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Genetic variability in Peregrine Falcon populations of the Western Palaearctic region

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Abstract We analysed variation in ten polymorphic microsatellite loci and a portion of cytochrome b gene of mitochondrial DNA in 65 samples from four populations of Peregrine Falcon (*Falco peregrinus peregrinus* and *F. p. brookei*) breeding in Northern and Southern Italy, Northern Spain and the Czech Republic to assess genetic diversity in the poorly investigated Western Palearctic region. We added to our cytochrome b sequences a dataset of previously published mtDNA sequences of other populations and subspecies to outline genetic variation in the region on a worldwide basis. Regarding mtDNA we identified 12 haplotypes from our 65 Peregrine Falcon samples, nine of which were new and three already known. The 52% of our samples, including all Italian and Czech specimens, belonged to the previously identified HI haplotype, another 22% of the samples, most of which were from Sicily, showed the new H1 haplotype, while the remaining 26% of the sample partitioned among the other 10 haplotypes. Allelic patterns and genetic structuring of microsatellites were similar to those of other European populations. Genetic differentiation in both mtDNA and microsatellites loci is almost absent and it is not possible to distinguish geographical groups according to taxonomic designation at the subspecies level.

Keywords: Genetic structuring, *Falco peregrinus brookei,* microsatellites, mitochondrial DNA, Peregrine Falcon

Összefoglalás A kevéssé kutatott Nyugat-Palearktikus régióban vizsgáltuk a vándorsólyom két alfajába (*Falco peregrinus peregrinus* and *F. p. brookei*) tartozó négy populáció (Észak-, és Dél-Olaszország, Észak-Spanyolország, Cseh Köztársaság) genetikai diverzitását. Ehhez tíz mikroszatellit lókusz variabilitását és a mitokondriális DNS citokróm b génjének szekvenciáit elemeztük 65 mintában, négy populációból, melyek az észak-és dél-olaszországi, az észak-spanyol és a cseh populációkból származtak. Az általunk szekvenált citokróm b szekvenciákat együtt elemeztük a már korábban publikált más populációk és alfajok szekvenciáival, hogy a genetikai diverzitást a teljes elterjedési területen tudjuk vizsgálni. A saját 65 vándorsólyom mintákból 12 haplotípust azonosítottunk, melyek közül kilenc volt új és három már ismert. A mintáink 52%-a, beleértve az összes olaszországi és csehországi egyedet, a már korábban publikált HI haplotípusba tartozott, a minták egy másik 22%-a, amik főleg Szicíliából származtak, az új H1 haplotípusba, míg a maradék 26% megoszlott a többi 10 haplotípus között. A mikroszatelliták vizsgálata alapján találtunk különbségeket az allélek megoszlásában, így van egyfajta genetikai mintázat más európai populációkkal összehasonlítva, ennek ellenére sem a mikroszatellit lókuszok, sem pedig a mitokondriális DNS alapján nem lehet egyértelmű földrajzi csoportokat elkülöníteni a taxonómiai besorolás szerinti alfaji szinten.

Kulcsszavak: genetikai térképezés, *Falco peregrinus brookei,* mikroszatelliták, mitokondriális DNS, vándorsólyom

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Introduction

The Peregrine Falcon *(Falco peregrinus)* is a nearly-cosmopolitan species with a large phenotypic variability, currently described in 19 subspecies across the world (White *et al*. 2013a). After a massive decline and several regional extinctions in the second half of the 20th century, the ban of organochlorine pesticides coupled with greater protection, reintroduction programmes and restrictions of illegal trading have stopped the species' decline and have boosted up the Peregrine Falcon populations of the Northern Hemisphere (Cade *et al*. 1988, White *et al*. 2013a).

The use of molecular markers that can be easily amplified by polymerase chain reaction (PCR) and then sequenced is nowadays a widespread tool for the reconstruction of phylogenetic relationships among avian taxa (Sibley 1994). The nucleotide sequence of the mitochondrial cytochrome b gene was one of the first markers used in this field, and original contributes related to the phylogeny of Peregrine Falcon and its allied species in the Falconiformes group have been reported in Helbig *et al.* (1994) and Wink *et al*. (1998, 2000). Microsatellites, referred to as tandem repeats of short DNA sequences, are genetic markers that show a high level of variation and have been employed for studying avian population structure and systematics (Ellegren 1992, Bruford & Wayne 1993, Nesje *et al*. 2000a). Actually, there are available several developed microsatellite markers specifically for the Scandinavian Peregrine Falcons, which were used to compare the population structure and genetic variability of *F. p. peregrinus* populations breeding in north Europe with some subspecies present in North America and Tasmania (Nesje *et al*. 2000b).

Molecular analyses of variable microsatellite markers and mitochondrial DNA supported the conservation actions taken in North Europe and North America (Nesje *et al*. 2000a, Tordoff & Reding 2001, Jacobsen *et al*. 2008, Johnson *et al*. 2010, Ponnikas *et al*. 2017), and the investigation of the genetic relationships among many subspecies, including some of remote oceanic islands (Nesje *et al*. 2000b, Talbot *et al*. 2011, White *et al*. 2013b, Bell *et al*. 2014). In Mediterranean areas of Western Palaearctic region, corresponding to most of *F. p. brookei* range, the past population collapse was less extended. For instance, both in Italy (Schenk *et al*. 1985, Allavena & Brunelli 2003) and Spain (Gainzarain *et al*. 2002) large populations (826–1048 breeding pairs in Italy, and 2384–2690 in Spain) fluctuated or increased locally and were threatened mostly by nest despoliation for illegal trading of eggs and chicks, and direct human persecution. As consequence, population structure and genetic variability of Peregrine Falcons living in these areas were poorly investigated. In this study, we characterize the genetic composition and structure of four populations breeding in North and South Italy, North Spain and the Czech Republic by using ten polymorphic microsatellites and a portion of cytochrome b gene of mitochondrial DNA. The Spanish population breeds in the *F. p.*

brookei/F. p. peregrinus contact zone (Zuberogoitia *et al*. 2009), both the North Italian and the Czech populations breed within the *F. p. peregrinus* range, while the South Italian breeds within the *F. p. brookei* range (Bryndová *et al*. 2012, White *et al*. 2013a).

Material and Methods

We genotyped quills of nuchal feathers (e.g. Horváth *et al.* 2005) and muscle tissues of documented nest-site origin coming from 65 specimens: 9 from Northern Spain (Biscay), 8 from the Czech Republic, 5 from Northern Italy (Piedmont and Emilia), and 43 from Southern Italy (1 from Campania, continental Italy; 42 from the island of Sicily). One nestling from each nest site was used *(Table 1)*. Feather samples were obtained from wild nestlings (Spain, Sicily, and the Czech Republic), while muscle tissues came from adult individuals found dead in different and well separated localities of North and continental Southern Italy. The sample from the Czech Republic is a subsample of wild Peregrine Falcons DNA already used in Bryndová *et al.* (2012).

We used ZR Genomic DNA II Kit™ for solid/liquid samples (Zymo Research) to extract and purify genomic DNA from samples. DNA samples were genotyped across 10 microsatellite markers originally designed for *Falco peregrinus* (Nesjie *et al.* 2000a): Fp13, Fp31, Fp46_1, Fp54, Fp79_4, Fp86_2, Fp89, Fp92_1, Fp107; and for *Accipiter gentilis* (Topinka & May 2004): Age5. We carried out two independent PCR replicates to check the absence of Allelic Drop Out (ADO) or false alleles (FA). Furthermore, we amplified and sequenced a 960 bp long fragment of cytochrome b gene in mtDNA using a combinations of primer pairs (L14841-H15149, L15132-H15516, L15489-H15915) according to protocols described in Bell *et al*. (2014), to which refer for further details. In both cases we used the following PCR protocol: a first denaturation step at 94 °C for 3 min; 35 cycles at 94 °C for 40 s, 55 °C for 40 s, 72 °C for 40 s; and a final step at 60 °C for 30 min. PCR products were then processed in an ABI 3130XL sequencer. We used Genalex 6.1 (Peakall & Smouse 2006) to estimate the allele frequencies by locus and population, mean number of alleles per locus (NA), observed (H_o) and expected unbiased (UH_e) heterozygosity and the related chi-square test for deviations from Hardy-Weinberg equilibrium (HWE). Pairwise F_{ST} (Weir & Cockerham 1984), which is a measure of among-population variance in allelic frequencies, and principal coordinate analysis (PCoA), which gives an ordination of all data points based on a covariance matrix with microsatellite data standardization, were calculated using GENETIX 4.05 (Belkhir *et al.* 1996–2004) and FSTAT (Goudet 2001). We aligned the mtDNA sequences of our samples together with 17 haplotype sequences of *F. peregrinus*, and one Sharp-shinned Hawk *(Accipiter striatus)* sequence retrieved from GenBank and published by Bell *et al.* (2014). The latter was used as outgroup as in Bell *et al.* (2014). We aligned the mtDNA sequences with Bioedit (Hall 1999), then we identified the haplotypes using Dnasp 5 (Librado & Rosas *al.* 2009). We clustered a Tamura and Nei genetic distance matrix using the neighbour-joining procedure in Mega 5 (Tamura *et al.* 2011) with internode bootstrap values determined after 1000 resampling steps. Eventually, we reconstructed the phylogenetic relationships among the mtDNA haplotypes using median-joining networks in Network 4.6 (Bandelt *et al.* 1999).

Table 1. List of specimens considered in the study of genetic diversity of the Western Palaearctic Peregrine Falcons. Sample: MU = muscle tissue, FE = feather; mtDNA haplotypes HE (GeneBank Accession No. KP863007), HI (KP863006) and HL (KP863014) described in Bell *et al*. 2014, while haplotypes H1-H9 found in the present study (MH837632-MH837640)

1. táblázat A vándorsólyom egyedek táblázata, amelyek mintáit használtuk a genetikai diverzitás felmérésére a nyugat-paleakrtikusi elterjedési területen. Sample: MU = izomszövet, FE = toll; Bell *et al.* (2014) által publikált mtDNS haplotípusok: HE, HI és HL, ebben a vizsgálatban talált új haplotípusok: H1-H9

Id Lab	Taxon	Area	Region	Sampling year	Sex	Age	Sample	Mt DNA haplotype
FPE62	*F. p. brookei*	South Italy	Campania	2005	M	NA	MU	HI
FPE63	*F. p. brookei*	North Italy	Emilia	2010	M	NA	MU	HI
FPE66	*F. p. peregrinus*	North Italy	Piedmont	2012	M	SAD	MU	HI
FPE69	*F. p. peregrinus*	North Italy	Piedmont	2007	M	JUV	MU	HI
FPE71	*F. p. peregrinus*	North Italy	Piedmont	2010	F	AD	MU	HI
FPE72	*F. p. peregrinus*	North Italy	Piedmont	2010	M	NA	MU	HI
FPE76	*F. p. peregrinus*	Czech Republic	Central	2011	M	JUV	FE	HI
FPE77	*F. p. peregrinus*	Czech Republic	Central	2011	F	JUV	FE	HI
FPE78	*F. p. peregrinus*	Czech Republic	Ústí nad Labem	2010	M	JUV	FE	HI
FPE79	*F. p. peregrinus*	Czech Republic	Central	2011	F	JUV	FE	HI
FPE80	*F. p. peregrinus*	Czech Republic	Hradec Králové	2011	F	JUV	FE	HI
FPE81	*F. p. peregrinus*	Czech Republic	Central	2010	M	JUV	FE	HI
FPE83	*F. p. peregrinus*	Czech Republic	Ústí nad Labem	2010	F	JUV	FE	HI
FPE85	*F. p. peregrinus*	Czech Republic	Central	2011	F	JUV	FE	HI
FBI19	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	H1
FBI6	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	HI
FBI7	*F. p. brookei*	South Italy	Sicily	2017	M	SAD	FE	H1
FPE1	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	H1
FPE102	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	H1
FPE105	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	H1
FPE13	*F. p. brookei*	South Italy	Sicily	2016	M	JUV	FE	H6
FPE15	*F. p. brookei*	South Italy	Sicily	2016	M	JUV	FE	H1
FPE16	*F. p. brookei*	South Italy	Sicily	2016	F	JUV	FE	HE
FPE20	*F. p. brookei*	South Italy	Sicily	2016	M	AD+	FE	H7
FPE47	*F. p. brookei*	South Italy	Sicily	2017	M	AD+	FE	HI
FPE48	*F. p. brookei*	South Italy	Sicily	2017	F	SAD	FE	HL
FPE49	*F. p. brookei*	South Italy	Sicily	2017	M	AD+	FE	HI
FPE5	*F. p. brookei*	South Italy	Sicily	2016	F	JUV	FE	H3
FPE50	*F. p. brookei*	South Italy	Sicily	2017	F	AD+	FE	HI

Id Lab	Taxon	Area	Region	Sampling year	Sex	Age	Sample	Mt DNA haplotype
FPE51	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	HE
FPE52	*F. p. brookei*	South Italy	Sicily	2017	F	SAD	FE	HL
FPE53	*F. p. brookei*	South Italy	Sicily	2017	F	SAD	FE	H4
FPE54	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	HL
FPE55	*F. p. brookei*	South Italy	Sicily	2017	M	SAD	FE	HI
FPE56	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	H8
FPE57	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	HI
FPE58	*F. p. brookei*	South Italy	Sicily	2017	M	SAD	FE	H5
FPE59	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	HI
FPE6	*F. p. brookei*	South Italy	Sicily	2016	F	JUV	FE	HI
FPE60	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	H1
FPE61	*F. p. brookei*	South Italy	Sicily	2016	M	JUV	FE	H9
FPE64	*F. p. brookei*	South Italy	Sicily	2016	F	AD+	FE	HI
FPE65	*F. p. brookei*	South Italy	Sicily	2016	F	JUV	FE	H9
FPE67	*F. p. brookei*	South Italy	Sicily	2005	F	AD+	FE	HI
FPE68	*F. p. brookei*	South Italy	Sicily	2005	F	–	FE	H1
FPE70	*F. p. brookei*	South Italy	Sicily	2017	F	SAD	FE	HI
FPE74	*F. p. brookei*	South Italy	Sicily	2016	M	SAD	FE	H1
FPE75	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	HI
FPE86	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	HI
FPE88	*F. p. brookei*	South Italy	Sicily	2017	–	JUV	FE	H1
FPE89	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	H1
FPE9	*F. p. brookei*	South Italy	Sicily	2016	M	JUV	FE	H1
FPE94	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	H6
FPE96	*F. p. brookei*	South Italy	Sicily	2017	M	JUV	FE	H1
FPE98	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	HI
FPE99	*F. p. brookei*	South Italy	Sicily	2017	F	JUV	FE	H1
FPE21	*F. p. brookei*	Spain	Biscay	2016	F	JUV	FE	HI
FPE25	*F. p. brookei*	Spain	Biscay	2016	M	JUV	FE	HI
FPE27	*F. p. brookei*	Spain	Biscay	2016	M	JUV	FE	HI
FPE30	*F. p. brookei*	Spain	Biscay	2016	F	JUV	FE	HI
FPE32	*F. p. brookei*	Spain	Biscay	2016	F	JUV	FE	HE
FPE33	*F. p. brookei*	Spain	Biscay	2016	F	JUV	FE	HE
FPE35	*F. p. brookei*	Spain	Biscay	2016	M	JUV	FE	HI
FPE37	*F. p. brookei*	Spain	Biscay	2016	F	JUV	FE	HI
FPE41	*F. p. brookei*	Spain	Biscay	2016	M	JUV	FE	H2

Results

Mitochondrial DNA

The analysis of the four populations of Peregrine Falcons revealed a low inter-population genetic variability between the considered subspecies (*peregrinus* and *brookei*), albeit an insular effect can be observed based on the genetic pattern of Sicilian population. We identified 12 haplotypes from the 65 Peregrine Falcon samples, nine of which were new haplotypes found for the first time and coded from H1 to H9, while the other three have been already described by Bell *et al*. (2014) *(Table 1)*. The 52% of our samples (n = 34) belonged to the previously published HI haplotype, and another 22% (n = 14) of the samples showed the new H1 haplotype, while the remaining 26% of the samples partitioned among the other 10 haplotypes. Both the whole Italian and the Czech samples belonged to the HI haplotype, while the Spanish Peregrine Falcons split up among two Bell's haplotypes HI (n = 6) and HE (n = 2), and one new H2 haplotype (n = 1). The Sicilian Peregrine Falcons showed the largest haplotype diversity as the 42 individuals split up among Bell's and new haplotypes unique for this population. The already identified Bell's haplotypes found in Sicily are: HI (n = 14), HL (n = 3) and HE (n = 2), whereas the new ones are: H1 (n = 14) and from H3 to H9, all these latter with n = 1, but H6 and H9 with n = 2 individuals.

The neighbour-joining network of the haplotypes found in a 960 bp fragment of cyt b mtDNA gene and considered in this study *(Figure 1)* reproduces the large heterogeneity between the geographic origin and the taxonomic designation of Peregrine Falcons. For instance, haplotype HI, the most shared among individuals, occurs across Canada *(tundrius)* and Europe *(peregrinus, brookei)*. Yet, in our sample this haplotype assembles Sicilian and South Italian Peregrines expected to be *brookei*, together with Spanish specimens from the *brookei/peregrinus* border zone of Biscay, plus two more Spanish *brookei* samples present in Bell *et al*. (2014). Such a *brookei* group of samples anyway cluster with North Italian and Czech Republic individuals expected to belong to the nominate subspecies *peregrinus*. 14 Sicilian samples were grouped in the new haplotype H1, which indeed was the second in order of frequency among the found haplotypes. Third in order of frequency comes the haplotype HQ, which is separated by one mutational step from H1, and is definitely the most cosmopolitan one, as groups together some Sicilian and Biscay Peregrine Falcons with the Australian *(macropus)*, the Argentinian *(cassini)*, the American *(tundrius, anatum)*, the African *(minor)* and others subspecies with unknown geographic origin *(babilonycus*, *pelegrinoides)*.

Microsatellites

The genetic intra-population diversity based on microsatellite analysis of 65 unrelated specimens (42 from Sicily, 9 from Spain, 8 from the Czech Republic, 6 from Italy) has been reported in *Figure 2* and *Table 2*. All loci were polymorphic. The number of alleles per locus ranges from 1 to 11 (in Spain: 2–8, in the Czech Republic: 2–7, in Italy: 1–8, in Sicily: 2–11) with a total mean±SE of 3.65 ± 0.319 alleles over the four populations, the highest number of different alleles was found in Sicily *(Figure 2)*. Average of allelic richness in the four population was 3.24, ranging from the minimum 3.03 in the Czech Republic to the maximum

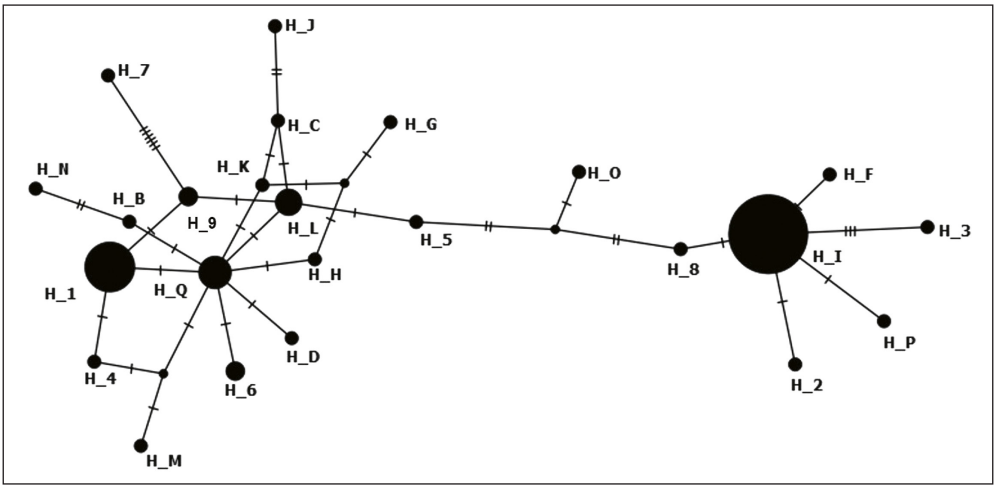

Figure 1. Haplotype neighbour-joining network of 960 bp segment of cytochrome b gene of mtDNA based on the 65 Peregrine Falcons considered in this study (the 9 new haplotypes are marked from H1 to H9) and the Bell *et al*. (2014) samples (15 haplotypes marked with H followed by letter) retrieved from GenBank. Three Bell's haplotypes (HI, HL and HE) were also found in our sample. The size of the nodes indicates the relative frequency of the corresponding haplotype in the dataset, as listed in *Table 1*. Small tracts show the mutational steps occurring between adjacent haplotypes

1. ábra A mtDNS citokróm b génjének 960 bázispár hosszú része alapján készült haplotípus hálózat. H1-H9: a 65 vándorsólyom minta alapján általunk talált haplotípusok, H-betűkkel jelölve: Bell *et al.* (2014) által közölt haplotípusok a GenBank-ból származó szekvenciái alapján. A mintáinkban a Bell *et al*. (2014) által közölt haplotípusok közül hármat találtunk meg (HI, HL and HE). A nóduszok méretei megfelelnek a haplotípusok relatív gyakoriságainak az *1. táblázat*ban közölt mintákban. Az ágakon lévő kicsi vonalak a mutációs lépések számát jelölik a haplotípusok között

3.40 in Italy. All populations have a comparable mean of different alleles with a frequency ≥ 5%, as well as of effective alleles, indeed the mean of these latter is slightly lower in the Czech Republic than in the other populations *(Figure 2)*. Contrariwise, Sicily has the highest mean of unique alleles with respect to the other populations *(Figure 2)*, depending from the absolute number of exclusive alleles (n = 10) found in the island, with respect to the Czech Republic (n = 3), Spain and Italy (both n = 2). Besides, Shannon diversity index, the observed (H_o), expected (H_e) and unbiased expected heterozygosity (UH_e) values were comparable among the four populations, with a relatively lower Shannon diversity value, and UH_e of the Czech population *(Table 2)*. Fixation index values are negative for Italy and the Czech Republic and positive for Spain and Sicily. This latter has the relatively higher reduction in heterozygosity when compared to Hardy-Weinberg expectations *(Table 2)*. As a matter of fact, none of the populations shows significant differences from Hardy-Weinberg equilibrium, with a fixation index ranging around a zero average value (–0.031).

As regards to genetic structuring, we have found a low amount of non-random mating in all populations, which shows F_{IS} values (after 1000 allelic permutations averaged over the ten loci in each population) negative in Czech Republic (–0.056) and positive in the others three areas (Spain = 0.082, Italy = 0.071, Sicily = 0.053). P-values were not significant for all pairwise comparisons between the F_{IS} values.

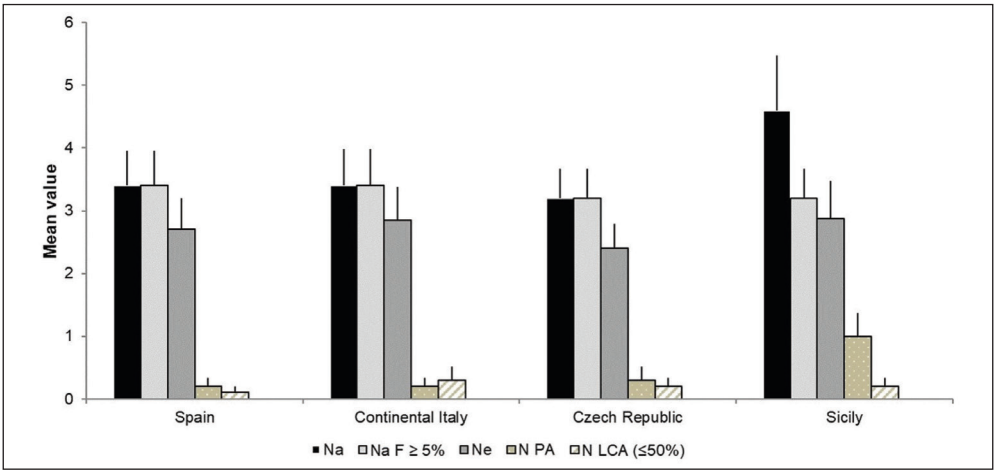

Figure 2. Allelic pattern across populations of Peregrine Falcon in areas of the Western Palaearctic. Na = N of different alleles; Na (F≥ 5%) = N of different alleles with a frequency ≥ 5%; Ne = N of effective alleles; N PA = N of alleles unique to a single population; N LCA (≤50%) = N of locally common alleles with a frequency of ≥5% found in 50% or fewer populations

2. ábra Vándorsólyom-populációk allél-mintázatai a Nyugat-Palearktiszban. Na = különböző allélek száma; Na (F≥ 5%) = különböző allélek száma, melyek gyakorisága ≥ 5%; Ne effektív allélek száma; N PA = csak egy populációban előforduló egyedi allélek száma; N LCA (≤50%) = lokálisan azonos allélek száma, melyek gyakorisága ≥5% csak a populációk 50%-ban, vagy kevesebben megtalálható

Table 2. Intra-population diversity and heterozygosity mean (± SE) values based on microsatellite analysis of 65 unrelated Peregrine Falcons. Mean over loci for each Peregrine Falcon population. SI = Shannon index; H_o = Observed Heterozygosity; H_e = Expected Heterozygosity; UH_e = Unbiased expected Heterozygosity; FI = Fixation index

2. táblázat Populáción belüli diverzitás és átlagos heterozigócia (átlag (mean) ± standard hiba (SE)) értékek a 65 nem rokon vándorsólyom mintáinak mikroszatellita elemzése alapján. A táblázat a lókuszok átlagos értékeit mutatja az egyes populációkban (Spain-spanyol, Continental Italy-kontinentális olasz, Czech Republic-cseh, Sicily-szicíliai, Total-összes). SI = Shannon index; H_o = megfigyelt heterozigócia; H_e = várt heterozigócia; UH_e = torzítatlan várt heterozigócia; FI = fixációs index

		SI	Ho	He	UHe	FI
Spain	Mean	0.973	0.533	0.546	0.578	0.011
	SE	0.146	0.079	0.061	0.065	0.091
Continental Italy	Mean	0.994	0.567	0.556	0.606	–0.004
	SE	0.161	0.097	0.073	0.079	0.096
Czech Republic	Mean	0.902	0.580	0.517	0.552	–0.162
	SE	0.123	0.048	0.051	0.055	0.081
Sicily	Mean	1.079	0.549	0.572	0.579	0.035
	SE	0.154	0.048	0.050	0.051	0.037
Total	Mean	0.987	0.557	0.548	0.579	–0.031
	SE	0.071	0.034	0.029	0.031	0.040

Pairwise values of F_{ST} statistics, as obtained from FSTAT (above diagonal values) and GENETIX (below diagonal values) software have been reported in *Table 3*. We reported results from both software for a careful approach as GENETIX implements a permutation-based procedure alternative to jack-knifing used in FSTAT to calculate statistical inference. According to both software, differences among populations are small and only the values between the Czech Republic and Sicily are statistically significant (marked with asterisk in *Table 3*), while only GENETIX recorded significant values also between Spain and the Czech Republic.

The analysis of molecular variance (AMOVA) shows that differences between the four populations are marginal as they explained only 3.3% of the total genetic variation, while intra-population difference was the 96.7% of the total genetic variation *(Table 4)*.

Eventually, the scatter of Principal Coordinates Analysis *(Figure 3)* shows how all the specimens spread across the first two factor axes (F1 and F2) that give a measure of the variance accounted for by the corresponding coordinates (eigenvectors), evidencing the lack of genetic structuring among the four studied Peregrine Falcon populations. Such a lack of genetic structuring keeps even considering the third axis (F3). The cumulative percentage of variance explained by the first 3 axes is equivalent to 31.36%.

Table 3. Pairwise F_{ST} statistics comparison between populations, as obtained from FSTAT (above diagonal values) and GENETIX (below diagonal values) software. Values marked with asterisk are statistically significant ($P \leq 0.05$)

3. táblázat Páronkénti F_{ST}-értékek a populációk (Spain-spanyol, Continental Italy-kontinentális olasz, Czech Republic-cseh, Sicily-szicíliai) összehasonlítására az FSTAT (diagonális feletti értékek) és a GENETIX (diagonális alatti értékek) programmal számolva. A csillagozott értékek statisztikailag szignifikánsak ($P \leq 0.05$)

	Spain	Continental Italy	Czech Republic	Sicily
Spain	–	–0.0173	0.0299	0.0151
Continental Italy	0.4083	–	–0.0022	0.0092
Czech Republic	0.0083*	0.1333	–	0.0317*
Sicily	0.0250	0.0750	0.0083*	–

Table 4. Analysis of molecular variance table showing the low differentiation among the populations with respect to the large individual variability. The related PhiPT statistics is = 0.033, with P = 0.022. The probability P (random ≥ data) for PhiPT is based on standard permutations across the full data set

4. táblázat A molekuláris variancia analízis táblázata, ami kevés variabilitást mutat a populációk között (Inter-population), összehasonlítva a nagy variabilitással a populációkon belül (Intra-population). A PhiPT érték = 0.033, és a hozzátartozó P-érték = 0.022. A PhiPT P-értkétt (random ≥ adat) standard permutációval számítottuk ki a teljes adathalmazra. df: szabadsági fok, SS: eltérés-négyzetösszeg, MS: eltérés-négyzetösszeg átlaga, Estimated Variance: becsült variancia

Source	df	SS	MS	Estimated Variance	Estimated Variance (%)
Inter-population	3	25.688	8.563	0.207	3.3
Intra-population	61	374.405	6.138	6.138	96.7
Total	**64**	**400.092**		**6.345**	**100**

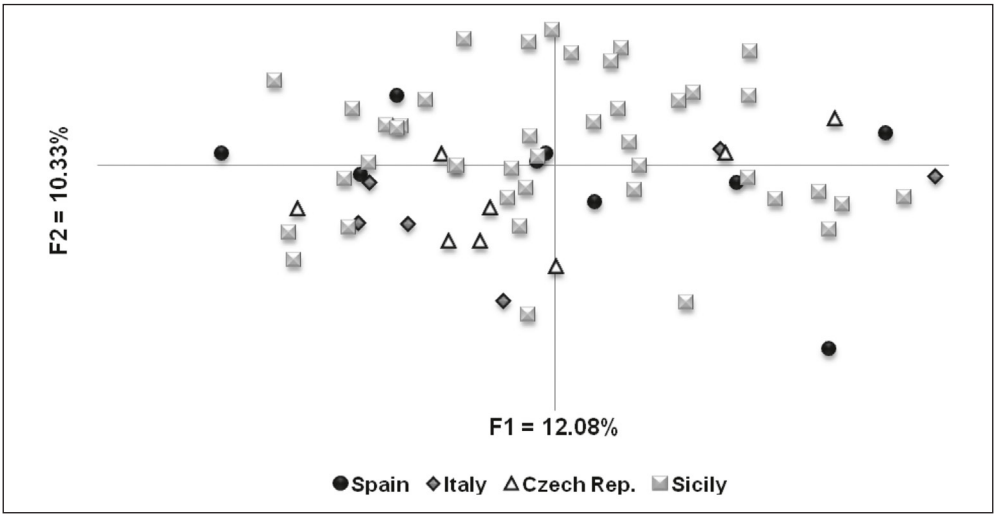

Figure 3. Scatter of the ordination method (Principal Coordinates Analysis by covariance matrix with data standardization) used to detect similarities of microsatellite data among the 65 unrelated Peregrine Falcons breeding in the four populations of Western Palaearctic. F1 and F2 are the factor axes that account for percentage of variation among specimens. No geographic grouping emerges from the scatter

3. ábra Főkomponens-analízis eredménye (PCoA) kovariancia mátrix alapján 65 nem rokon vándorsólyom minta adatait standardizálva a négy nyugat-palearktikus populációból

Discussion

We reported here for the first time molecular data from wild Peregrine Falcons living in Sicily, continental Italy and Northern Spain (Biscay). Low sample sizes in some of the considered populations might affect some results, as likely occurs for the higher Shannon diversity index of the Sicilian population, which had also the larger sample size. Despite this limitation, the estimation of intra-population diversity and allelic patterns provided an adequate genetic condition in the Spanish and Italian studied populations as the low-medium number of alleles and medium level of observed heterozygosity values were observed, but coupled with statistically not significant F_{IS} values and departure from HW equilibrium. These data fall within the species' variability range as they are consistent to other Peregrine Falcon populations. For instance, Nesje *et al.* (2000a) found 2–11 alleles per locus with a mean ± SE of 4.25 ± 0.81, mean ± SE H_o value was equal to 0.452 ± 0.080 and H_e was 0.512 ± 0.073 when examining genetic relationships among Peregrine Falcon populations in Southern Norway. In a much larger comparison of subspecies across the world, Nesje *et al*. (2000b) found 3–18 alleles per locus with H_o ranging from 0.405 to 0.490 in all populations (except the Tasmanian with H_o = 0.146). Jacobsen *et al.* (2008) comparing Southern Scandinavian and Northern Fennoscandic populations with different origin have found the H_o to decrease from 0.53 ± 0.07 in the historical population to 0.46±0.08 in the reintroduced population. Likewise, the H_e value's range was from 0.56 ± 0.07 in the historical to 0.50 ± 0.07 in the reintroduced population, anyway both heterozygosity decreases were not statistically significant.

The complete dataset (n = 30) of wild Peregrine Falcons that Bryndová *et al.* (2012) have analysed gave slight different values with respect to the subsample (n = 8) considered here *(Table 2)*. For instance, the mean H_o was 0.546, while the mean H_e was 0.632, and the allelic richness per locus ranged from 2.69 to 6.51 in the complete dataset. In addition, the whole Czech sample showed deviation from Hardy-Weinberg equilibrium in the form of heterozygote excess in the locus fp31, besides to a significant higher inbreeding coefficients F_{IS}.

As regard the genetic structuring, we have found a low amount of non-random mating comparable to the historic Scandinavian population (F_{IS} = 0.08 in Jacobsen *et al.* 2008) and quite less than contemporary reintroduced wild populations (F_{IS} = 0.14 in Jacobsen *et al.* 2008 and F_{IS} = 0.139 in Bryndová *et al*. 2012). The relatively higher F_{IS} value in our samples was found in the Biscay population from Spain. It is enquiring that the signal of non-random mating among individuals, although low, is larger in a continental than in the insular population of Sicily. Interchange of adults in the Biscay population was documented within a radius of about 360 km (Zuberogoitia *et al*. 2009), a value that would maintain genetic connections with other Spanish and South-western French populations. It remains an open question whether such a non-random mating signal could be depending by chance from the specific sample here used, or from the peculiar condition of the Biscay population, at the border between the Spanish '*brookei*' and the French '*peregrinus*' ranges (Zuberogoitia *et al*. 2009). Otherwise, first investigation on natal dispersal has not yet recorded emigration from Sicily (see Bondì *et al*. 2018), though we cannot still exclude immigration from continental Italy. This condition goes hand in hand with the large number of exclusive alleles found in the Sicilian falcons, so to allow supposing a quite close population. If this would be the case, the population of Peregrine Falcons in this island, currently estimated at 240–250 pairs (see Bondì *et al*. 2018), would be large enough to limit non-random mating among individuals.

Despite the significant F_{ST} differences between the Sicilian and the Czech populations, and also between the Spanish and the Czech based on only the GENETIX results, the AMOVA showed no differentiation among populations, with only a 3.3% of inter-population genetic variation. Similarly, Jacobsen *et al*. (2008) showed that differences between their four Scandinavian populations explained only 5% of the total genetic variation.

The low differentiation among populations is confirmed yet by the analysis of cytochrome b mitochondrial DNA sequences that produced a haplotype network, which was not concordant with geographic origin and taxonomic designation of the specimens. Previous works (White *et al*. 2013a, Bell *et al*. 2014, Johnson *et al*. 2017) have already noted such a discrepancy, suggesting that historical and recent dispersal, combined with rapid morphological evolution, could have contributed to the lack of phylogenetic concordance between mitochondrial DNA variation and geographic origin of the Peregrine Falcon. Both the Biscay and Sicilian populations could well describe this situation. The former has been suggested to show a character introgression with the close French *F. p. peregrinus* population that would produce variable phenotypes (Zuberogoitia *et al.* 2009) and the signals of non-random mating would stabilize pairs maintaining phenotypical variation (*Figure 4)*. While the Sicilian population appears to be quite isolated as judging from the presence of an exclusive and highly frequent H1 haplotype and the lack of dispersal (see Bondì *et al*. 2018). Despite this presumed insular condition, Sicilian Peregrine Falcons show quite large deviation from the expected *brookei*

Figure 4. Individual phenotype variability of Biscay Peregrine Falcons. Above, a male *brookei* phenotype which is paired to a female *peregrinus* phenotype (below). Photo by Iñigo Zuberogoitia

4. ábra Egyedi fenotípusos változatosság a Biscay-i vándorsólymokban. Felül: hím *brookei* fenotípusú egyed, ami párt alkot egy tojó *peregrinus* (alul) fenotípusú egyeddel. Fotó: Iñigo Zuberogoitia

Figure 5. Individual phenotype variability of Sicilian Peregrine Falcons. Four adult females collected in different years and localities of Sicily arranged from the most *F. p. peregrinus* (left) to the most *F. p. brookei* (right) similar phenotype, above in ventral and below in dorsal view. Courtesy of Carmagnola Museum, Turin. Photo by Giovanni Boano

5. ábra Egyedi fenotípusos változatosság a szicíliai vándorsólymokban. Négy öreg tojó, amelyek különböző években és helyeken lettek gyűjtve Sziciliában *F. p. peregrinus* (bal) és *F. p. brookei* (jobb) jellegeket mutat. Felül hasi, alul háti nézetben. Carmagnola Museum, Torinó. Fotó: Giovanni Boano

phenotype *(Figure 5)* and mitochondrial sequences consistent with continental populations that would admit gene flow with *peregrinus* populations. Further genomics analyses (e.g. Johnson *et al*. 2017) of both the Biscay and Sicilian and their neighbouring populations, together with natal dispersal studies may further clear these aspects.

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