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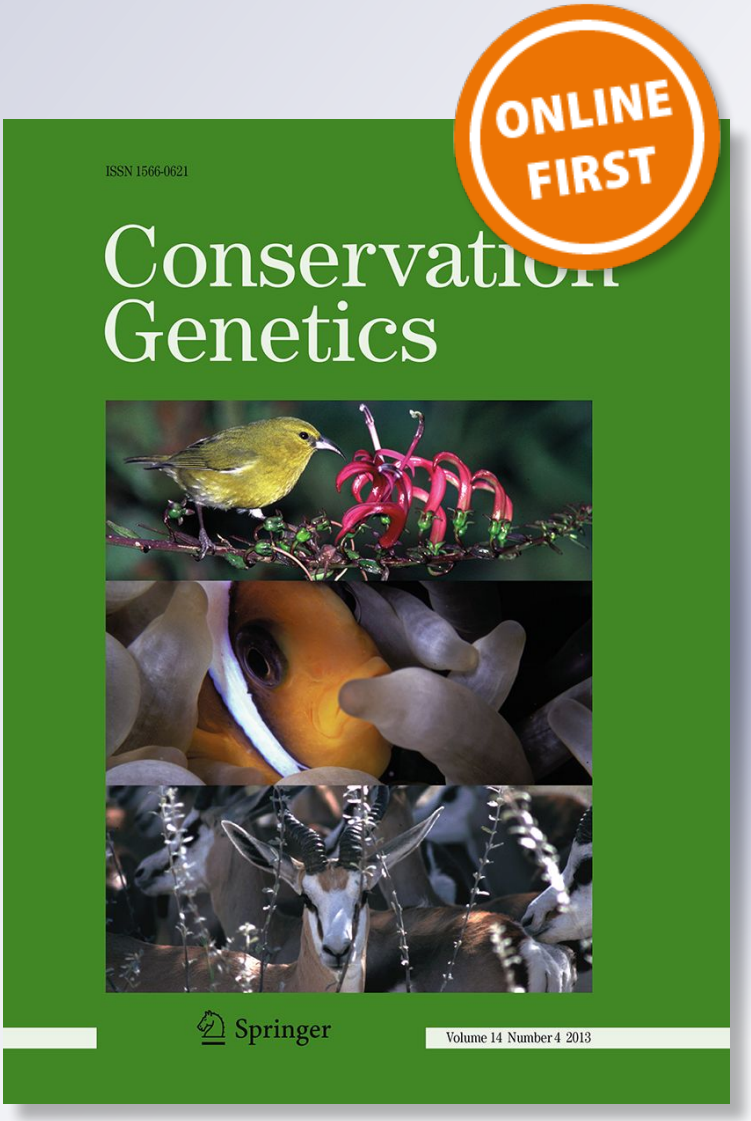

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RESEARCH ARTICLE

Genetic connectivity among osprey populations and consequences for conservation: philopatry versus dispersal as key factors

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Abstract

Genetic variability and population structure in osprey were studied using DNA microsatellite markers. Special emphasis was placed on the subspecies living in the Afro-Palearctic (*Pandion haliaetus haliaetus*). For comparative purposes, American osprey subspecies (*P. h. carolinensis, P. h. ridgway*) and Indo/Australian subspecies (*P. h. cristatus*) were included in this analysis. Twenty DNA microsatellite loci were analysed across a total of 200 individuals. Cluster analysis of genetic distances generally grouped populations of osprey in accordance with their subspecific designation and with previous results from mtDNA analysis. Ospreys from America and Australia were clearly separated from *P. h. haliaetus* suggesting a more ancient isolation which prevented recent gene flow across these groups. Within *P. h. haliaetus*, significant genetic differentiation was found between populations in northern and southern Europe, suggesting that the Afro-Palearctic group is structured into two interconnected entities (Mediterranean and continental Europe). Population structuring was supported by an assignment test and by analysis of allele-sharing among individuals. At the Mediterranean scale, no significant differences of allelic information were found between populations. Behaviours such as dispersal, migration and philopatry seem to have played simultaneously and in contrary directions in shaping the genetic structure and diversity of populations. Our results provide essential information for reconstructing gene flow and genetic variability among osprey populations at different scales, which call for caution in the proactive management and conservation of the species, namely in the Mediterranean area.

Keywords Nuclear molecular markers · *Pandion haliaetus* · Gene flow · Population structure · Migration · Mediterranean

Introduction

The preservation of natural levels of connectivity between populations lends to multiple benefits to their conservation (Crooks and Sanjayan 2006). For example, the natural ranging behaviour of animals facilitate the exchange of genetic material among isolated populations, which enriches the genetic variability of the populations itself and, in turn, mitigate the potential deleterious effects of inbreeding depression (Höglund 2009). In the long term, connectivity between populations allows species to adapt and evolve to changing environmental conditions and persist over time (Crooks and Sanjayan 2006; Ladle and Whittaker 2011). Consequently, understanding the genetic structure and connectivity of populations is of fundamental importance to establish appropriate conservation plans, particularly for those populations which are threatened and deserve specific management measures (Cresswell 2014).

If on one hand, the genetic structure of populations is known to be mostly determined by evolutionary forces (e.g. natural selection, mutations, genetic drift), on the other hand it is also influenced by behavioural processes (Nesje et al. 2000; Agudo et al. 2011). Intensity of gene flow may differ between species according to several behavioural factors which sometimes act antagonistically. One factor is dispersal

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that is defined as the permanent movements an individual makes from its place of birth to its first breeding site (natal dispersal) or between successive breeding sites (breeding dispersal) (Clobert et al. 2001). Dispersal may allow colonization or recolonization of favourable habitats/vacant sites (Hanski and Giplin 1997), thus resulting in homogenization of the gene pool between populations (deficit or excess heterozygotes; Salanti et al. 2005). Migration, defined as the widespread behaviour by which an animal periodically moves from one region to another (Berthold 2001), can interact synergistically with dispersal in the regulation of allelic frequencies between the different populations, because individuals may choose to breed at a site they have previously visited during migratory journeys. On the other hand, philopatry (the behaviour by which individuals tend to return to their natal area to reproduce once sexually mature) is an antagonist factor to dispersal, since it favours a local sub-structuring by preventing gene flow between populations. Therefore, it is expected that strictly philopatric species will show strong population genetic structure, characterized by many private alleles and heterozygote deficiency. For example, in the migratory Egyptian vulture (*Neophron percnopterus*) genetic isolation might be mostly due to a strong philopatric behaviour of the species rather than because of geographical barriers such as the Mediterranean Sea that do not operate as an obstacle to gene flow (Agudo et al. 2011). In this sense, the genetic structure of populations of species with a high philopatric behaviour should be similar to species living in islands (MacArthur and Wilson 1967) and behaviours operating as barriers (e.g. high territoriality, philopatry) may have an important role in explaining the genetic structure of such populations. Therefore, antagonistic behaviours (e.g. dispersal vs. philopatry) can often act concurrently, being more or less prominent but affecting the ultimate genetic aspect of populations. Understanding the role of such behaviours and their concomitant effects on population dynamics is crucial to guide and develop effective actions of conservation for threatened species/populations that have experienced strong decline after centuries of persecution by humans (e.g. raptors; Newton 2003).

In this context the osprey, *Pandion haliaetus*, is of notable interest as, both resident and long-distance migratory populations are known across its worldwide distribution range (Poole 1989; Monti et al. 2015). Moreover, this raptor has large capacity for natal dispersal that varies according to sex (sex-biased dispersal with females dispersing over a greater distance than males; Martell et al. 2002) but also has a strong philopatric behaviour (Poole 1989). Hence, the osprey is an interesting biological model for investigating how genetic pools were structured among different populations by these antagonistic factors. At regional scales, dispersal movements, migration and genetics of this species are still poorly known, preventing the full understanding of their ecology and in turn arising many questions about the long-term maintenance of populations. For example, the absence of connectivity (thus the absence of gene flow) between Mediterranean populations living on islands and those from mainland (continental Europe), could lead to a significant loss of genetic diversity in the former because of their low numbers and related risks of extinction.

In this study, we aimed at quantifying the connectivity between osprey populations by means of 20 polymorphic microsatellites that have been specifically developed for *Pandion haliaetus*. We estimated the degree of genetic divergence between populations at a global level first, then within the Western Palearctic, with a special interest for the Mediterranean area. We postulated that both geographic (e.g. sea, islands) and behavioural (e.g. philopatry, territoriality) factors could act as barriers to gene flow between populations, thus affecting the ultimate genetic structure of populations. In particular, we addressed whether: (1) the levels of genetic diversity are lower for Mediterranean populations (which live in a fragmented habitat at sea, potentially limiting gene flow between breeding nuclei) than for mainland populations of Europe (which live in a continuous forested habitat, potentially promoting connectivity); (2) a sex-biased dispersal would have had any influence in shaping the genetic structure of populations, independently to their geographical origin; and (3) the demographic declines recorded for some Mediterranean populations would shed light on past population bottlenecks and suggest future measures to adopt.

Materials and methods

Sampling and DNA extraction

A total of 200 individual-samples were collected from many localities heterogeneously distributed over the extensive species distributional range (Fig. 1; Table 1; Monti et al. 2015). Osprey samples were collected from breeding birds or collected during the breeding season in the breeding grounds (no sample came from wintering areas). Chicks were also sampled at nest, thus before dispersal. DNA was extracted from blood (preserved in alcohol in the Queen buffer or dried on filter paper), feathers, muscles or toe-pad fragments using the Qiagen "DNeasy ® kit Blood and tissue" (Ref. 69506, Qiagen Inc.). The extraction protocol of DNA was adapted according to the type of sample. Once extracted, the quality and quantity of DNA samples were checked by electrophoresis using an aliquot of 5 µl DNA of each individual and of a size reference marker (Thermo scientific GeneRuler ™ DNA Ladder, Fermentas), upon an agarose gel containing 1% ethidium bromide (an intercalating fluorescent DNA).

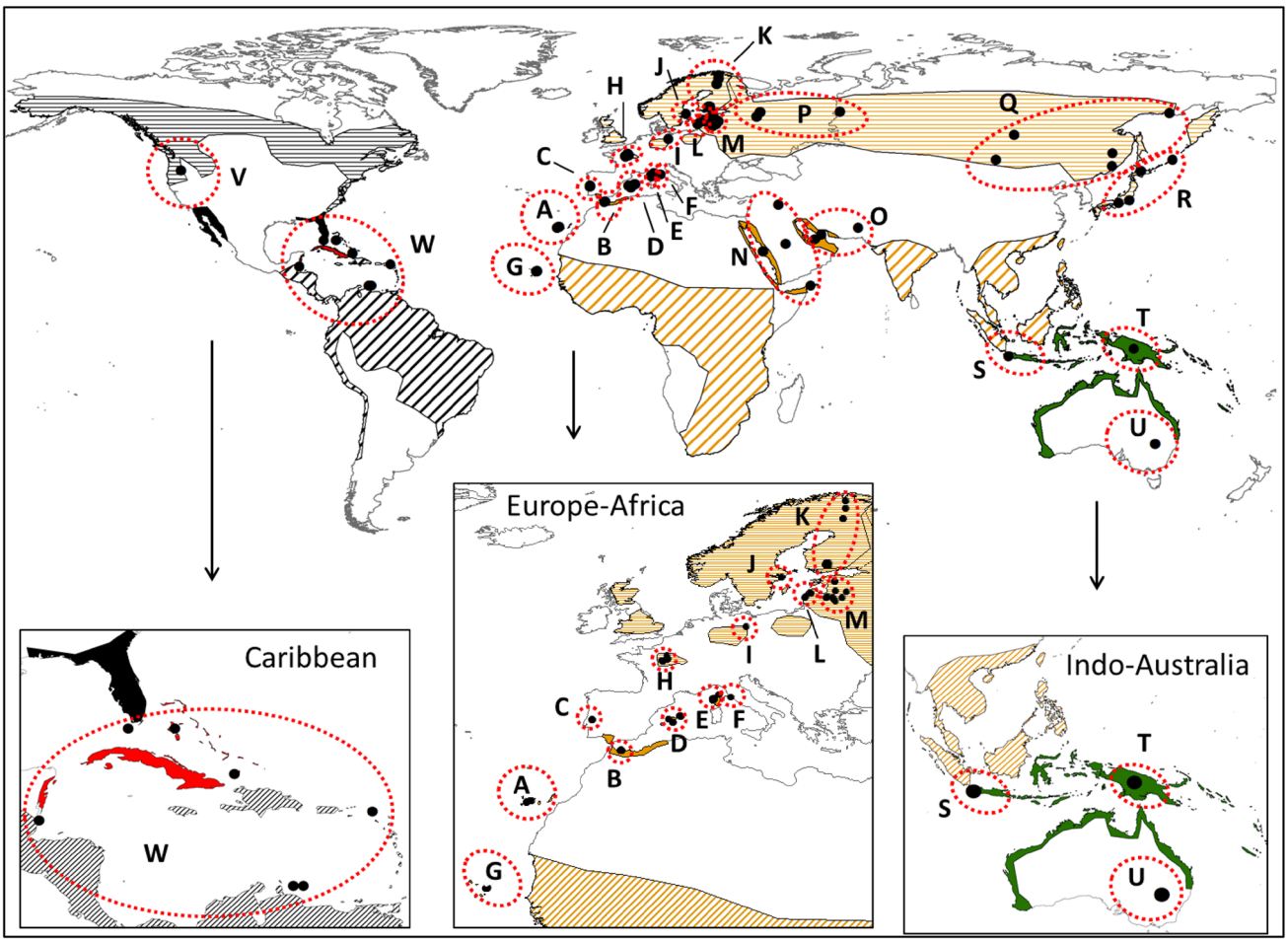

Fig. 1 Geographical distribution of *Pandion haliaetus*. Ranges for the four-recognized subspecies are in different colors: black for *carolinensis*, red for *ridgwayi*, orange for *haliaetus* and green for *cristatus*. Horizontal stripes are for breeding areas, skew lines for wintering areas and color-filled zones represent areas with sedentary populations. Circles symbolize sample locations. In the small boxes (from left to right) three zones are zoomed in: Caribbean, Mediterranean and Indo-Australasian areas. Red dotted circles include different populations as defined in this study (see "Materials and methods" and Table 1 for population letter codes). (Color figure online)

Microsatellites genotyping and sexing of individuals

A microsatellite library was built for *Pandion haliaetus* by the biotechnology company Genoscreen, using the method of high-throughput pyrosequencing (GS FLX ®, Roche Diagnostics ®; Malausa et al. 2011). A total of 411 loci have been validated as containing a microsatellite motif showing different repeated units: di-nucleotides (272 loci), trinucleotides (112 loci), tetra-nucleotides (19 loci), penta-nucleotides (6 loci) and hexa-nucleotides (2 loci). Considering that markers with more complex patterns and multiple repetitions are likely to have a higher polymorphism (Frankham et al. 2002), we selected 40 loci (24 tri-, 10 tetra-, 4 penta- and 2 hexa-nucleotide motifs) that were tested for amplification and polymorphism.

Amplification reactions contained 5 µl of QIAGEN Mix ®, 1 µl of forward and reverse primers, 1 µl natif DNA and 2 µl H_2O. Amplification products were detected on an ABI Prism 3130 Sequencer (Applied Biosystems) at the platform "Génotypage-Séquençage" of the Labex CeMEB (Montpellier, France). Multiplexed PCRs were performed with 5 µl of a multiplex mix (Master Mix, Qiagen), 1 µl of a primer solution at the concentration of 2 pM for each primer, 3 µl of water and 1 µl of ADN. PCR best conditions are an initial denaturation at 95 °C for 15 min followed by 30 cycles of denaturation at 94 °C for 30 s, annealing at 48–56 °C for 90 s, and extending at 72 °C for 1 min with a final elongation step at 60 °C for 30 min. PCRs were realized with 5 µl of a multiplex mix (Master Mix, Qiagen), 1 µl of solution of primer concentrated to 2 µl ml^{-1} for all loci in multiplex, 3 µl of water and 1 µl of ADN. Genotyping of individuals was determined by two independent readings with the Genemapper software version 3.7 (Applied Biosystems).

Table 1 Description of the 200 samples of *Pandion haliaetus* used in this study

Locality	N (sexed samples)	N_males	N_females	Origin	Sample type
A—Canary Islands	10 (4)	3	1	Fresh	Blood (filter paper) + feathers (dry)
B—Morocco	6 (3)	1	2	Fresh	Blood (filter paper)
C—Portugal (extinct pop.)	7 (0)	NA	NA	Ancient/fresh	Blood (alcool) + eggs shell
D—Balearic Islands	23 (18)	14	4	Fresh	Blood (alcool + filter paper)
E—Corsica	29 (19)	8	11	Fresh	Blood (filter paper) + feathers (dry)
F—Italy	3 (2)	1	1	Fresh	Blood (filter paper)
G—Cape Verde Islands	8 (0)	NA	NA	Fresh	Blood (filter paper) + feathers (dry)
H—France	17 (12)	7	5	Ancient/fresh	Feathers (dry + alcool)
I—Germany	1 (0)	NA	NA	Ancient	Eggs shell
J—Sweden	1 (0)	NA	NA	Fresh	Feathers (dry)
K—Finland	13 (13)	7	6	Fresh	Feathers (dry)
L—Latvia	15 (10)	7	3	Fresh	Feathers (dry)
M—Estonia	8 (7)	3	4	Fresh	Feathers (dry)
N—Middle East	7 (0)	NA	NA	Ancient/fresh	Toe pad + blood (alcool)
O—Persian Gulf	3 (0)	NA	NA	Fresh	Blood (alcool)
P—West Russia	9 (0)	NA	NA	Ancient/fresh	Blood (filter paper)
Q—East Russia	12 (0)	NA	NA	Ancient	Toepad
R—Japan	5 (0)	NA	NA	Ancient/fresh	Toe pad + muscle (alcool)
S—Indonesia	1 (0)	NA	NA	Ancient	Toepad
T—New-Guinea	1 (0)	NA	NA	Ancient	Toepad
U—Australia	9 (0)	NA	NA	Fresh	Blood (alcool)
V—North America	3 (0)	NA	NA	Fresh	Blood (alcool)
W—Caribbean	9 (0)	NA	NA	Ancient	Toepad
Total	200 (88)	51	37		

For each locality: total number of samples (N) and number of sexed animals into parentheses, number of males and females, tissue origins and sample type

Molecular sexing of ospreys was performed through PCR-amplification of the CHD (chromo-helicase DNA binding protein) gene using the P2 and P8 primers (Griffiths et al. 1998). This PCR allowed the amplification of the CHD-Z copy (about 350 pb) for the male (ZZ) and of CHD-Z and CHD-W (about 400pb) for the female (ZW), thus leading to only one fragment for males and to two fragments for females when PCR-products are run on a 2.5% agarose gel. The number of sexed animals per locality as well as the number of males and females is provided in Table 1.

Genetic analyses and population structure

The software MICRO-CHECKER v.2.2.3 (Van Oosterhout et al. 2004) was used to estimate null allele frequencies for each locus and population. Test for linkage disequilibrium (LD) was performed between pairs of loci using GENETIX 4.05 (Belkhir et al. 2004), considering that two loci were in disequilibrium when $p < 5\%$.

Several genetic diversity indices were calculated with GENALEX v6.4 (Peakall and Smouse 2006): the percentage of polymorphic loci (P%), the average number of alleles per locus (Ar), the number of private alleles (Ap), the observed heterozygosity (*Ho*), the expected heterozygosity (*He*) under the Hardy–Weinberg equilibrium (HWE), the inbreeding coefficient (F_{IS}). The probability associated with the F_{IS} (deviation from HWE) was calculated with GENEPOP4.3 (Rousset 2008) using a Markov chain dememorization of 10,000, 100 batches and 10,000 iterations per batch. The pairwise fixation index (F_{st}) and the associated probability were calculated between different groups and populations using the software FSTAT (Goudet 2002).

We used a Principal Component Analysis (PCA) to represent the general organization of the global genetic variability of the sampling considered. PCA was conducted using the software R v.2.15.1 (packages ade4 and adegenet) to rank individuals depending on their genetic proximity.

The number of genetic units within *Pandion haliaetus* has been evaluated with the Bayesian method implemented in Structure v. 2.1 (Pritchard et al. 2000; Falush et al. 2003). This software estimates the number of populations (K), maximizing the likelihood L(K) of the data observed from the likelihood values of the model parameters. The simulations under the admixture model were performed with a Monte

Carlo Markov Chain (MCMC) runs of 2×10^6 iterations with a burn-in period of 3.5×10^5. The number of cluster (K) tested ranged from 1 to 15 at most and, for each K value, analyses were repeated 15 times to test for the stability of the results. The determination of the most likely number of genetic group was estimated by the value of maximum likelihood as well as by the Evanno's method which is based on the calculation of the Delta K function (Evanno et al. 2005). An analysis under the Locprior model was also performed for the Palearctic group using the same characteristics for other parameters as previously described.

BayesAss v 1.3 (Wilson and Rannala 2003) was used to assess the direction and rate of contemporary gene flow between populations. To assure consistent and accurate estimates, we performed 10 different runs with different seed numbers. The MCMC was run for 1×10^8 iterations with a burn-in of 3×10^7 generations and a sampling frequency of 1000. To find the run that provides the best fit we calculated the Bayesian deviance according to Meirmans (2014) and we chose the run with the lowest deviance.

Results

Microsatellite characteristics and variability

Among the 40 selected loci, a correct amplification (a single band at the expected size) of these loci was first checked by PCR using non-labeled primers (cold primers). We kept 28 out of the 40 loci tested. Subsequent tests performed with fluorochrome-labeled primers (hot primers) confirmed the validity of 27 loci after reading their electrophoretic profile. The 27 loci were scored and associated in six multiplex (M1: 4 loci, M2: 5 loci, M3: 6 loci, M4: 4 loci, M5: 4 loci and M6: 4 loci; see Table 2). For one locus (Balbu19), PCR has been realized in monoplex because the amplification temperature was different from other loci and then added to M6 for genotyping. Characteristics and accession numbers of the 27 loci are given in Table 2.

Twenty out of the 27 loci were polymorphic for the 200 individuals analysed. Monomorphic loci (Balbu10, Balbu33, Balbu34, Balbu36, Balbu39, Balbu6 and Balbu38) were not included in the population structure analyses since they provide no discriminant information. Analysis with the software MICRO-CHECKER showed that 2 loci (Balbu28 and Balbu35) had null alleles for one population (N + O and K, respectively); the locus Balbu17 for two populations (K and P); and the locus Balbu23 for 5 populations (K, H, L, N + O, and U). No linkage disequilibrium (LD) was detected.

At the intra-population level, indices of diversity (Table 3) were calculated for 16 localities (excluding Indonesia and New Guinea where only one individual has been sampled). The lowest percentage of polymorphic loci was observed for Australia (35%), Cape Verde Islands (35%) and Canary Islands (20%), whereas the maximum value recorded was 70% for Finland + Sweden, Latvia and West Russia. The mean allelic richness per locus (Ar) ranged from a minimum of 1.30 for the Canary Islands to a maximum of 2.2 for Finland + Sweden and Latvia. Mediterranean populations (Morocco, Balearic Islands and Corsica) showed intermediate values of Ar = 1.8. In total, 24 private alleles were detected for the entire osprey complex. However, the majority of these private alleles were recorded in osprey populations of America (12) and Australia (5), whereas Mediterranean populations did not return any private alleles. Significant deviations from Hardy–Weinberg equilibrium (heterozygote deficits) were detected in 9 of the 16 populations (Table 3). F_{IS} values ranged from a minimum of 0.011 for the population of Portugal, to a maximum of 0.324 for those of the Cape Verde Islands (Table 3).

Population structure and differentiation

Analyses with Structure were conducted at several scales. A first analysis was run on the total dataset (200 individuals from all over the world) with a K variable value shifting from 1 to 15. The likelihood curve L(K) and those of delta (K) gathered with Evanno's method returned a maximum value of 2 clusters. The graph of genetic assignation with K = 2 (Figure S1A, Supplementary information) showed marked structuring distinguishing individuals from Americas (together with only one individual from Portugal, population code = C) and Australia (dark grey cluster in Figure S1A) within a group and the remaining other samples from the rest of the world in another group (light grey cluster). To have a more detailed view of this structuration, we repeated the analysis for each of the two clusters obtained. The analysis on the group containing Australians, Americans and one Portuguese individual (N = 22) was run with a K value shifting from 1 to 8, while the analysis of the Palearctic group (rest of the world: N = 178) with a K value from 1 to 13. In both cases, the likelihood curve L (K) and the delta (K) gave a maximum value of K = 2. This first group (Figure S1B, Supplementary Information) was hence split in two subgroups with Americas + Portugal from one side (light grey cluster) and Australian on another side (dark grey cluster). For the Palearctic group (excluding the individual from Portugal), Structure analyses returned the same results whatever the model used (Admixture or Locprior) indicating that this group was also split into two sub-units (Fig. 2): individuals from the Mediterranean basin (Morocco, Corsica, Portugal, Italy, Canary Islands and Balearic Islands; dark grey cluster = MEDITERRANEAN) and individuals coming from continental Europe, Cape Verde Islands and Arabian Peninsula (dark grey cluster = EURASIA). Hybrid individuals (HYB) between these two groups have been identified in

Table 2 Characteristics of the 27 microsatellite markers developed for *Pandion haliaetus*: size and length of the repeated motif, GenBank accession number, 5′–3′ Forward and Reverse primers, PCR annealing temperature (Tm), colour code of the fluorochrome (F*) used for each forward primer and code indicating loci sharing multiplex PCR reactions

Locus	Repeat motif	Accession no	Forward primers	Reverse primers	Tm (°C)	F*	Multiplex
Balbu11	3–6 (CATA)	MG066657	5′-TGTTACCTTGTTTTGGGAGGA-3′	5′-CATGGAAGATGGAGATGCAA-3′	50	VIT	M1
Balbu15	4–5 (ATGT)	MG066660	5′-TTTTGCCAAATCCAAGTTCTG-3′	5′-TGTTGTTGAGGTAAGCTGCG-3′	50	PET	M1
Balbu18	9 (TTG)	MG066662	5′-AAACAAAACCCAATTTCTGGC-3′	5′-TGCCATCCAAGAGTAGTGGC-3′	50	VIT	M1
Balbu40	6 (TGT)	MG066679	5′-TGATAAACCTGTTAGGCCTTCAA-3′	5′-CATGGACTTTGATGTTTGCAT-3′	50	FAM	M1
Balbu12	6 (ATAG)	MG066658	5′-TGCTCAGATACTACAGAAGTGAAGTG-3′	5′-TCACTGGCTTTGGAAGAACC-3′	55	VIC	M2
Balbu25	8 (GGT)	MG066666	5′-GCAGAGGTACCTGGTGCTTT-3′	5′-CCAAAATAGTCTACAACAGGCAA-3′	55	PET	M2
Balbu30	7 (GGA)	MG066670	5′-GCAGCCAATTAAGGTCTTATTAGAGAG-3′	5′-CACTCTGCCTAGAAACATCACTG-3′	55	VIC	M2
Balbu35	6 (AAC)	MG066674	5′-GGGCAAAATTGGGATATACTG-3′	5′-TGAACTACTTGTACTGGGGGAGA-3′	55	FAM	M2
Balbu37	6 (TTG)	MG066676	5′-TGGATTTAAACTGCACTCCG-3′	5′-CAGTAGCAGGACTGAGGGCT-3′	55	NED	M2
Balbu14	5 (AGAT)	MG066659	5′-TTTGACTTCTAGCTTTGCATTAGA-3′	5′-CTGGTGCAGAGGCCTTTATG-3′	53	FAM	M3
Balbu21	9 (ACA)	MG066664	5′-TGCATTTGCTAGAAGTAGCCC-3′	5′-GGATGGTCCTCAGCTTGATT-3′	53	NED	M3
Balbu23	8 (GGA)	MG066665	5′-AGATCAGGCCAAGCGTCTC-3′	5′-GGATTGAAGTGTTGTGATGACC-3′	53	VIC	M3
Balbu28	7 (GGA)	MG066668	5′-TTCTTGCTTGCCAGTGTGAC-3′	5′-GGGCTGGTGAATTTTCTAGG-3′	53	PET	M3
Balbu29	7 (GTT)	MG066669	5′-CTTCAAAGTGACGCAAGTCAA-3′	5′-TGTGCAACTTCCTACAATAATGC-3′	53	NED	M3
Balbu31	7 (AGG)	MG066671	5′-CAAGTGCCTTGGGACTTTGT-3′	5′-GCTCTCAGGGATGGGTGATA-3′	53	PET	M3
Balbu10	6 (TATG)	MG066656	5′-CATTTCAGGCATTTTAGCAGG-3′	5′-GGCAGGATCTCACTGAAGGA-3′	53	NED	M4
Balbu17	11 (AAC)	MG066661	5′-TCAGTCTTCCACCAAGGAATG-3′	5′-TCTGACAGCACTCAGCATACAA-3′	53	FAM	M4
Balbu33	6 (TCC)	MG066672	5′-CTTTTAATTCAATAAATCCTACCCC-3′	5′-ATAAGTGGAGAATTCTGTCCAGATG-3′	53	VIC	M4
Balbu34	6 (ACA)	MG066673	5′-ATAGCAGATGAAGGAGGCGA-3′	5′-TTGATTTATGAAGAGGAATGGGA-3′	53	PET	M4
Balbu02	5 (CTCCTT)	MG066653	5′-TCTCCAGCAAGCTACACCCT-3′	5′-TGCTGAGCTGTATGGTAGACAAA-3′	53	PET	M5
Balbu26	8 (TGT)	MG066667	5′-GGGCCATGTTATCAAGAGGA-3′	5′-GGGGCTCTGTTCTAACACTTT-3′	53	NED	M5
Balbu36	6 (AAC)	MG066675	5′-AGAGGCAGTCATTGAACCTCA-3′	5′-AATTCTTCCCACCTGACCAC-3′	53	FAM	M5
Balbu39	6 (TGT)	MG066678	5′-TCTCGGTTTCCCTAACTATAGAACA-3′	5′-TGGTTTAGAAATGTGCTGTTTCA-3′	53	VIC	M5
Balbu05	6 (GTTTT)	MG066654	5′-TGGCAATGCTACCACTGAAG-3′	5′-TGGCAGCTCTAGGAAAGTAAAGA-3′	53	NED	M6
Balbu06	5 (GTTTT)	MG066655	5′-ACCTCAGCTGTCCCTTTTCT-3′	5′-CCAAAGGTCTTCCTGTTTGC-3′	53	VIC	M6
Balbu38	6 (TCC)	MG066677	5′-GAAACAGGAAATGCATCCCTTA-3′	5′-CAACAGCTGTGTTTGAATCTGG-3′	53	VIC	M6

Table 2 (continued)

Locus	Repeat motif	Accession no	Forward primers	Reverse primers	Tm (°C)	F*	Multiplex
Balbu19	9 (AAC)	MG066663	5′-ACACTGGAAGTTGCCAAA GC-3′	5′-TCACCCAATGGGGTAAAG AT-3′	50	FAM	postM6

Table 3 Summary of genotypic data calculated for 16 populations of *Pandion haliaetus*

Pop	N	P%	Ar	Ap	Ho	He	F_{IS}
Canary Islands	10	20	1.3	0	0.105	0.083	− 0.272
Morocco	6	45	1.6	0	0.167	0.167	− 0.028
Portugal	7	45	1.35	0	0.140	0.129	0.011*
Balearic Islands	22	45	1.75	0	0.141	0.160	0.103
Corsica + Italy	32	55	1.85	0	0.176	0.199	0.090
Cape Verde Islands	8	35	1.35	0	0.071	0.103	0.324*
France + Germany	18	65	2	0	0.189	0.221	0.102**
Finland + Sweden	14	70	2.2	2	0.204	0.254	0.158**
Latvia	15	70	2.2	0	0.270	0.281	0.040*
Estonia	8	65	2.1	1	0.281	0.272	− 0.051
Middle East + Persian Gulf	10	55	1.75	1	0.122	0.194	0.217*
West Russia	9	70	2.00	0	0.228	0.247	0.030*
East Russia	12	50	1.80	1	0.203	0.205	0.023
Japan	5	60	1.75	2	0.260	0.222	− 0.128
Australia	9	35	1.6	5	0.108	0.135	0.160*
North America + Caribbean	12	60	2.00	12	0.172	0.239	0.195**

N sample size, *P%* percentage of polymorphism, *Ar* average number of alleles per locus, *Ap* number of private alleles, *H0* observed heterozygosity, *He* expected heterozygosity, F_{IS} inbreeding coefficient, F_{IS} significance *P < 0.05, **P < 0.001

different places: Cape Verde Islands (G), continental France (H), Finland (K), and Latvia (L) for the EURASIA group and Portugal (C) and Corsica (E) for the MEDIT group that appeared more homogenous. Thus, ospreys appeared structured in four groups geographically well-defined: America, Australia and two in the Palearctic (Mediterranean and Eurasia).

To assess the importance of sex-biased gene flow in the structure observed, the sex of individuals (known for 37 females and 51 males of the Palearctic group; see Table 1) has been indicated on the Structure plot (Fig. 2). The aim was to determine if migrants between the two groups included more females than males. To this purpose an individual was considered as introgressed when more than 50% of its genetic composition was composed by the genetic background of the other group than the one it was supposed to belong. Ten individuals have been identified as introgressed (indicated by an asterisk on Fig. 2), representing five individuals for each identified group (Mediterranean and Eurasia). These individuals represented two females and three individuals of unknown sex for the Mediterranean group and two females, one male and two unknowns for the Eurasian group. In total, 11% of females (4/37) were identified as introgressed versus 2% of males (1/51).

PCAs were carried out on the 20 polymorphic microsatellite loci and counted 76 variables (total number of alleles expressed in the set of loci). An analysis (Figure S2 in Supplementary Information) was performed on the four major groups previously identified. The two first axes explained 19 and 11% of the variance, respectively. The PCA distinguished three main distinct genetic groups: Australia (U), America + Caribbean (V + W) and the Palearctic group (all other populations) among which the Mediterranean and Eurasian groups partly overlapped. The individual from Portugal previously identified as belonging to the American group with Structure here appeared in the middle of the space.

Population differentiation was assessed by calculating pairwise F_{st} values, estimated for the four major groups (America + Caribbean, Australia, Mediterranean and Eurasia) as well as between 14 Palearctic populations, excluding those with < 5 individuals (Italy, Germany, Sweden, Persian Gulf, Indonesia and New Guinea) (Table S1 in Supplementary Information). The highest values of genetic differentiation, recorded for Australia and America, indicated that these populations were clearly differentiated from those of the Palearctic. All F_{st} values were highly significant between the major four groups with the smallest value (0.12) being recorded between the Palearctic Mediterranean and Eurasian

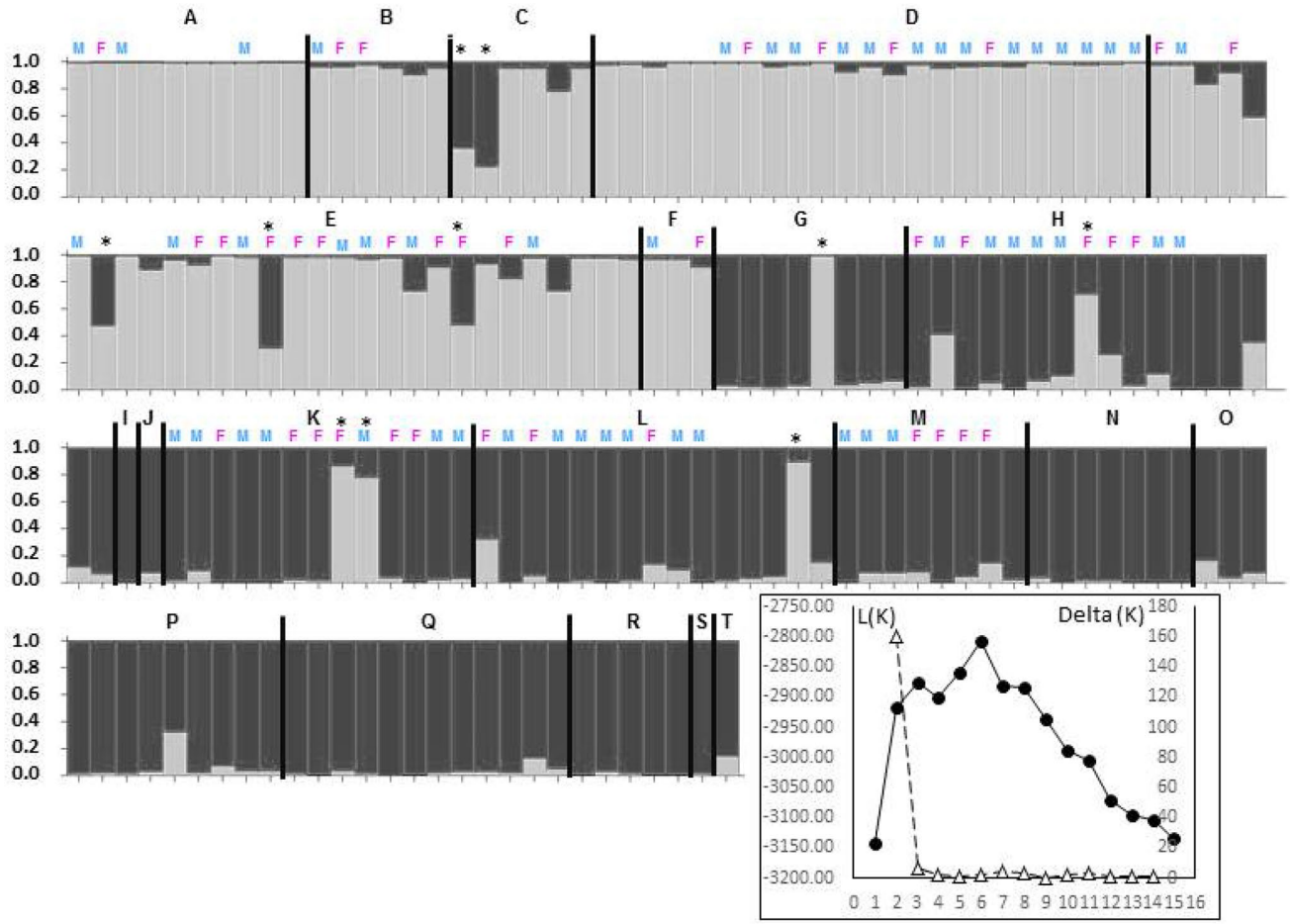

Fig. 2 Genetic assignation of ospreys from the Palearctic (N = 178) using Structure. In inset curves showing mean of the natural logarithm of the likelihood L(K) (left y-axis) and of delta(K) (right y-axis) computed following the Evanno's method (2005) using 15 replicate runs for each K (x-axis). Barplots show assignment of individuals to clusters for K = 2. The y-axis represents the probability to belong to a certain cluster, while on the x-axis is reported each population (code name given in Table 1) delimited by a black solid vertical line. Each colour represents a cluster and each vertical bar a single individual. Sex of individuals (*F* female, *M* male) is indicated when known. An asterisk denotes individuals that have been considered as introgressed. (Color figure online)

groups. Within the 14 Palearctic populations, the F_{st} values ranged from −0.03 (between Morocco and Balearic Islands) to 0.52 (between Canary Islands and Cape Verde Islands) and 45% of F_{st} are significant, thus showing large amplitude in genetic differentiation between populations.

Contemporary gene flow between populations

Migration rates were first estimated between the four main lineages (Australia, America, Mediterranean and Eurasian Palearctic) (Table 4). For the 10 replications, the deviance

Table 4 Migration rate (mean and standard deviation into parentheses) between the four major groups evidenced for the osprey, estimated as the fraction of individuals in population i (group of destination in column) that are migrants (per generation) derived from population j (group of origin in line)

to \ from	Mediterranean	Eurasia	Australia	America
Mediterranean	*0.98* (*0.01*)	0.01 (0.01)	0.004 (0.004)	0.008 (0.005)
Eurasia	**0.04 (0.02)**	*0.95* (*0.02*)	0.003 (0.003)	0.003 (0.003)
Australia	0.026 (0.02)	0.025 (0.02)	*0.92* (*0.03*)	0.025 (0.02)
America	0.021 (0.02)	0.021 (0.02)	0.021 (0.02)	*0.94* (*0.03*)

The highest value for the migration rate is indicated in bold. The proportion of non-migrants in each group is indicated in italics

varied from 4751.38 to 4751.925, indicating that all runs converged properly (Meirmans 2014). The proportion of non-migrants was high for each group and varied from 0.92 (Australia) to 0.98 (Mediterranean). The highest migration rate was 0.04 from the Mediterranean to the Eurasian groups, meaning that about 4% of this group was composed of migrants from the Mediterranean.

A second analysis was performed between the 14 populations from the Palearctic (Table S2). As previously, the 10 runs showed a good convergence and the run with the lowest deviance (3451.161) was selected. Among the Mediterranean group, a significant proportion of migrants per generation (> 5%) was observed from Corsica to Morocco, Balearic Islands and Portugal as well as from Balearic Islands to Canary Islands, Morocco and Corsica. Among the Eurasian group, a significant migration rate (> 5%) was identified from Latvia to Finland and West Russia as well as from East Russia to Japan (Table S2). Some populations, such as Balearics, Corsica, Latvia and France (but with lower rates) appeared as major donor of migrants (source populations).

Discussion

We produced 27 new microsatellites specifically developed for the osprey, among which 20 loci were recorded polymorphic when analyzed for 200 individuals from all over the world. These microsatellites can thus be added to the 26 markers previously obtained by Dawson et al. (2015).

Genetic differentiation at global scale

At the global scale, the osprey appeared to be genetically split in three main groups: Australasia, America and Palearctic between which gene flow is almost non-existent. Isolation has been probably promoted by oceanic barriers, which might have prevented connectivity and gene flow between these groups, as also suggested by F_{st} values among populations. These outcomes are only in partial accordance with the taxonomic classification of osprey subspecies based on morphological characters (Cramp and Simmons 1980), but rather were in line with results found in analyses carried out with mtDNA (Monti et al. 2015), although microsatellites did not discriminate the fourth phylogeographical clade in eastern Asia found in the later study. Worldwide, the average allelic richness values (Ar) of the osprey complex swung between 1.3 and 2.4, that is quite low compared to other studies on long-lived raptor species, sharing similar migratory traits. For instance, a study on the genetic structure of a partially migratory American kestrel (*Falco sparverius*), based on five microsatellite loci and 211 individuals sampled across 13 populations in North America, showed Ar values between 3 and 5.2 (Miller et al. 2012). In Egyptian vulture (*Neophron percnopterus*), a long-lived migratory species, with a marked philopatric behaviour, Ar values ranged between 2.42 and 3.08 from 22 loci analysed for 176 individuals across six populations of the Iberian Peninsula (Agudo et al. 2011). In the cosmopolitan Peregrine Falcon (*Falco peregrinus*), mean Ar values ranged between 1.4 and 4.4, on the basis of 12 microsatellite loci studied for 146 individuals from ten populations distributed over the world (Nesje et al. 2000).

Moreover, the heterozygote deficit detected in nine populations could be due to a decrease in the observed heterozygosity produced by the mixture in a given population of individuals genetically heterogeneous (i.e. Wahlund effect). Another cause could be an assortative mating, especially for insular populations (e.g. Balearic Islands, Cape Verde Islands).

The particular case of the Portuguese sample (being the only one showing an affinity with American samples) should be considered with caution. By looking at its genotype it has just one locus showing allele from America, the rest of the genotype is clearly from the Palearctic group. This old specimen was definitely collected in Portugal, but the exact origin was not clear: it could have come from Portuguese islands such as the Azores, in the middle of the Atlantic, where at least one young osprey originating from north America have been recently observed (Strandberg 2013). However, all the other six Portuguese samples (belonging to the same collection) are within the Mediterranean group.

Population connectivity in the western palearctic

Two sub-entities geographically featured were found in the Palearctic, with a first sub-group from Japan to north-east Europe (EURASIAN group) and a second sub-group in the Mediterranean area (MEDIT group). Although the two sub-groups were interconnected, the estimated gene flow appeared limited and asymmetric (mainly from Mediterranean to Eurasia; see Table 4). Factors influencing such genetic diversity and structuration were probably the result of migratory and dispersal strategies on the one hand, and to the strong phylopatric behaviour, on the other hand. These results corroborate recent findings of differences in migratory behaviour in osprey populations of the Western Palearctic, via individual GPS tracking (Monti et al. 2018). Despite recent mitochondrial DNA analyses grouped all the Western Palearctic osprey populations within only one evolutionary clade (Monti et al. 2015), the existence of two sub-entities identified by means of microsatellite analyses is a novel result which could be due to: (a) variation in mutation rate between microsatellites and mtDNA that respond to diverse temporal evolutionary scales; (b) different types of genetic transmission (i.e. mtDNA transmitted exclusively via maternal).

The clustering analysis revealed signs of introgression between the two geographic clusters (hybrid individuals presenting both colours on Fig. 2). The estimated migrations rates between 14 Palearctic populations (Table S2) indicated that exchange of migrants was geographically restricted between mainly Mediterranean (Corsica, Morocco, Balearic Islands, Canary Islands and Portugal) and Western (Latvia, Finland and West Russia) or Eastern (East Russia and Japan) Eurasian populations. Exchanges between the Mediterranean and Eurasian sub-groups appeared at a very low rate (less than 4%) and limited to some populations (from Balearic Islands and Corsica to Estonia, Latvia and Cape Verde Islands; see below about the role of sex in shaping genetic structure).

A possible explanation for this genetic structuration at the scale of the Palearctic could be related to the migratory behaviour of different populations. Three main flyways are known in the Palearctic for ospreys to reach African wintering grounds (Fig. 3): (i) a western flyway passing through the strait of Gibraltar until sub-Saharan wintering grounds followed by north-western ospreys (e.g. Alerstam et al. 2006); (ii) a central flyway through Corsica-Sardinia and Italy (e.g. Bai and Schmidt 2011); and (iii) an eastern flyway through Middle East and Red Sea (e.g. Newton 2010). Accordingly, northern ospreys migrating along the western flyway and wintering in west Africa (the coast of Senegal-Mauritania being a major wintering site) may have colonized Cape Verde Islands, assisted by easterly trade winds, and thus would explain the dominance of Eurasia-like genotypes in Cape-Verde archipelago. A similar process could have happened for populations residing in the Red Sea area (that are predominantly with Eurasia genotype) across the eastern flyway. These birds living in tropical environment would have lost their migratory behaviour subsequently. On

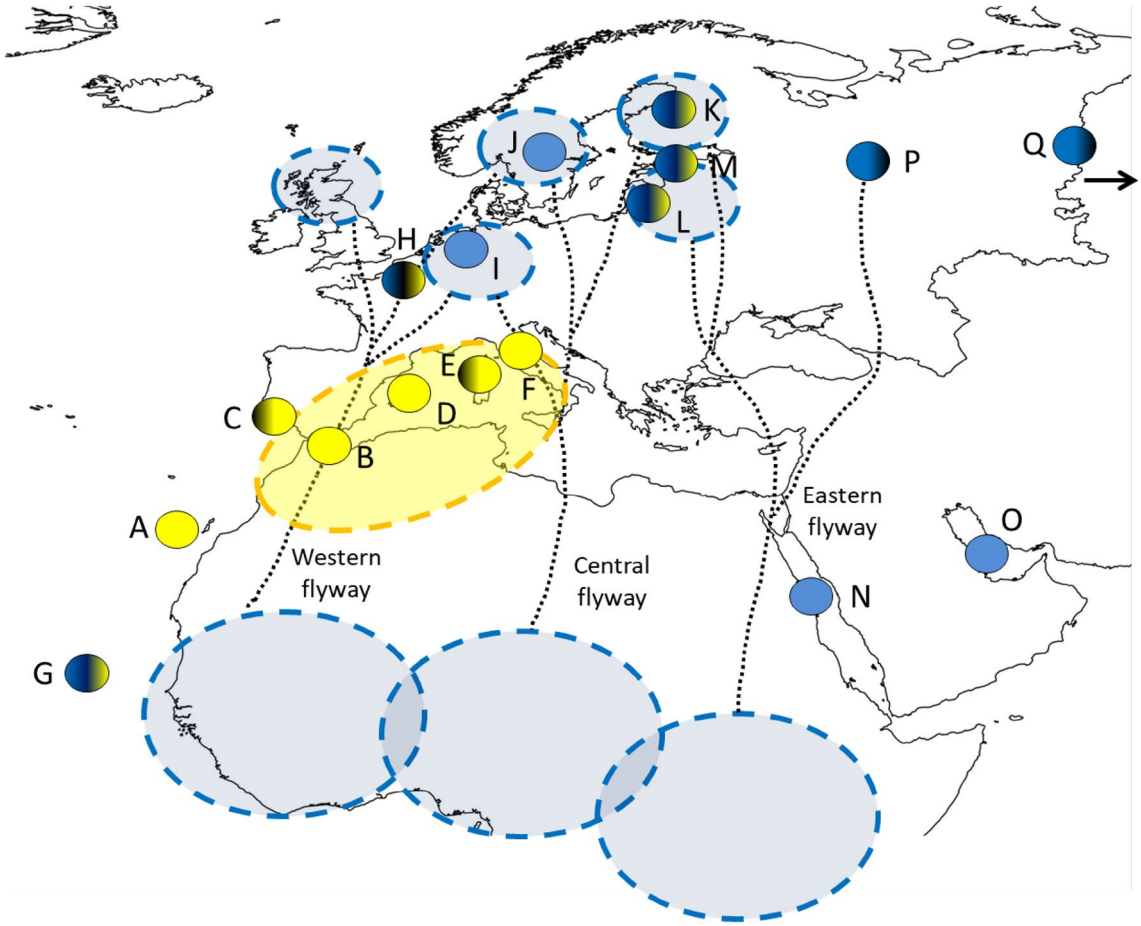

Fig. 3 Graphic representation of genetic structuration of osprey populations in the Western Palearctic. Each dot indicates a sampled population, defined by a letter as reported in Table 1. The colour of each dot varies between blue for the Eurasian genetic group (continental Europe), yellow for the MEDIT group (Mediterranean) and black for HYB (hybrids) individuals sharing both of the allelic information. Dotted areas and lines represent main migration flyways in the Western Palearctic (blue for long-distance migrant osprey from continental Europe, yellow for short distance migrants or resident osprey from Mediterranean). The flyways and wintering areas shown here are adapted from: Klaassen et al. (2008), Zwarts et al. (2009), Väli and Sellis (2016), Mackrill (2017), Monti et al. (2018). (Color figure online)

the other hand, osprey from Canary Islands share the same Mediterranean genotype with the populations from Balearics, Morocco and Corsica, and thus were probably originating from birds from the Mediterranean basin. Within the Mediterranean, no genetic differentiation was found between the different populations. The absence of structuration at this level might be due to the fact that movements of individuals are probably not much affected by the sea crossing (Monti et al. 2018), which seems not to operate as a physical barrier (e.g. Horton et al. 2014). In this sense, osprey populations living in the Mediterranean still seem to be connected by gene flow.

The role of sex in shaping genetic structure

Sexual differences in dispersal patterns within a species or population are important for reducing the degree of inbreeding (Limiñana et al. 2012). Despite a strong philopatric behaviour, the osprey shows a certain degree of dispersal which allows populations to maintain genetic variability and admixture. As for other bird species (see Greenwood 1980 for a review), including raptors (e.g. Cadahía et al. 2009; Hernández-Matías et al. 2010; Limiñana et al. 2012), dispersal in ospreys is sex-biased in favour of females that tend to cover greater distance than males, which are more philopatric (Martell et al. 2002; Monti et al. 2014). In osprey, males, once sexual maturity is reached, tend to return to their natal area to settle: there they build and defend a nest where they attract a female in dispersal, then, once mated, providing the female with food during most of the breeding season (Poole 1989). On the other hand, females tend to move further and to breed outside the natal areas, thus showing a higher dispersal ability.

Our Bayesian analyses performed with Structure indicated that more females (about 11%) than males (2%) have been identified as highly introgressed, and thus can be considered as migrants. Therefore, our results suggest that females contribute much to maintain a certain degree of admixture between populations. However, more data would be necessary (particularly by sexing more introgressed individuals) to confirm that a high capacity for genetic exchange between osprey populations, at the Palearctic scale, is mostly driven by female individuals.

Such behavioural mechanism favouring a low degree of population genetic structuring has been described also in the Montagus harrier (*Circus pygargus*; García et al. 2011). Conversely, philopatric males (which showed a more marked structure in two clusters) tend to play a fundamental role in maintaining a population genetic structure, characterized by many private alleles and heterozygote deficiency. In the osprey, these antagonistic behaviours between sexes (philopatry vs. dispersal) seem to play concurrently in shaping genetic structure and diversity at different scales. To better understand their respective influence on the global genetic structuring, it is mandatory to investigate migratory strategies and dispersal patterns of individuals from different populations and along different migratory flyways.

Conservation issues

Our results provide essential information for management and conservation of the species and call for the definition of at least 2–4 Management Units (MU) in the Western Palearctic. It becomes obvious that ospreys from the Mediterranean basin behave differently than ospreys from Continental Europe (Monti et al. 2018), and the gene flow is limited (but not null) between these populations. Mediterranean ospreys are mostly resident or migrate over short distances within the Mediterranean basin (Monti et al. 2018), while all continental ospreys are long-distance migrants wintering in tropical Africa. Therefore, these two populations could be defined as 2 MUs for immediate conservation actions. While populations from northern and central Europe are globally increasing with several thousand breeding pairs (Schmidt-Rothmund et al. 2014), populations from Mediterranean are threatened with less than 80 breeding pairs, with various trends between localities (Monti 2012). Our results also show that the resident ospreys from Cape Verde Islands and Middle-Est (Red Sea and Persian Gulf) belong to a different subgroup as the Mediterranean birds. Hence these populations should deserve 2 other MUs.

Conservation of ospreys in Mediterranean should target recolonization of the former breeding range on islands (e.g. Sardinia, Sicily) as well as on continental shores (Italy, France, Spain, Tunisia). To achieve this goal by natural recolonization, birds can be attracted to new breeding sites by installing artificial nests, as it worked for increasing the population in Corsica (Bretagnolle et al. 2008), but it is mostly efficient for new sites at short distances from the source population. For recolonization of sites distant of several kilometers, reintroductions by means of translocations of chicks have proved to be much more efficient (Dennis and Dixon 2001; Monti et al. 2014). However, our results call for caution when choosing the source population: it must preferentially belong to the same MU, to account for the difference in genetic structure and migratory behaviour (Monti et al. 2018). This was the solution chosen for reintroducing osprey in Tuscany, Italy, with a source population from nearby Corsica (in the same MU; Monti et al. 2014). Yet in the current years, the populations in Mediterranean islands are probably too small and vulnerable to tolerate the translocation of several chicks per year (e.g. Monti et al. 2013). However, it should be mentioned that translocating migratory birds from source populations in areas where different genetic structure (MU) or even migratory strategies have been detected, might have ecological consequences and

promote new behaviours in newly established populations. Conservation programmes should take into account population connectivity and respect genetic structure that can best maintain processes and potential for evolutionary change.

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Supplementary Information

Table S1: Values of F_{st} between 16 populations (14 from Palearctic, Australia, America + Caribbean) obtained from 20 microsatellites loci of *Pandion haliaetus*. In inset F_{st} values between the four major groups. *: p<0.05; **: p<0.005; ***: p<0.0005.

	Canary Is.	Morocco	Portugal	Balearic Is.	Corsica	Cape Verde	France	Finland	Latvia	Estonia	Middle East	East Russia	West Russia	Japan	Australia
Canary Is.															
Morocco	0.13*														
Portugal	0.37	0.12													
Balearic Is.	0.12**	-0.03	0.15												
Corsica	0.15***	-0.01	0.09	0.04											
Cape Verde	0.52	0.35	0.47	0.35**	0.35***										
France	0.35***	0.13*	0.20	0.18***	0.13***	0.38**									
Finland	0.31***	0.10	0.14	0.16***	0.10***	0.25**	0.06*								
Latvia	0.29***	0.07	0.11	0.14***	0.07***	0.26**	0.04*	-0.01							
Estonia	0.3**	0.07	0.17	0.13***	0.09***	0.23	0.09**	0.03	0.00						
Middle East	0.49*	0.26	0.25	0.34**	0.26***	0.40	0.2**	0.12	0.10	0.15					
East Russia	0.39	0.13	0.22	0.18**	0.10*	0.36	0.10*	0.01	0.01	0.04	0.24				
West Russia	0.36***	0.15*	0.14	0.23***	0.15***	0.34**	0.05*	0.03	0.01	0.09	0.14*	0.10			
Japan	0.48	0.23	0.41	0.3**	0.21**	0.50	0.15*	0.13	0.10	0.13	0.24	0.12	0.16		
Australia	0.75**	0.63*	0.65	0.63***	0.57***	0.73*	0.52***	0.49***	0.48***	0.52**	0.59	0.53	0.50**	0.59	
America	0.69**	0.59	0.65	0.64***	0.60***	0.64	0.55***	0.51**	0.51***	0.51*	0.56	0.53	0.53**	0.54	0.64*

	Mediterranean	Eurasia	Australia
Eurasia	0.12***		
Australia	0.6***	0.44***	
America	0.63***	0.50***	0.63***

Table S2: Migration rate (mean and standard deviation into parentheses) between the 14 populations from the Palearctic.

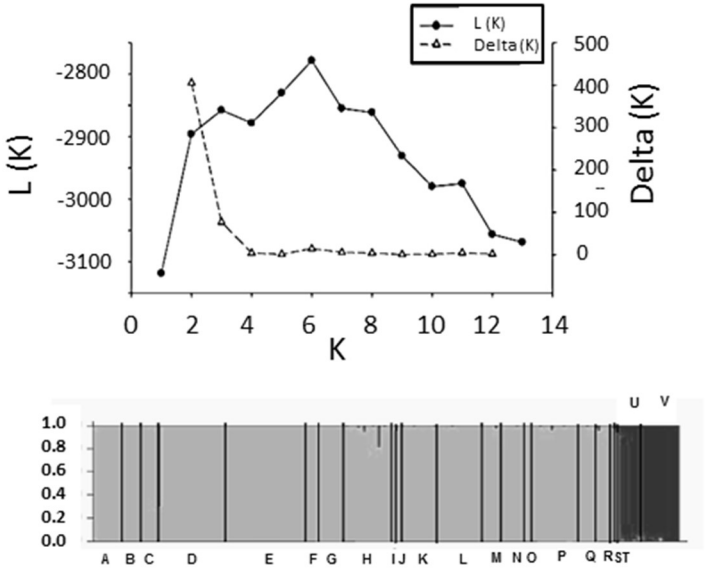
to \ from	0: Canary islands	1: Morocco	2: Portugal	3: Balearic islands	4: Corsica	5: Cape Verde	6: France	7: Finland	8: Latvia	9: Estonia	10: Middle East	11: West Russia	12: Est Russia	13: Japan
0: Canary islands	0.6805 (0.0133)	0.0138 (0.0132)	0.0140 (0.0134)	0.1505 (0.0341)	0.0160 (0.0155)	0.0140 (0.0135)	0.0142 (0.0136)	0.0138 (0.0133)	0.0138 (0.0133)	0.0140 (0.0133)	0.0140 (0.0134)	0.0139 (0.0133)	0.0139 (0.0133)	0.0138 (0.0132)
1: Morocco	0.0167 (0.0159)	0.6835 (0.0160)	0.0167 (0.0160)	**0.0685 (0.0383)**	0.0476 (0.0347)	0.0185 (0.0178)	0.0224 (0.0210)	0.0183 (0.0175)	0.0207 (0.0200)	0.0174 (0.0166)	0.0168 (0.0161)	0.0181 (0.0173)	0.0181 (0.0175)	0.0167 (0.0159)
2: Portugal	0.0170 (0.0161)	0.0167 (0.0158)	0.6833 (0.0158)	0.0334 (0.0302)	**0.0623 (0.0394)**	0.0195 (0.0187)	0.0249 (0.0234)	0.0210 (0.0207)	0.0262 (0.0257)	0.0189 (0.0184)	0.0177 (0.0169)	0.0222 (0.0206)	0.0199 (0.0195)	0.0169 (0.0161)
3: Balearic islands	0.0090 (0.0088)	0.0090 (0.0088)	0.0089 (0.0088)	0.8044 (0.0620)	**0.0728 (0.0590)**	0.0097 (0.0095)	0.0129 (0.0130)	0.0105 (0.0104)	0.0127 (0.0133)	0.0104 (0.0103)	0.0092 (0.0089)	0.0102 (0.0101)	0.0111 (0.0110)	0.0092 (0.0089)
4: Corsica + Italy	0.0078 (0.0076)	0.0076 (0.0075)	0.0077 (0.0075)	**0.0962 (0.0575)**	0.7497 (0.0586)	0.0087 (0.0086)	0.0224 (0.0332)	0.0140 (0.0160)	0.0240 (0.0327)	0.0096 (0.0112)	0.0082 (0.0081)	0.0124 (0.0135)	0.0238 (0.0335)	0.0078 (0.0077)
5: Cape Verde	0.0152 (0.0145)	0.0150 (0.0144)	0.0153 (0.0146)	0.0369 (0.0253)	0.0207 (0.0191)	0.7217 (0.0458)	0.0374 (0.0432)	0.0274 (0.0340)	0.0202 (0.0221)	0.0200 (0.0236)	0.0212 (0.0269)	0.0175 (0.0193)	0.0164 (0.0170)	0.0152 (0.0145)
6: France + Germany	0.0109 (0.0106)	0.0108 (0.0105)	0.0107 (0.0104)	0.0215 (0.0194)	0.0292 (0.0269)	0.0203 (0.0206)	0.7190 (0.0391)	0.0267 (0.0271)	0.0415 (0.0378)	0.0203 (0.0182)	0.0126 (0.0126)	0.0329 (0.0332)	0.0324 (0.0306)	0.0111 (0.0110)
7: Finland + Sweden	0.0123 (0.0120)	0.0123 (0.0118)	0.0123 (0.0119)	0.0227 (0.0198)	0.0222 (0.0205)	0.0160 (0.0160)	0.0317 (0.0287)	0.6981 (0.0294)	**0.0590 (0.0418)**	0.0168 (0.0164)	0.0138 (0.0136)	0.0337 (0.0300)	0.0361 (0.0316)	0.0129 (0.0125)
8: Latvia	0.0115	0.0115	0.0114	0.0195	0.0392	0.0148	0.0357	0.0383	0.7016	0.0189	0.0128	0.0334	0.0396	0.0117

	(0.0112)	(0.0111)	(0.0111)	(0.0177)	(0.0293)	(0.0145)	(0.0317)	(0.0356)	(0.0305)	(0.0186)	(0.0126)	(0.0304)	(0.0351)	(0.0114)
9: Estonia	0.0153	0.0152	0.0153	0.0407	0.0394	0.0185	0.0257	0.0258	0.0353	0.6836	0.0174	0.0235	0.0287	0.0157
	(0.0146)	(0.0145)	(0.0146)	(0.0297)	(0.0308)	(0.0181)	(0.0236)	(0.0238)	(0.0292)	(0.0164)	(0.0168)	(0.0218)	(0.0248)	(0.0150)
10: Middle East + Persian Gulf	0.0159	0.0158	0.0159	0.0167	0.0177	0.0444	0.0276	0.0346	0.0271	0.0188	0.7048	0.0233	0.0213	0.0161
	(0.0152)	(0.0151)	(0.0152)	(0.0159)	(0.0168)	(0.0461)	(0.0323)	(0.0378)	(0.0291)	(0.0189)	(0.0395)	(0.0247)	(0.0243)	(0.0154)
11: West Russia	0.0128	0.0129	0.0128	0.0154	0.0191	0.0165	0.0344	0.0370	**0.0647**	0.0160	0.0148	0.6951	0.0353	0.0133
	(0.0123)	(0.0125)	(0.0123)	(0.0147)	(0.0181)	(0.0165)	(0.0318)	(0.0350)	**(0.0459)**	(0.0156)	(0.0148)	(0.0247)	(0.0324)	(0.0129)
12: East Russia	0.0145	0.0146	0.0145	0.0200	0.0315	0.0176	0.0444	0.0257	0.0434	0.0182	0.0153	0.0266	0.6986	0.0151
	(0.0140)	(0.0139)	(0.0138)	(0.0185)	(0.0282)	(0.0169)	(0.0360)	(0.0257)	(0.0354)	(0.0177)	(0.0147)	(0.0262)	(0.0265)	(0.0148)
13: Japan	0.0176	0.0175	0.0177	0.0176	0.0192	0.0183	0.0242	0.0226	0.0330	0.0178	0.0182	0.0255	**0.0661**	0.6847
	(0.0165)	(0.0166)	(0.0168)	(0.0167)	(0.0183)	(0.0176)	(0.0245)	(0.0235)	(0.0337)	(0.0169)	(0.0173)	(0.0279)	**(0.0452)**	(0.0174)

Figure S1: Genetic assignation of ospreys using STRUCTURE. On the left-hand side, curves showing mean of the natural logarithm of the likelihood L(K) (left y-axis) and of deltaK (right y-axis) computed following the Evanno's method (2005) using 15 replicate runs for each K (x-axis). On the right-hand side, graphs showing assignment of individuals to clusters: the y-axis represents the probability to belong to a certain cluster, while on the x-axes is reported each population (code

name given in Table 1) delimited by a black solid vertical line. Each colour represents a cluster and each vertical bar a single individual. Analysis were carried out on: A – all individuals; the whole dataset with ospreys from all around the world (N = 200; K = 2); B – individuals from Australia, Caribbean and America (N = 22; K = 2).

A – All Individuals



B – Individuals from Australia + America

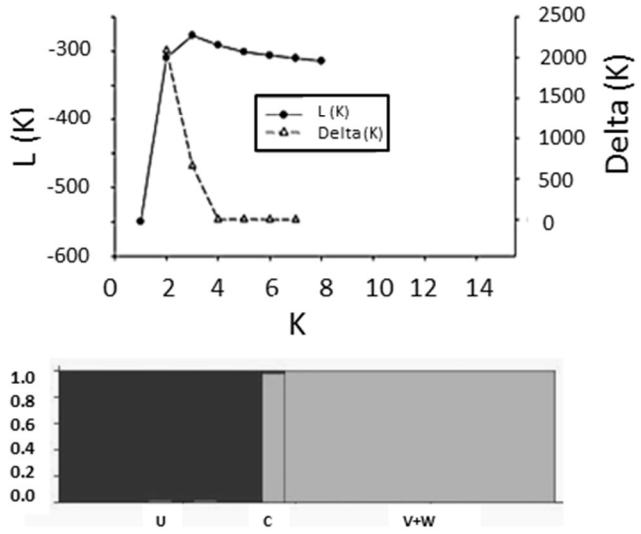


Figure S2: PCA based on the polymorphic character of 20 microsatellites loci (76 alleles) of osprey from 16 populations (N=200). Genetic groups were violet for Australia, blue for America + Caribbean and grey (Mediterranean) / orange (Eurasia) for the two Palearctic groups.