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Fine-scale genetic structure in a high dispersal capacity raptor, the Montagu's harrier (*Circus pygargus*), revealed by a set of novel microsatellite loci

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Abstract

The Montagu's harrier (*Circus pygargus*) is a semi-colonial raptor species widely but patchily distributed across the Palearctic region with recorded cases of philopatry and presence of extra-pair copulation. In order to assess Montagu's harrier spatial genetic structure and contemporary gene flow, we developed 16 new microsatellite markers using 454 pyrosequencing. Genotypes of 117 chicks sampled in a 200 × 300 km farmland area in Central Western France were analyzed to characterize genetic polymorphism at each locus and regional and fine-scale genetic structure. Fourteen markers were found polymorphic, with a number of alleles ranging from 3 to 11. The expected and observed heterozygosities ranged from 0.36 to 0.856 and from 0.35 to 0.868, respectively. A single genetic unit was found at the regional scale with higher genetic similarity observed at a small spatial scale (up to 10 km). Our results are consistent with overall large-scale juvenile and adult dispersal together with small-scale male philopatry. Cross-species amplification of this set of microsatellites makers has been successful in two closely related harrier species: the marsh harrier (*Circus aeruginosus*) and the Hen harrier (*Circus cyaneus*) for which 14 and 12 markers were polymorphic, respectively. These new microsatellite markers could be used to study the population genetic structure, contemporary gene flow and parentage analyses in these three species and to conduct microsatellite-based demographic inferences on the Montagu's harrier.

Keywords Raptor · Spatial genetic structure · Relatedness · Microsatellites · *Circus pygargus* · *Circus cyaneus* · *Circus aeruginosus*

Introduction

Dispersal plays a fundamental role in population biology and conservation because it influences the dynamics, the genetic structure and the persistence of populations (Clobert et al. 2001). Since migration tends to homogenize allelic frequencies among populations, species with high dispersal capacity are usually found to be genetically homogeneous over large spatial scales. However, even for species with high dispersal capacity, fine-scale genetic structure may still result from biological processes such as mating system or social behavior. As for example, in iteroparous species with sex-biased dispersal, genetically related individuals (parent–offspring, brothers or sisters) from the philopatric sex might breed close by resulting in fine-scale genetic structure (e.g. Temple et al. 2006; Gauffre et al. 2008).

Montagu's harrier, *Circus pygargus* (Linnaeus, 1758), is a Palearctic raptor with an estimated population of 54,500–92,200 breeding females in Europe. It is currently

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listed as "least concern" according to BirdLife International (2018), though negative population trends have been reported in France (Le Rest et al. 2015). This ground-nesting breeder originates (and still lives in) steppe habitats, but has adapted well to farmlands during the twentieth century (Arroyo et al. 2002). The species is particularly vulnerable to harvesting of cereal fields, its major breeding habitat in Western Europe (Santangeli et al. 2015b). This semi-colonial breeder is widely but patchily distributed across the Palearctic region and undertakes long-distance migrations to either Africa or India (Arroyo et al. 2004). Despite Montagu's harrier displays long distance, female-biased, natal dispersal (Chadoeuf et al. 2017), it was shown based on five tagging surveys that a small proportion of individuals present philopatry (up to 5% birds breed within 10 km of the natal site in Spain, see Limiñana et al. 2012).

Despite Montagu's harrier being a well-studied raptor, only a handful of genetic studies has been conducted on the species. A first phylogeographic study based on two mitochondrial markers found low genetic diversity and weak genetic differentiation at the scale of the species whole distribution range, suggesting an intensive gene flow in Europe (Garcia et al. 2011). A more recent study evaluated polymorphism in the hypervariable domain of the mitochondrial control region in Europe and found more genetic diversity but still weak genetic structure (Rutkowski et al. 2015). However, while mitochondrial markers are suited to investigate the historical processes generating patterns of genetic variation among populations (Manel et al. 2003), the study of contemporary processes affecting genetic variation at a local or regional scale requires more variable genetic markers such as microsatellites (Wang 2010; Goldstein and Schlotterer 1999).

Indeed, microsatellites allow cost-effective investigation of spatial genetic structure and gene flow over recent timescale (e.g., < 100 years), which might be critical in the context of conservation challenges raised by human activities. They are popular in landscape genetics, which promotes individual-based approaches to uncover fine-scale genetic structure (Storfer et al. 2007). A first set of microsatellite markers has already been recently developed and successfully tested for parentage analyses (Janowski et al. 2014). In another study based on microsatellites adapted from other species, authors compared genetic diversity between two populations from Poland and Spain (Rutkowski et al. 2014). They did not find differences in genetic diversity and, although significant, the level of genetic differentiation between these two populations was low, consistently with previous phylogeographic studies. Interestingly, Rutkowski et al. (2014) also reported one case of extra-pair paternity, which was suspected in the species since it has a relatively high frequency of extra-pair copulations (Mougeot et al. 2001). Altogether, these genetic studies suggest both historical and contemporary gene flow across Europe in the Montagu's harrier. However, to date, no specific study has investigated regional and fine scale genetic structure, while local genetic patterns might result from extra-pairs paternity or philopatry.

The aim of this work was to characterize regional and fine scale genetic variation and structure in the Montagu's harrier using individual-based analyses. We first report the development of 16 new microsatellite markers for the Montagu's harrier and cross-species amplification in two closely related species, the marsh harrier, *Circus aeruginosus*, and Hen harrier, *Circus cyaneus*. Second, based on 117 chicks sampled in 117 nests across Central Western France, Montagu's harrier regional spatial genetic structure was assessed using the newly designed microsatellite markers. Third, fine-scale genetic structure was explored using spatial autocorrelation analyses. Both male philopatry and potential existence of extra-pair paternity would translate into increased genetic similarity at a fine-scale (e.g. <10 km). In case of extra-pair paternity, an excess of half sibs would be expected, while if genetically related individuals (parent–offspring, brother–sister) breed close by consecutively to male philopatry, an excess of cousins would be expected.

Materials and methods

Genetic sampling

Montagu's harrier sampling was carried out in June and July 2008 in a 200 × 300 km farmland area of Central Western France (Fig. 1), as part of research program aiming at investigating the demographic parameters of this species in France (Santangeli et al. 2015a; Chadoeuf et al. 2017; Bourrioux et al. 2018). During the survey program, 117 harrier nests were visited and we took nestlings blood samples by puncturing the brachial vein of a wing. Only one chick per brood was analyzed to avoid presence of siblings in our sample. Blood samples were stored at 4 °C while in the field, then frozen until DNA extraction.

Marsh harriers and Hen harriers were sampled following the same methodology, i.e. during nest visits performed for similar research programs on these two species in four sites of Central Western France: Ré island, Poitevin marshes, Rochefort marshes and Brouage marshes (see map in Sternalski et al. 2013).

Microsatellite library

Microsatellite markers were isolated using 454 pyrosequencing. For genomic library construction, we extracted DNA from 12 Montagu's harrier individuals, originating from the sampling (see above), using DNeasy Blood and Tissue

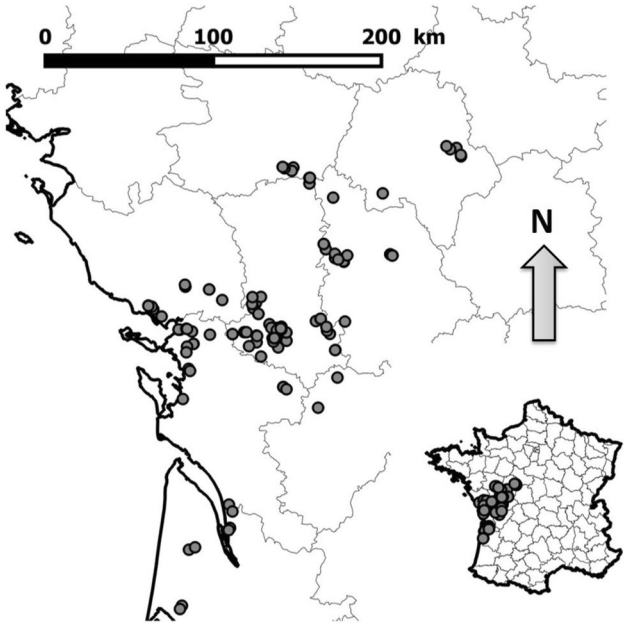

Fig. 1 Map of locations of the 117 Montagu's harrier sampled in central Western France

Kit (Qiagen™) according to the manufacturer's protocol. We then verified that these extraction products displayed equimolar amounts of DNA using a Nanodrop ND-1000. From this pool of DNA, Genoscreen (Lille France) (http://www.genoscreen.fr) constructed a microsatellite-enriched genomic library through a partial 454 GS FLX sequencer run with Titanium chemistry, as described in Malausa et al. (2011). Briefly, total DNA was mechanically fragmented and enriched for TG, TC, AAC, AAG, AGG, ACG, ACAT and ACTC repeat motifs.

A total of 29,327 reads were obtained, of which 6900 contained microsatellite motifs. Sequences were analyzed with the QDD software (Meglecz et al. 2010). Among the 810 primer pairs designed by the manufacturer to amplify fragments containing a microsatellite motif, and on the basis on our criteria (PCR product > 100pb and number of repeats ≥ 5), we selected 48 primer pairs and tested their polymorphism on 24 individuals, including 12 Montagu's harriers, 9 marsh harriers and 3 Hen harriers. Forward primers were indirectly fluorochrome labeled (6-FAM) by adding a 19-bp M13 tail at their 5′end (5′-CACGACGTT GTAAAACGAC-3) (Schuelke 2000; Boutin-Ganache et al. 2001). Each amplification reaction was performed in a total volume of 10 µL: 5 µL of AmpliTaq Gold® 360 Master Mix 2x (ref: 4398881, Life technologies), 0.25 µL (1 µM) of forward primer tagged with the M13 tail (final concentration: 0.025 µM), 0.25 µL (10 µM) of reverse primer (final concentration: 0.25 µM), 0.25 µL (10 µM) of fluorescent dyed M13 tail (final concentration: 0.25 µM) and 2 µL (10 ng/µL) of genomic DNA (final concentration: 2 ng/µL). The PCR was performed using a denaturation step of 10 min at 95 °C, followed by 7 cycles of 30 s at 95 °C, 30 s at 62 °C with a decreasing of 1 °C per cycle, 30 s at 72 °C, then by 30 cycles of 30 s at 95 °C, 30 s at 55 °C, 30 s at 72 °C, followed by 8 cycles of 30 s at 95 °C, 30 s at 56 °C, 30 s at 72 °C and finally 6 min at 72 °C. PCR products were diluted 1:60 before sequencing, mixed with Formamide (ref: 4311320, Life technologies) and LIZ 500 size standard (ref: 4322682, Life technologies). Fragments were separated using an ABI 3730 genetic analyzer (Life technologies) Alleles were scored using GeneMapper® v 4.0 (Applied Biosystems) and checked visually. Primer pairs were eliminated from further development when (1) they failed to amplify, (2) they amplified multiple fragments, or (3) genotype scoring was deemed unreliable. Finally, 16 candidate loci were kept for characterizing polymorphism on populations. We performed a BLAST analysis to compare the sequences of these 16 loci against already published sequences. Our sequences did not overlap with previously published microsatellite loci from the Montagu's harrier or closely related species (see details in Table S1, Supplementary Information).

In a second step, in order to maximize efficiency and minimize cost, five different labeled fluorochromes were used (6_FAM, HEX, PET, VIC or NED) to pool amplification products in 4 poolplexes according to their amplified fragment sizes and dyes. Type of dye was chosen in order to analyze simultaneously loci of similar allelic size and to avoid overlapping between loci with the same dye. The set of 16 loci was amplified (following the same conditions as above) and sequenced on 117 Montagu's harriers, 17 Marsh harriers and 24 Hen harriers, all sampled in Western France.

Genetic diversity for three harrier species

For each species, the number of alleles per locus was recorded and deviation from the Hardy–Weinberg equilibrium (HWE) was estimated over all individuals using the exact test implemented in GENEPOP 4.7 (Rousset 2008), for each locus and globally. Unbiased expected (H_e, Nei 1987), observed (H_o) heterozygosities and, to quantify deviation from HWE, the Weir & Cockerham's estimate of F_{IS} (Weir and Cockerham 1984) were also calculated using GENEPOP. We tested genotypic linkage disequilibrium for each pair of loci. When needed, we used a false discovery rate correction for multiple tests (FDR, Benjamini and Hochberg 1995) with a nominal level of 5%. Polymorphism information content (PIC) and individual identity probability for each marker and over the whole marker set were estimated with CERVUS 3.0 (Kalinowski et al. 2007). Presence of null alleles was checked by assessing whether homozygotes excess might be due to null alleles with MICRO-CHECKER 2.2.3 (Van Oosterhout et al. 2004) and null alleles mean frequencies were estimated following Van Oosterhout et al. (2006).

Montagu's harrier genetic structure in Central Western France

First, Montagu's harrier population genetic structure across Central Western France was assessed with Bayesian clustering using Structure Version 2.2 (Pritchard et al. 2000), with the admixture model and correlated allele frequencies (Falush et al. 2003). To determine the number of clusters (K) within our data set, ten replicate runs of 2×10^6 Markov chain Monte Carlo (MCMC) iterations, after an initial burn-in period of 5×10^5 iterations, were performed for values of K ranging from one to ten. Results were summarized using the standard pipeline on the Clumpak web server (Kopelman et al. 2015). The most likely number of clusters (K) was explored using the estimated logarithm of likelihood (LnP(D)) and the Evanno et al. (2005) ΔK method that finds the point of greatest change in the distribution of LnP(D) with Structure Harvester Version 0.6.92 (Earl and vonHoldt 2012).

Second, Isolation-by-distance (IBD) was analyzed by regressing the pairwise genetic distance against log-transformed spatial distances between individuals (Rousset 2000), and tested using Mantel test (10,000 permutations) with Genepop. Confidence intervals around the slopes of the regressions were estimated by bootstrapping over loci.

Third, the spatial pattern of genetic variation was investigated using spatial autocorrelation analyses that assess the genetic similarity between pairs of individuals at different distance classes, thus providing results on the scale at which spatial pattern may be found. Using Spagedi 1.5 (Hardy and Vekemans 2002), two relatedness coefficients were computed, the kinship coefficient of Loiselle et al. (1995) and the relationship coefficient of Queller and Goodnight (1989), among pairs of individuals using six distances classes (0–10, 10–50, 50–100, 100–150 and 150–200 km). Distance classes were defined to explore small and large scale pattern of genetic structure with at least 400 pairs of individuals in each distance class (i.e. excluding the 171 pairs distant by > 200 km). For each distance class, significant deviation of spatial autocorrelation from a random distribution of genotypes was tested by 10,000 random permutations of individual locations.

Results and discussion

Genetic diversity for three harrier species

Among the 16 loci tested, 15 were polymorphic and have been correctly amplified and genotyped in the Montagu's harrier global dataset (Table 1). There was a heterogeneous level of polymorphism with number of alleles per locus ranging from three to 11 (mean = 7, Table 1). Five loci showed PIC value less than 0.5 (Table 1), and the average PIC value across all loci was 0.58. After FDR correction, one pair of loci, BC04 and BC24, showed significant linkage disequilibrium. Since BC24 was monomorphic for 94% of the individuals genotyped and thus uninformative (PIC 0.104), we decided to exclude this locus from further analyses. Hence, without BC24, the mean expected (H_E) and observed (H_O) heterozygosities were 0.659 and 0.666 and average PIC value across all loci raised to 0.62. There was no significant overall heterozygosity deficit in the total sample ($P = 0.166$, global $F_{is} = 0.01$), and no loci showed significant deviation from HWE after FDR correction. The average probability that the set of loci will fail to differentiate between two randomly selected individuals (NE-I) was 4.29×10^{-13} (see Table 1 for locus by locus values). Null alleles were not detected at any loci (Table 1). The level of genetic diversity in our study ($H_O = 0.666$) was similar to those measured over 100 chicks collected in southern Germany in a previous study based on 16 microsatellite markers developed on the Montagu's harrier ($H_O = 0.69$, Janowski et al. 2014). By contrast, the level of genetic diversity was much lower in the two population studied by Rutkowski et al. (2014) using microsatellites from other species ($H_O = 0.28$ and 0.34 in the Spanish and Polish populations, respectively). This might results from the use of microsatellites from other species as many experiments applying cross-species amplification of microsatellites indicated a lack of amplification, monomorphism and/or lower level of polymorphism in the target species as compared to the source species (see Rutkowski et al. 2014).

Among the 16 loci tested for cross-species amplification, 14 and 12 were found to be polymorphic and have been correctly amplified and genotyped in the marsh harrier and the Hen harrier, respectively (Table 2). The average number of alleles per locus was 3.56 and 5.56 for marsh and Hen harriers, respectively. After FDR correction, no pairs of loci showed significant linkage disequilibrium in both species. Mean H_E/H_O were 0.496/0.496 and 0.471/0.450 for marsh and Hen harriers, respectively. There was no departure from HWE in the marsh harrier and Hen harrier total samples ($P = 0.237$, $F_{is} = -0.003$ and $P = 0.967$, $F_{is} = 0.035$, respectively). No loci showed significant deviation from HWE for the marsh harrier and the Hen harrier, respectively, while null alleles were not detected at any loci in either species (details in Table 2). In both species, the success of cross-amplification was high, suggesting close phylogenic relation. The slightly larger number of loci isolated from Montagu's harrier that cross-amplified in the marsh harrier is consistent with these two species more closely related in harriers phylogeny compared to the Hen harrier (Oatley et al. 2015).

Table 1 Characterization of 16 polymorphic microsatellite loci developed for *Circus pygargus* and global diversity statistics

Locus	NCBI SRA acc. no.	Primer sequence (5′–3′)	Repeat motif	Allele sizes range	Dye	Sequencing group	Genetic diversity (n = 117)								
							N_A	H_O	H_E	PIC	NE-I	F_{IS}	HWE p	Null	Null F
BC02	MK028972	F: CAAACTTGGCATCTAGAATCT GAA	(AC)8	105–115	FAM	P1	6	0.35	0.36	0.338	0.432	0.028	0.5	No	0.003
		R: TGATGGAGTCAGTTGCTATGG													
BC03	MK028973	F: ATGTTCGTGCAAGACCAGATT	(CTAT)9	96–124	FAM	P2	8	0.846	0.803	0.773	0.067	−0.054	0.557	No	−0.032
		R: CCCATGTGCGGCTTTAGA													
BC04	MK028974	F: ATCACCATGGAGAAGCAACC	(CA)8	109–115	FAM	P3	3	0.692	0.643	0.566	0.204	−0.076	0.606	No	−0.041
		R: GCTAAGTGCATCCCTTCTGC													
BC05	MK028975	F: AAATGTCTGGCCTGAGGAGT	(TGGA)6	113–125	FAM	P4	4	0.521	0.504	0.449	0.301	−0.034	0.304	No	−0.039
		R: TATGTGTCCATTTCCTCCCC													
BC07	MK028976	F: CAACACAGTAAATGCATCCCA	(AC)8	117–125	VIC	P1	5	0.397	0.471	0.403	0.348	0.159	0.004	No	0.066
		R: CAGAGTCATCATTTTGCCACA													
BC09	MK028977	F: TAACAGCAGGAACACCAAGG	(GAG)5	120–141	VIC	P2	8	0.718	0.682	0.624	0.158	−0.053	0.5	No	−0.039
		R: CCTTCTCACTCTCCACAGGC													
BC10	MK028978	F: CATCTAGGGTGTCTTTGGGC	(TG)10	113–125	VIC	P4	9	0.803	0.85	0.826	0.043	0.055	0.93	No	0.021
		R : GTCATCACGATGCACACCA													
BC13	MK028979	F: GATGCTCAGCTTCACTGTGG	(AC)9	136–150	NED	P1	5	0.402	0.418	0.382	0.375	0.039	0.015	No	0
		R: CAGAAAGAAAGGGCAGAGTAGG													
BC15	MK028980	F: ATTGAGTTCTCATGTCTGGGG	(ATAG)11	142–171	VIC	P3	8	0.769	0.82	0.792	0.058	0.062	0.146	No	0.031
		R: AGGTTCTCTTTCATTTGCTTA AAA													
BC23	MK028981	F: TTCCCATGGATGGACTTCTC	(GGAT)9	174–198	PET	P1	6	0.692	0.67	0.612	0.166	−0.033	0.516	No	−0.016
		R: TCATGTAAAGAAGATGACAGT TTTGA													
BC24	MK028982	F: AGTGCACTGCCTGGCTTTTA	(ACA)5	184–190	NED	P2	4	0.114	0.109	0.104	0.799	−0.047	1.000	No	−0.059
		R: TTGTTGTCTGGTTCATCTCATTG													
BC27	MK028983	F: TTCAAAATTATTGGAAGTAAA ATAGCA	(AAC)17	NA	NED	P4	1	–	–	–	–	–	–	–	–
		R: GAACACTCTGTATACCCTTGG ACA													
BC40	MK028984	F : TGCACCTGCAGAAGAGGTTA	(CTT)14	284–320	PET	P2	11	0.769	0.836	0.812	0.048	0.079	0.415	No	0.039
		R : CGCTTTGGAAACAAGCTACC													
BC41	MK028985	F : GGCTCCTGAGCTTGAGGAAT	(TAGA)16	254–294	NED	P3	11	0.868	0.856	0.837	0.037	−0.014	0.921	No	−0.01
		R : CGTTTGCTTAGTTGGCTGTTC													

Table 1 (continued)

Locus	NCBI SRA acc. no.	Primer sequence (5′–3′)	Repeat motif	Allele sizes range	Dye	Sequencing group	Genetic diversity (n = 117)							
							N_A	H_O	H_E	PIC	NE-I	F_{IS}	HWE p	Null	Null F
BC44	MK028986	F : AAACACCAAATATCAGCC TGAAG R : TGCTGGTAGCAGTGTAGGCA	(ACA)10	293–314	PET	P4	8	0.615	0.624	0.563	0.202	0.014	0.875	No	0.007
BC46	MK028987	F : TTCAAATGGGAAACTGGAGG R : GGCAATCAGGGGATTAGAAC	(TTG)11	297–315	PET	P3	9	0.778	0.783	0.749	0.081	0.079	0.757	No	0.005

Locus name, GenBank Accession Number, forward (F) and reverse (R) primer sequences, number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), polymorphism information content (PIC) value, average non-exclusion probability for identity of two unrelated individuals (NE-I), F_{IS} value, uncorrected exact *p*-value of the Hardy–Weinberg equilibrium test (HWE-p), assumption of null alleles using Microchecker (Null) and estimation of null allele frequencies using Van Oosterhout method (Null F)

Montagu's harrier genetic structure in Western France

Analyses were performed on 14 loci, thus excluding BC24 and BC27 (see above). The most likely value of *K* from the STRUCTURE analysis based on the method of Evanno et al. (2005) was three (Table S2 in Supplementary Information). However, none of the 117 individuals could be assigned to a given cluster for any *K* value from 2 to 6, as each individual had a similar probability of belonging to each cluster, suggesting no genetic structure in the dataset. Consistently, the estimated logarithm of likelihood for data was highest for $K = 1$, confirming the presence of a single genetic cluster in our dataset. This lack of regional structure is consistent with a previous microsatellite-based study, which found very low level of genetic differentiation between two populations from Poland and Spain ($F_{ST} = 0.016$; Rutkowski et al. 2014).

Spatial genetic autocorrelation analysis between pairs of individuals showed marginally significant positive kinship values and significant positive relationship values for the first distance class only (0–10 km), then genetic similarity between individuals slightly decreased with no further trend with spatial distance (Fig. 2). This absence of spatial genetic structure beyond 10 km was confirmed by the IBD analysis that indicated non-significant relationship between genetic differentiation and spatial distance between pairs of individuals (Slope = 0.002, 95% CI: [−0.0052 to 0.0102], Mantel test P-value = 0.3).

The higher genetic similarity observed in the first distance class (up to 10 km) could be explained by different mechanisms. First, the species is semi-colonial and promiscuity may increase the probability of extra pair paternity (EPP, Griffith et al. 2002; Rutkowski et al. 2014). This could either originate from breeding males copulating with neighboring female(s) or floating individuals, unpaired sexually mature males, observed around colonies (pers. obs.). Second, although rare (Väli 2017), polygyny may occur, thus resulting in a similar pattern as EPP. These two mechanisms would result in an excess of half siblings (relatedness = 0.25) at fine-scale. On the other hand, sex-biased natal dispersal in the species (Chadoeuf et al. 2017) results in higher male philopatry, and brothers could be found breeding relatively close by. Male natal philopatry would also result in increasing inter-chicks relatedness at a fine-scale with an excess of cousins (relatedness = 0.125). In addition, this long-lived species develop various adult dispersal strategies including adult breeding philopatry with both males and females returning to their first breeding site from year to year. Combined with male natal philopatry this would similarly increase fine-scale inter-chicks relatedness with chicks from parents—sons breeding close by (relatedness = 0.125). Histogram of the distribution of relatedness

Table 2 Diversity statistics of 16 loci for the Marsh harrier and the Hen harrier

Locus	N_A	H_O	H_E	F_{IS}	HWE-p	Null	Null F
Marsh harrier (n = 17)							
BC02	2	0.529	0.449	−0.180	0.609	No	−0.116
BC03	5	0.875	0.769	−0.138	0.009	No	−0.094
BC04	2	0.059	0.059	0.000	NA	No	−0.030
BC05	2	0.471	0.427	−0.103	1.000	No	−0.072
BC07	5	0.588	0.750	0.216	0.077	No	0.083
BC09	2	0.412	0.511	0.194	0.624	No	0.079
BC10	5	0.882	0.779	−0.132	0.569	No	−0.094
BC13	4	0.294	0.268	−0.096	1.000	No	−0.153
BC15	1	–	–	–	–	–	–
BC23	4	0.588	0.570	−0.032	0.625	No	−0.045
BC24	1	–	–	–	–	–	–
BC27	4	0.643	0.637	−0.009	0.776	No	−0.018
BC40	3	0.471	0.590	0.203	0.541	No	0.087
BC41	7	0.625	0.721	0.133	0.109	No	0.051
BC44	4	0.563	0.627	0.103	0.787	No	0.041
BC46	6	0.941	0.787	−0.196	0.061	No	−0.130
Hen harrier (n = 24)							
BC02	3	0.125	0.121	−0.030	1.000	No	−0.064
BC03	7	0.708	0.708	0.000	0.769	No	0.009
BC04	2	0.333	0.337	0.011	1.000	No	0.004
BC05	2	0.458	0.438	−0.046	1.000	No	0.034
BC07	4	0.478	0.507	0.057	1.000	No	−0.015
BC09	15	0.833	0.895	0.069	0.358	No	0.020
BC10	8	0.667	0.771	0.135	0.278	No	0.000
BC13	3	0.417	0.434	0.040	1.000	No	0.000
BC15	1	–	–	–	–	–	–
BC23	6	0.625	0.679	0.080	0.595	No	0.000
BC24	1	–	–	–	–	–	–
BC27	8	0.714	0.858	0.168	0.080	No	−0.053
BC40	1	–	–	–	–	–	–
BC41	18	0.917	0.938	0.022	0.604	No	0.062
BC44	1	–	–	–	–	–	–
BC46	9	0.917	0.849	−0.080	0.714	No	0.000

Locus name, number of alleles (N_A), observed heterozygosity (H_O), expected heterozygosity (H_E), uncorrected exact *p*-value of the Hardy–Weinberg equilibrium test (HWE-p), assumption of null alleles using Microchecker (Null) and estimation of null allele frequencies using Van Oosterhout method (Null F)

among pairs of individuals at distance < 10 km showed a higher proportion of pairs with relationship coefficient comprised between 0.1 and 0.2 (Fig. 3). This pattern strongly suggests male philopatry as the mechanism leading higher genetic similarity at fine-scale in the Montagu's harrier. Similar spatial structure linked to philopatry and dispersal strategies has been found in other raptors species (e.g., Ortego et al. 2008). Further specific assessment of the underlying mechanism(s) through dedicated work using an extended dataset including adults genetic material is however required.

Conclusion

In this study, 16 microsatellite markers were developed and 14 of them were polymorphic and suitable for population genetics analyses in the Montagu's harrier. In addition, 14 and 12 of these 16 markers tested for cross-species amplification were found to be polymorphic in the marsh harrier and the Hen harrier, respectively. These markers may be used to study the population genetic structure, contemporary gene flow and for parentage analyses in these

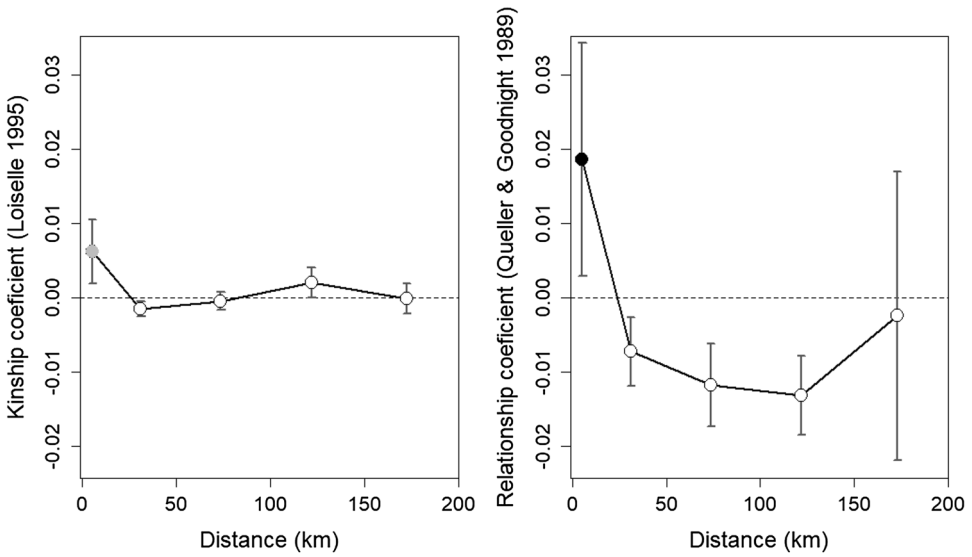

Fig. 2 Autocorrelograms showing the Loiselle kinship coefficient (Loiselle et al. 1995) and the relationship coefficient (Queller and Goodnight 1989) as a function of distance (expressed in km) on pairs of Montagu's harrier individuals of central Western France. The first distance class represents pairwise comparisons between individuals distant from less than 10 km (average 5.3 km), while subsequent distance classes are 10–50, 50–100, 100–150, 150–200 and 200–320 km. Filled dots indicate marginally significant (grey) and significant (black) departure from the 95% CI for the null hypothesis of a random distribution of genotypes determined by 10,000 random permutations of individual locations. SE computed by bootstrapping over loci is plotted for each class

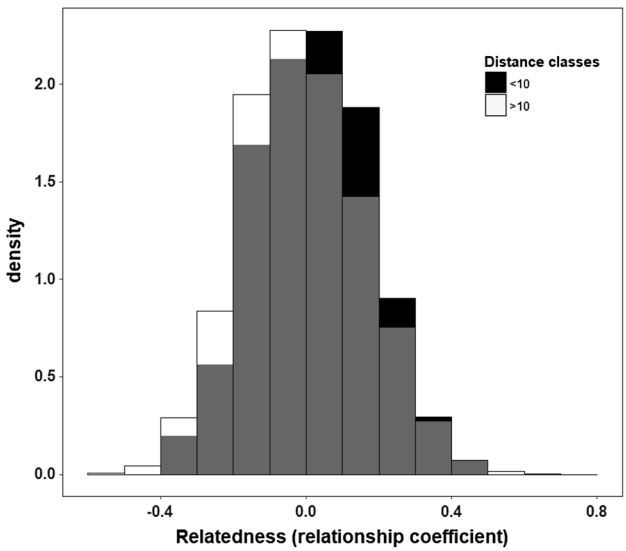

Fig. 3 Histogram of the distribution of the relationship coefficient (Queller and Goodnight 1989) among pairs of individuals separated by < 10 km (black) and between 10 and 100 km (white). Grey area corresponds to overlap between the two distance classes

three species. In particular, this set of new polymorphic markers together with previously designed microsatellite markers (Janowski et al. 2014) might be useful to conduct microsatellite-based demographic inferences that require large sets of markers (i.e. >> 20; Leblois et al. 2014).

The absence of genetic structure for the Montagu's harrier in Central Western France (up to > 300 km) suggests intense and large-scale gene flow, which is expected for a species with high natal dispersal (Chadoeuf et al. 2017), as well as rather high adult dispersal (Arroyo et al. 2004). This result is consistent with a previous microsatellite-based study showing low genetic differentiation between Poland and Spain (Rutkowski et al. 2014), and previous genetic studies based on mitochondrial DNA revealing important historical gene flow (Garcia et al. 2011; Rutkowski et al. 2015). Additional samples should be examined to determine contemporary Montagu's harrier spatial genetic structure at the scale of its distribution range using landscape genetic approaches. Despite the lack of regional genetic structure, our fine scale investigation of genetic patterns at different distance classes indicated a highest relatedness among chicks distant by less than 10 km, likely resulting from male philopatry. However, EPP cannot be excluded as another mechanisms accounting for this fine scale pattern and analyzing chicks from same nests is required to properly test this hypothesis.

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Compliance with ethical standards

Conflict of interest Cecile Ribout declares that she has no conflict of interest. Alexandre Villers declares that he has no conflict of interest. Stephanie Ruault declares that she has no conflict of interest. Vincent Bretagnolle declares that he has no conflict of interest. Damien Picard declares that he has no conflict of interest. Karine Monceau declares that she has no conflict of interest. Bertrand Gauffre declares that he has no conflict of interest.

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