population experienced a recent bottleneck or is presently decreasing. However, verification of decreased genetic diversity in Germany requires further studies, especially those including samples from other regions of the country.

We found low genetic differentiation among the investigated regions. Moreover, some geographically isolated breeding areas (e.g., northeastern Poland and central Spain) were included in a common genetic group. This confirms previous observations based on other mitochondrial and nuclear markers (García et al. 2011; Rutkowski et al. 2014). Small genetic differentiation, estimated using both maternally (García et al. 2011; this study) and bi-parentally inherited

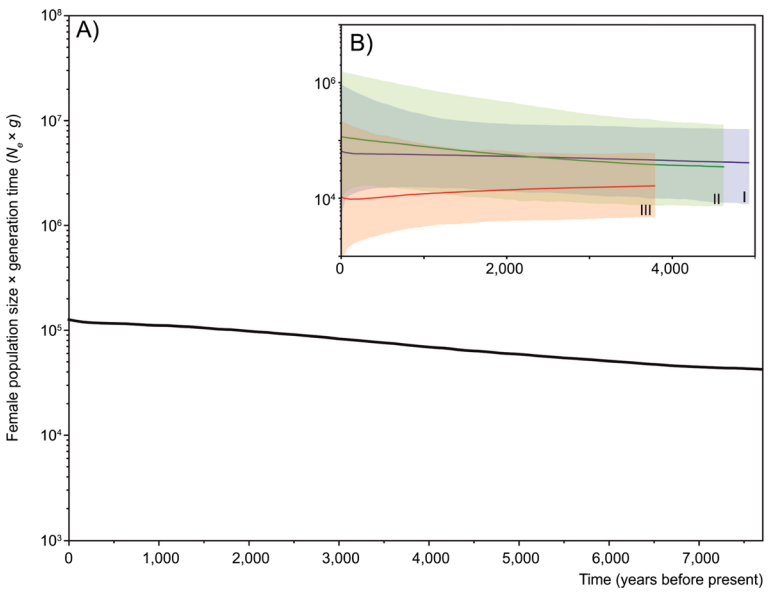

Fig. 3 Bayesian Skyline plot (BSP) for the European population of Montagu's harrier. A per-lineage substitution rate of 7.4 % Ma^{-1} was assumed to estimate the timescale using BEAST software. **a** BSP analysis for all samples pooled. Median effective population size at present time was estimated at 31,600 females (95 % CI ~260–251,800) assuming an average generation time of 4 years. **b** Results of separate BSP analyses for three genetic groups indicated by SAMOVA results (*I*: SPA-C&POL-NMP&POL-SP; *II*: CZR-M&POL-S; *III*: GER-B). Median effective population sizes at the present time, assuming an average generation time of 4 years, are estimated for 16,000 females for group I (95 % HPD, 147–143,500), 29,000 females for group II (184–273,600) and 2630 females for group III (8–33,800)

markers (Rutkowski et al. 2014), suggests that this is not a sex-specific pattern. As stated by García et al. (2011), a low level of population structure in the European population probably reflects the long-distance migration behaviour of the species. The authors indicated food availability during winter and wind conditions as the expected reasons for gene pool mixing between the two main migratory routes. This could explain the lack of significant differentiation among breeding populations from western (Spain) and eastern Europe (northeastern Poland), despite the fact that the birds follow different migratory routes (Trierweiler et al. 2006).

Although many migratory bird species with wide geographical distribution are composed of different subpopulations using distinct migratory routes, the lack of a clear differentiation between the migratory flyways of the Montagu's harrier is not an exception (e.g., Liu et al. 2012; Kraus et al. 2013). Moreover, it was shown that the species presents low philopatry and a high capacity for natal dispersal in both sexes—the behaviour favouring gene flow among populations (Limiñana et al. 2012). Low genetic differentiation is frequently observed in birds (Crochet 2000), including many Accipitridae (Martínez-Cruz et al. 2004; Hull et al. 2008a; Takaki et al. 2009; Bourke et al. 2010). Recent phylogenetic study of harriers has also suggested that a low level of genetic structuring could be characteristic for many other members of the *Circus* genus (Oatley et al. 2015). However, even for widespread raptors that are highly mobile, the population's genetic structure might be substantial if regional variation in habitat occurs (Hull et al. 2008b; Lerner et al. 2009). Presently, the European breeding population of Montagu's harrier nests mainly in crop fields, and the agricultural landscape dominates in many parts of the continent, so breeding habitat availability seems to be widespread. Obviously, the mobility of Montagu's harrier, its capability of long distance dispersal, the broad availability of breeding habitats (intensive farming) and low level of philopatry prevent substantial genetic differentiation.

As with our results, the majority of polymorphisms observed by García et al. (2011) were due to single-nucleotide substitutions (mean k=1.47, compared to k=1.43 in our study). Hence, our study confirmed that the European breeding population presents a high number of mitochondrial haplotypes, differing mainly by single substitutions. Indeed, the nucleotide diversity of the Montagu's harrier found in our study seems to be lower than values reported for the hypervariable domains of the control region in other European Accipitridae (Godoy et al. 2004; Martínez-Cruz et al. 2004; Hailer et al. 2007; Honnen et al. 2010; García et al. 2012; Langguth et al. 2013; Ponnikas et al. 2013). Higher nucleotide diversity in these species could be interlinked with the presence of different mitochondrial lineages in Europe. Indeed, two distinct haplogroups were found in European populations of the white-tailed eagle (Hailer et al. 2007; Honnen et al. 2010; Langguth et al. 2013; Ponnikas et al. 2013), bearded vulture (Godoy et al. 2004) and black vulture *Aegypius monachus* (Poulakakis et al. 2008). The presence of two haplogroups is usually connected to the existence of eastern and western glacial refugia, from which the species had expanded. After postglacial range expansion, the two lineages admixed, leading to the current lack of geographic separation of different lineages, as was shown for some European raptors (e.g. Godoy et al. 2004; Hailer et al. 2007; Langguth et al. 2013). A shorter isolation period during the interglacial phases of steppe-associated species compared to temperate fauna led to smaller genetic differentiation between refugia, as did the larger areas of refugia resulting from the wide distribution of periglacial steppes during glacial periods (Hailer et al. 2007; García et al. 2011). In our study, we did not find separate lineages of Montagu's harrier. The two 'core' haplotypes differ only by one substitution; hence, their origin in different glacial refugia is rather unlikely. It is possible that Montagu's harrier, as opposed to the white-tailed eagle or vultures, colonized Europe from a single glacial refugium. Although the limited geographic range of sampling in our study prevents us from identifying this refugium, the low genetic differentiation between Spanish and central European harriers suggests that it could be located within the Iberian Peninsula.

Conclusions

We found high genetic diversity in the European breeding population of Montagu's harrier, reflecting the large population size of the species. Despite some reduction in the number of occupied territories and the number of breeding pairs in the near past, interlinked with the gradual disappearance of natural nesting habitats (wetlands and river valleys), the size of the population probably did not reach the critical value required to significantly decrease the diversity of the genetic pool. Montagu's harrier shifted to a new breeding habitat (the agricultural landscape) quickly enough to retain a high level of genetic variability. However, we found decreased genetic variability related to a recent bottleneck or current decrease of population size in Germany's breeding population. The genetic structure of European breeding populations was weakly pronounced, which agrees with the low level of philopatry and high dispersal ability of Montagu's harrier. The analysis of mtDNA genealogy indicated two frequent, closely related haplotypes and many low-frequency and/or unique haplotypes, differing from them by a small number of substitutions. There was no clear connection between the position of the haplotype in the genealogy and its geographical distribution. We speculate that, in agreement with previous research, this pattern reflects the short time of isolation during interglacial periods and the wide distribution of steppes during glaciation.

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