

37. OTDK Biológia Szekció

Title

Genetic structure and diversity of eastern imperial eagle
populations across Eurasia

Author

Ádám Tisza

Eötvös Loránd Tudományegyetem, Department of Systematic Zoology and Ecology

Biology MSc II

Supervisors

Dr Szilvia Páztory-Kovács

University of Veterinary Medicine Budapest, Department of Zoology, Molecular
Ecology Research Group

Assistant research fellow

Dr Krisztián Szabó

University of Veterinary Medicine Budapest, Department of Zoology, Molecular
Ecology Research Group

Assistant research fellow

Eötvös Loránd University

Budapest, 2024

Table of contents

Summary.....	3
Introduction	4
Conservation genetics in large raptors	4
Eastern imperial eagle and its population structure	5
Aims	8
Materials and methods.....	9
Sample collection and DNA extraction	9
Individual genotyping	10
Statistical analysis	11
Results	13
Population diversity	13
Population structure	14
Discussion.....	18
Acknowledgements	21
Author contribution	22
References	23

Summary

The eastern imperial eagle (*Aquila heliaca*) is a long-lived raptor species with a wide distribution that follows the forest-steppe belt in Eurasia. The global breeding population is estimated to be 8,100-11,900 pairs and the species is deemed 'Vulnerable'. Russia and Kazakhstan possess the largest populations, which are migratory, while the western populations are mostly sedentary and separate into five geographic localities. In this research, I analysed 204 individuals collected from nine geographical populations across its distribution range (Western and Eastern Carpathian Basin, Northern Macedonia, Thrace, Anatolia, South Caucasus, Crimea-Ukraine, Volga-Ural, Altai). I amplified eight nuclear microsatellite loci for all samples, then I determined their genotypes. In the analyses, this dataset was supplemented with previously genotyped other seven loci. My results suggest a population structure with four distinct clades: 1. Carpathian Basin, 2. Northern Macedonia, 3. Thrace-Anatolia and 4. the unit of eastern populations. Out of these, the first three are all divergent, which could be the outcome of both historical bottlenecks and strong philopatric behaviour, although there is no sign of inbreeding. They all bear some resemblance to the eastern clade, which possesses the highest genetic diversity, indicating long-time stable extensive populations in the Caucasus and in the Altai Mountains.

Introduction

Conservation genetics in large raptors

Population decreasing effects, such as habitat fragmentation and degradation or human persecution can inherently cause the deterioration of genetic diversity by drift and inbreeding depression. Both pose serious threats in nature conservation (Frankham, 1995; Hedrick, 2000). Consequently, assessing the genetic variability of potentially endangered populations is often essential for successful conservation planning.

With the advance of molecular technologies and the rising issue of biodiversity loss, conservation genomics is starting to replace conservation genetics, since it allows researchers to determine whole genome sequences and study a substantially wider range of questions (Allendorf et al., 2010; Formenti et al., 2022; Ouborg et al., 2010; Pertoldi & Randi, 2018). Nonetheless, microsatellites have been highly popular genetic markers in numerous fields of biology for several decades, and despite of their limitations compared to genomics, they are still a dependable tool for conservation biology to assess population structure and genetic diversity, although not as fine-scaled (Abdul-Muneer, 2014; Hauser et al., 2021; Lemopoulos et al., 2019; Sunde et al., 2020). Furthermore, while genome sequencing is available only for high quality DNA samples, microsatellites can be more conveniently amplified from non-invasively collected, more degraded samples as well, which is often the only way to effectively sample adult raptors (Vili et al., 2013).

Even though birds of prey are highly efficient at dispersing, geographically distinct populations of multiple European raptors possess high genetic differentiation (Kretzmann et al., 2003; Nebel et al., 2019; Nemesházi et al., 2016). Martínez-Cruz et al. (2007) showed that the fragmentation of the Spanish imperial eagle (*Aquila adalberti*) in the past century resulted in genetically structured subpopulations that was non-existent in the past. Rudnick et al. (2005) found that in an eastern imperial eagle (*Aquila heliaca*) population in Kazakhstan the individuals are genetically monogamous and do not show inbreeding avoidance, even though the species is highly philopatric (Horváth et al., 2018b; Karyakin, 2020). Isolation often inherently leads to inbreeding, thus to the deterioration of the gene pool, therefore, detecting fragmentation and high differentiation of isolated populations is one of the most important aims of conservation genetics (Ralls et al., 2018). Even though the eastern imperial eagle is a widely studied species of conservation interest, no study yet has focused on exploring the broad genetic diversity of the species or the potential genetic isolation of European populations.

Eastern imperial eagle and its population structure

The eastern imperial eagle (EIE) is a long-lived raptor species with a distribution that spans from Eastern Austria to the Transbaikal region in Russia, and from Southern Ural to Turkey (Demerdzhiev et al., 2011; Karyakin, 2020). Forest steppes are its preferred habitat type, since they include broad open grasslands appropriate for hunting as well as trees or forest edges for nesting. The global breeding population is said to be 2,500-9,999 individuals by the IUCN (BirdLife International, 2019), but according to (Karyakin, 2020) most researchers estimate the number of all breeding pairs to be at 8,100-11,900. In Europe, the species is sorted into the 'Least Concern' category because of its nowadays increasing population trends (BirdLife International, 2021), but the global population is deemed 'Vulnerable' (BirdLife International, 2019), mainly due to critical declines experienced in the northern and easternmost part of its distribution (Ryabtsev & Katzner, 2007; Karyakin et al., 2011). Nonetheless, roughly two thirds of the global population, can be found in Russia and Kazakhstan (Karyakin, 2018). These eastern populations are migratory and winter in the Middle East, Southeastern Asia, and Eastern Africa, whereas the western populations are mostly sedentary (Horváth et al., 2018b; Horváth, 2022). Subadult individuals are prone to wander long distances or winter in the South before reaching adult age (Rudnick et al., 2008), but they almost always return to their natal population to breed (Horváth et al. 2018b; Karyakin, 2020).

The western part of the EIE's distribution consists of five separate populations, which are present in the Carpathian Basin, Northern Macedonia, Eastern Thrace (part of Bulgaria and European Turkey), Anatolia (Asian Turkey) and the South Caucasus (Horváth, 2022), see detailed data in Table 1. Out of these, the one in the Carpathian Basin is the largest one.

Geographic locality	Estimated number of breeding pairs	Population trend	Source
Carpathian Basin	455-480	increasing	Horváth et al., 2023
Hungary	380	increasing	Horváth et al., 2023
Austria	20-23	increasing	Schmidt & Horal, 2018
Czech Republic	6-8	increasing	Schmidt & Horal, 2018
Slovakia	45-50	increasing	Danko & Mihók, 2022
Romania	1-3	increasing	Milvus group, 2016
Serbia	1-5	unknown	Hám, 2022
Northern Macedonia	25-35	stable	Lisichanets, 2017, 2014
Thrace	85-95	increasing	(Demerdzhiev et al., 2022; Lazarova et al., 2020)
Bulgaria	62	increasing	Lazarova et al., 2020
European Turkey	45-50	increasing	(Demerdzhiev et al., 2022)
Anatolia	300-400	unknown	Horváth, Béres, et al., 2018
South Caucasus	130-190	increasing	(Abuladze, 2013; Sultanov et al., 2011; Ukolov et al., 2018)
Georgia	30-40	increasing	(Abuladze, 2013)
Azerbaijan	100-150	increasing	(Sultanov et al., 2011; Ukolov et al., 2018)
Crimea-Ukraine	55-60	increasing*	(Karyakin, 2020; Vetrov & Milobog, 2008)
Eastern Ukraine	50	increasing*	(Karyakin, 2020; Vetrov & Milobog, 2008)
Crimean Peninsula	5-10	unknown	(Karyakin, 2020; Vetrov & Milobog, 2008)
Volga-Ural	1608-1687	stable/increasing	(Karyakin, 2020)
Volga-Kama	908-962	stable/increasing	(Karyakin, 2020)
South Ural	700-725	stable/increasing	(Karyakin, 2020)
Altai	770-797	stable	(Karyakin, 2020)

Table 1 Population numbers and trends of sampled EIE localities. The sampled geographic populations are highlighted in bold font. *Increasing population trend of Crimea-Ukraine is valid until 2020, it is unknown since then.

The EIE had already been present in the Carpathian Basin thousands of years ago based on fossil data (Jánossy, 1981) and it has been a confirmed breeding species since the beginning of reliable data collection at the end of the 19th century (Vasvári, 1938). In the 20th century it started to retreat into mountainous areas, and by the end of the 1970s the population suffered a drastic decline (10-15 pairs remained), which was presumably due to human disturbance, persecution, and secondary poisoning (Haraszthy et al., 1996). With increasing conservation and decreasing hunting/poisoning, the population strengthened in the 1990s and even started to inhabit lowland plains again (Bagyura et al., 2002). To date, there are only a handful of breeding pairs remaining in mountain areas, while lowland populations are increasing (Horváth et al., 2011; 2022). The population divides into western and eastern parts, which have been proved to be genetically different in Slovakia (Vili et al., 2009). The Slovakian population also suffered a decline and managed to recover, although it is smaller and shows a lower rate of increase (Danko & Mihók, 2022; Karyakin, 2020). After the increase in Hungary and Slovakia, the EIE started to breed in Austria and the Czech Republic at the end of the 1990s (Horal, 2011), Romania in 2016 (Milvus group, 2016), and Serbia in 2020 (Hám, 2022).

The Northern Macedonian population had a severe decrease in the second half of the 20th century as well, with hardly any nests observed, most likely due to severe habitat degradation (Karyakin, 2020). During this time, similar declines had been observed in the entire Balkan Peninsula, which caused the supposed extinction of the species in multiple countries, for instance Croatia, Bosnia and Herzegovina, and Moldova (Bagyura et al., 2002; Kotrošan & Hatibović, 2012; Škorpíková et al., 2014). Thrace is in the proximity of Northern Macedonia and the population there also survived this period, but the two are isolated from each other (Karyakin, 2020).

In the Asian part of Turkey, Anatolia, the species also suffered a decline at the end of the 20th century but continued to nest in Northern Anatolia (Karyakin, 2020) and recovered by 2017 (Horváth et al., 2018a). In more southern parts of the country the EIE is replaced by the steppe eagle (*Aquila nipalensis*) due to the lack of trees in the habitat. The two species have been proved to hybridize with each other, producing fertile offsprings (Horváth et al., 2018a), which allows the possible foundation of a hybrid zone. Distribution of the EIE in the northeastern part of the country advances towards Georgia but is separated from the populations there by high mountain areas (Karyakin, 2020). The South-Caucasian population of Georgia and Azerbaijan

has a vague connection to the eastern part of the distribution, while the Ukraine population is in direct connection with it (Karyakin, 2020).

Aims

The aim of this study was to assess the genetic structure and diversity of western non-migrating and three eastern migrating populations across the species' distribution using 15 microsatellite loci. I provide diversity parameters for all sampled populations, thorough analysis of population structure, and discuss the potential biological processes behind the current pattern.

Materials and methods

Sample collection and DNA extraction

Shed feathers of breeding individuals (n=182) and armpit feathers of chicks (n=22) were collected in 2006-2017, from the western (n=31) and eastern (n=29) part of the Carpathian Basin, Northern Macedonia and Southern Serbia (n=21), Thrace (n=31), Anatolia (n=26), Georgia and Azerbaijan (n=23), Ukraine and the Crimean Peninsula (n=6), the Volga-Ural regions (n=7), and the Altai Mountains (n=30), see Fig. 1. Shed feathers were non-invasively collected around nests, which has been proved to be dependable for sampling the local breeding pair instead of occasional intruders (Jakab, 2015), and chick feathers were plucked during ringing procedure. Shed feathers were stored in plastic bags in dark and cool temperature to preserve DNA and were processed as soon as possible, whereas plucked feathers were stored at -20 °C in microtubes filled with 96% ethanol (Vili et al., 2013).

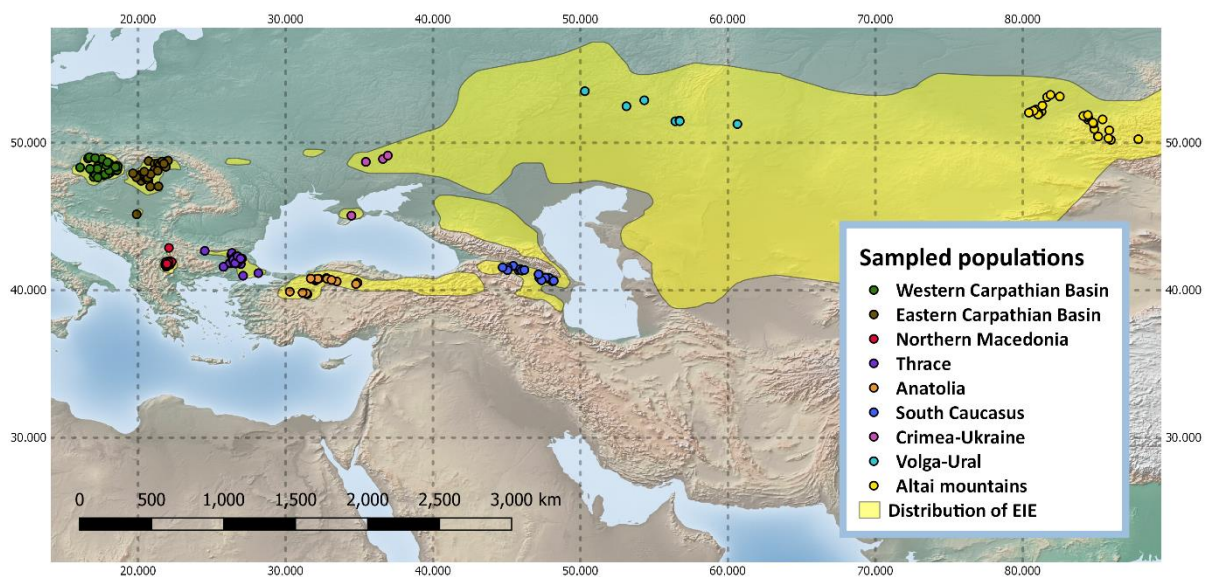


Fig. 1 Location of samples collected and breeding distribution of the EIE according to Karyakin (2020).

Genomic DNA was extracted from 204 feather samples with Omega E.Z.N.A.® Tissue DNA Kit (Omega Bio-tek Inc.) using the original instructions but adding 10 µl of dithiothreitol during the digestion step (Weigmann, 1968). In shed feathers, DNA was extracted from the blood clot in the superior umbilicus (Horváth et al., 2005), while in plucked chick feathers, the tip of the calamus was digested. Extracted DNA were stored in -20 °C marked with individual codes.

Individual genotyping

194 sampled individuals were previously genotyped with seven microsatellite loci, out of which two are tetranucleotide loci designed for eastern imperial eagle: IEAAAG09/G09, IEAAAG11/G11 (Busch et al., 2005), and five are dinucleotide loci originally designed for Spanish imperial eagle (*Aquila adalberti*): Aa02, Aa35, Aa36, Aa39, Aa43 (Martínez-Cruz et al., 2002). I genotyped additional 10 sampled individuals as well with the same seven loci. To increase the resolution and reliability of results, I genotyped all 204 samples with an additional eight loci. Four of them are dinucleotide loci designed for Spanish imperial eagle (Aa26, Aa49, Aa53, Aa57; Martínez-Cruz et al., 2002), two are dinucleotide loci designed for white-tailed eagle (*Haliaeetus albicilla*; Hal04, Hal10; Hailer et al., 2005), and two are trinucleotide loci designed for Japanese golden eagle (*Aquila chrysaetos japonica*; AQJ10, AQJ120; Goossens, 2015; Sato et al., 2015).

Forward primers were modified with fluorescent dyes on the 5' end: FAMTM for Aa02, Aa39, Aa43, G09, G11, and AQJ120, HEXTM for Aa35, Aa36, and AQJ10, NEDTM for Aa49, Aa53, Hal04, and Hal10, PETTM for Aa26 and Aa57. PCR was executed in a 10 µl volume, containing 10-70 ng of template DNA in a 3 µl solution, 2 µl 5xFIREPol® Master Mix (Solis BioDyne), 1 µl mix of the forward and reverse primer (5 pmol/µl), and 3 or 4 µl nuclease-free water based on if the reaction was done in simplex or duplex amplification. Aa26 and Aa57, Aa35 and Aa43, Aa36 and Aa39, Aa49 and Aa53, G09 and G11, Hal04 and Hal10 were amplified as duplexes, while Aa02, AQJ10 and AQJ120 were amplified as simplexes. PCR cycles were used as they were published in the original papers by Hailer et al. (2005) and Busch et al. (2005) or in case of the Aa and AQJ loci they were slightly modified from (Martínez-Cruz et al., 2002), using a touchdown annealing approach in order to decrease the chance of mispriming (Table 2).

	Hal loci (Hailer et al., 2006)			G loci (Busch et al., 2005)			Aa and AQJ loci (Martínez-Cruz et al., 2002)		
	No. of cycles	T	Period length	No. of cycles	T	Period length	No. of cycles	T	Period length
Denaturation		95°C	2 min		95°C	2 min		95°C	2 min
Touchdown cycles									
1. Denaturation	-	-	-	7	95°C	30 sec	17	95°C	30 sec
2. Annealing		-	-		66-60°C	30 sec		66-60°C	30 sec
3. Elongation		-	-		72°C	30 sec		72°C	30 sec
Regular cycles									
1. Denaturation	37	95°C	30 min	31	95°C	30 sec	21	95°C	30 sec
2. Annealing		57°C	45 min		60°C	30 sec		60°C	30 sec
3. Elongation		72°C	45 min		72°C	30 sec		72°C	30 sec
Final extension		72°C	7 min		72°C	7 min		72°C	7 min

Table 2 PCR protocols of the used microsatellite markers.

I performed gel electrophoresis to check the quality of amplification, using 2% agarose gel dyed by EcoSafe (Pacific Image Electronics Co., Ltd) with 100 V voltage for 45 minutes. Following that, we sent the PCR products to a service laboratory where capillary electrophoresis was used to determine the fragment lengths with an ABI 3500XL sequencer (Applied Biosystems, using Gene Scan TM -500LIZ TM). The software OSIRIS 2.16 (NCBI) was used to identify the previously done seven loci of 194 individuals, and I used it for the eight loci of 204 and seven loci of 10 individuals as well.

Statistical analysis

Possible occurrence of null alleles and allelic dropouts were checked with MICROCHECKER 2.2.3 (Van Oosterhout et al., 2004). GENALEX6.5 (Peakall & Smouse, 2006) was used to calculate observed (H_o) and expected (H_e) heterozygosity, pairwise fixation indices (F_{st}) and to perform Principal Coordinate Analysis (PCoA) on the population level. Computation of Pairwise F_{st} and

PCoA were done without the data of Crimea-Ukraine and Volga-Ural populations due to their low sample sizes.

Further calculations, such as number of private alleles, allelic richness, inbreeding coefficient (F_{is}), and PCoA on individual level (including individuals of all populations) was performed with the 'adegenet' package (Jombart, 2008) in the R 4.3.1 statistical environment (R Core Team, 2023). The 'genepop' package (Rousset et al., 2017) was used to test for Hardy-Weinberg equilibrium (HWE) and linkage disequilibrium (LD), for which multiple comparison of p-values was adjusted with Bonferroni corrections. I also performed a Mantel test using the package 'ade4' (Dray & Dufour, 2007) to see if there is a significant correlation between geographical distance (using the centroid points of coordinate data per population) and genetic distance (using the pairwise F_{st} values generated by GENALEX).

STRUCTURE 2.3.4. (Falush et al., 2003) was used to further analyse population structure. A burning period of 250,000 and 750,000 Markov Chain Monte Carlo (MCMC) replications were applied, and K (number of genetic clusters) was set between 1-8 with three independent iterations. A Discriminant Analysis of Principal Components (DAPC) was also performed to estimate the number of genetic clusters with the abovementioned 'adegenet' package. Finally, to detect first-generation migrant individuals, GENECLASS2 (Piry et al., 2004) was used by a bayesian approach (Rannala & Mountain, 1997) and with a probability computation of 10,000 individuals.

Results

Population diversity

No evidence of null alleles or allelic dropouts were detected with MICROCHECKER, as seen in Table 3. Crimea-Ukraine and Volga-Ural values should be interpreted with caution due to the small sample sizes. Out of all populations, the Altai mountains possessed both the highest number of alleles and private alleles, whereas the Western Carpathian Basin and Crimea-Ukraine had none of the latter. Allelic richness was also highest for the Altai mountains with Volga-Ural as well, while Thrace had the lowest value. H_o and H_e values were quite similar and generally high for all populations. There was no sign for high level of inbreeding, as F_{is} values are close to zero, except for Volga-Ural. Linkage disequilibrium was not detected between loci after applying Bonferroni corrections. Despite of the high heterozygosity values, only two populations proved to significantly differ from HWE, Thrace ($p=0.014$) and Anatolia ($p<0.001$). To account for the Wahlund effect, which means that a subpopulation has reduced genetic diversity, while the genotype frequencies of the whole population remain constant, I also checked if the two population together were in HWE, but they still were not ($p<0.001$).

Population	Sample size	Number of alleles	Null alleles	Private alleles	Allelic richness	H_o	H_e	F_{is}	LD*	HWE
Western Carpathian Basin	31	77	0	0	3.83	0.68	0.70	0.025	no	yes
Eastern Carpathian Basin	29	81	0	2	3.81	0.70	0.68	-0.032	no	yes
North Macedonia	21	77	0	4	3.77	0.68	0.68	-0.003	no	yes
Thrace	31	79	0	2	3.63	0.67	0.66	-0.028	no	no
Anatolia	26	79	0	2	3.85	0.64	0.68	0.043	no	no
South Caucasus	23	81	0	10	3.91	0.70	0.71	0.008	no	yes
Crimea-Ukraine	6	60	0	0	3.83	0.76	0.72	-0.064	no	yes
Volga-Ural	7	72	0	5	4.24	0.66	0.73	0.157	no	yes
Altai mountains	30	92	0	13	4.02	0.67	0.70	0.057	no	yes

Table 3 Summary of population diversity measures, based on the 15 loci dataset. Prominent results are highlighted, LD* is reported after Bonferroni corrections.

Population structure

According to pairwise F_{st} values (Fig. 2), the two parts of the Carpathian Basin (CBW-CBE), the two southernmost populations (THR-ANA), and Caucasus and Altai (CAU-ALT) are the less differentiated from each other. The Carpathian Basin bears more resemblance to the distant Altai and South Caucasus than to any of the closer ones (Northern Macedonia, Thrace, and Anatolia). The highest value of differentiation is between Thrace and the Eastern Carpathian Basin. Despite of their close geographic distance, Thrace also differs relatively highly from Northern Macedonia.

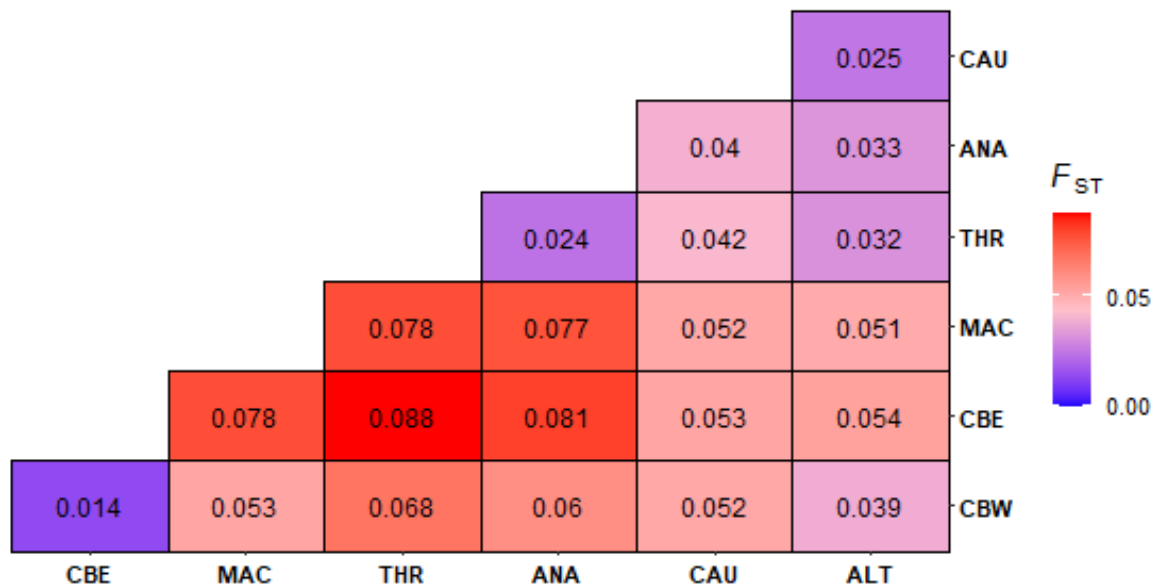


Fig. 2 Pairwise F_{st} values of the geographic populations (Abbreviations are the following: CBW – Western Carpathian Basin, CBE – Eastern Carpathian Basin, MAC – Northern Macedonia, THR – Thrace, ANA – Anatolia, CAU – South Caucasus, and ALT – Altai mountains).

The PCoA, calculated from the pairwise genetic distances of all individuals, shows that individuals from different populations highly overlap with each other (Fig. 3). Nonetheless, the Carpathian Basin, Northern Macedonia, and Thrace-Anatolia all diverge from the centre of the overlap where the rest of the populations lie.

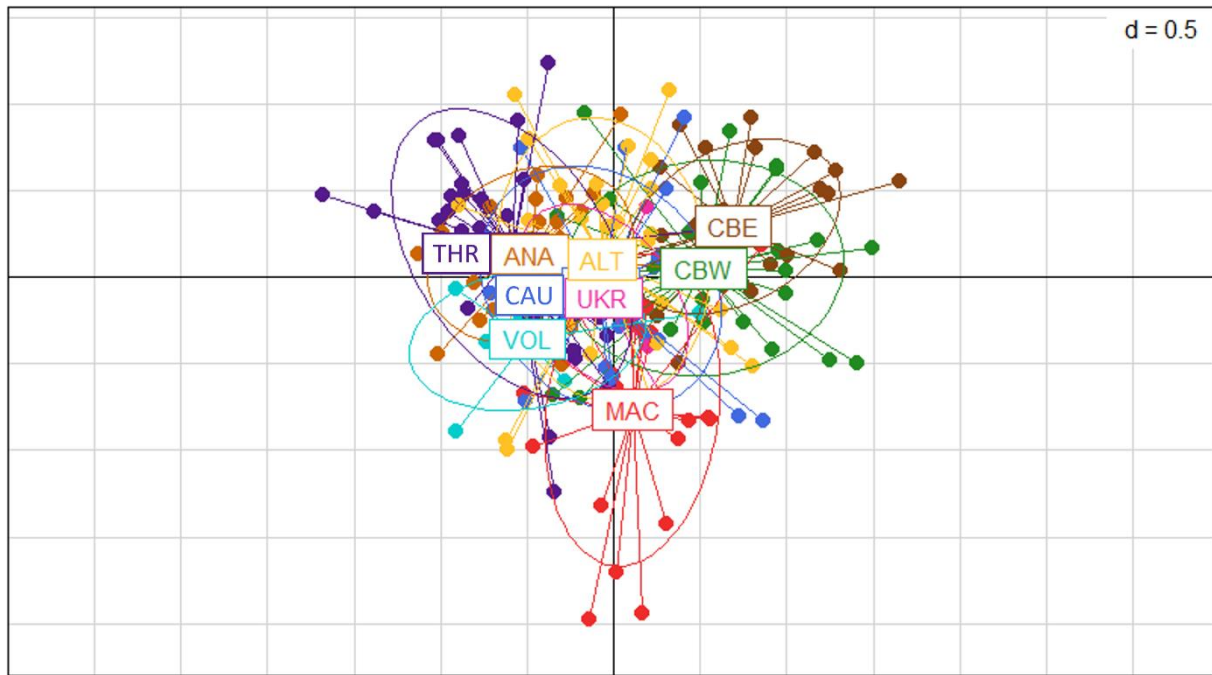


Fig. 3 Principal Coordinate Analysis, based on pairwise genetic distances between sampled EIE individuals (different colours refer for individuals in different geographic populations (CBW – Western Carpathian Basin, CBE – Eastern Carpathian Basin, MAC – Northern Macedonia, THR – Thrace, ANA – Anatolia, CAU – South Caucasus, UKR – Crimea-Ukraine, VOL – Volga-Ural, and ALT – Altai mountains).

On the population level (excluding Crimea-Ukraine and Volga-Ural data due to low sample sizes), PCoA shows the same pattern, with South Caucasus and the Altai mountains being in the middle, and the other populations splitting into divergent clades: the Western and Eastern Carpathian Basin, Northern Macedonia, and Thrace-Anatolia (Fig. 4).

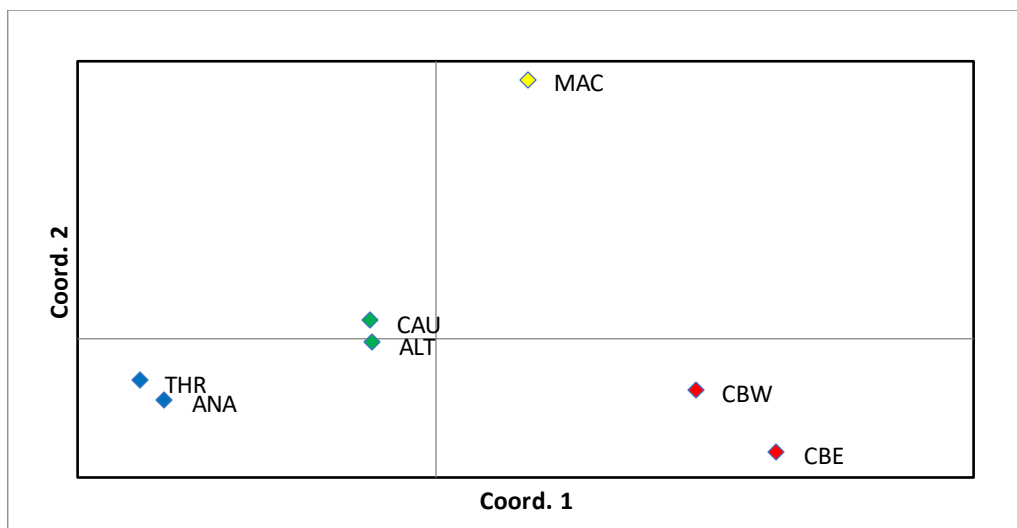


Fig. 4 Principal Coordinate Analysis based on the F_{st} values between the geographic EIE populations. (Abbreviations refer to the geographical populations: CBW – Western Carpathian Basin, CBE – Eastern Carpathian Basin, MAC – Northern Macedonia, THR – Thrace, ANA – Anatolia, CAU – South Caucasus, and ALT – Altai mountains).

The simulations performed in STRUCTURE yielded the highest likelihood with four clusters (K=4). DAPC showed cruder, but highly similar results. The geographic populations can be sorted into four main clades: Carpathian Basin, Northern Macedonia, Thrace-Anatolia, and the eastern clade (CAU, UKR, VOL, ALT). Even though both parts of the Carpathian Basin mainly belong to one cluster, the eastern part shows less diversity. Northern Macedonia shares its gene pool with the Carpathian Basin and Thrace to some degree but shows clear signs of isolation. Thrace-Anatolia bears more resemblance to the eastern clade, indicating some level of gene flow. The eastern populations (particularly the Altai Mountains) are almost evenly admixed between all four clusters, except the Volga-Ural, where the cluster one (shown in red on Fig. 5) was missing.

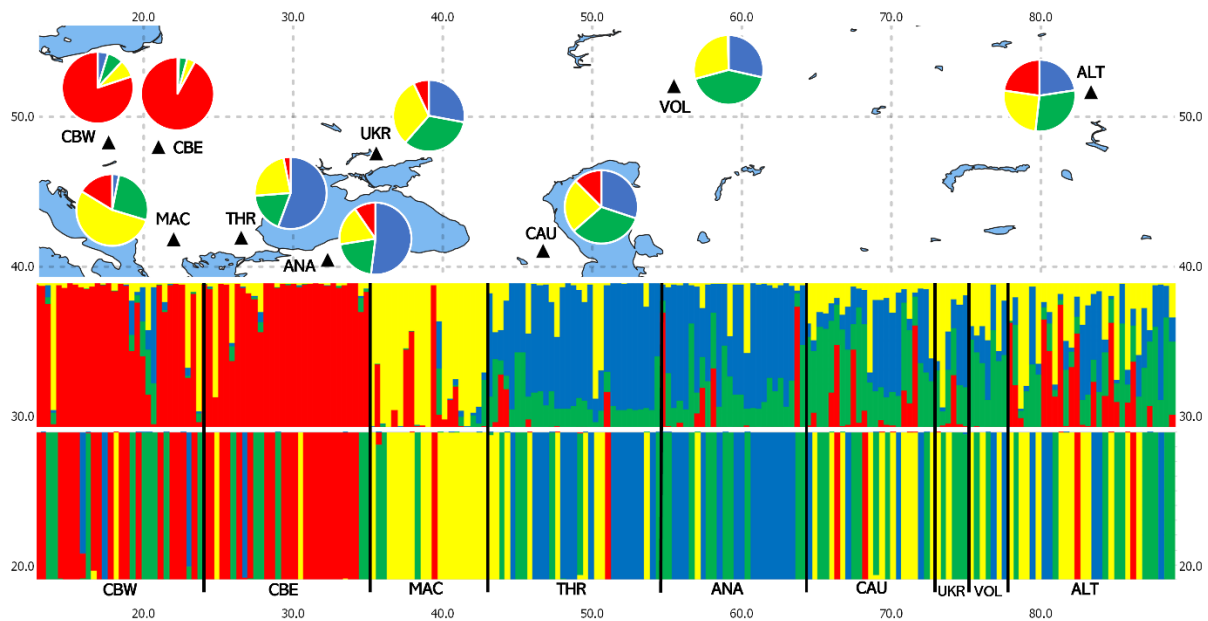


Fig. 5 Clustering of EIE samples (K=4) as inferred by STRUCTURE (above) and DAPC (below).: (Abbreviations refer to the geographical populations: CBW – Western Carpathian Basin, CBE – Eastern Carpathian Basin, MAC – Northern Macedonia, THR – Thrace, ANA – Anatolia, CAU – South Caucasus, UKR – Crimea-Ukraine, VOL – Volga-Ural, and ALT – Altai mountains. On the graphical output figures, each vertical bar represents an individual (n=204). Pie charts show the total ratios of assigned STRUCTURE genetic clusters of individuals in each geographic population. Geographic populations were marked with their centroid coordinate.

The Mantel test yielded a nonsignificant p-value (0.6966), indicating that there is no significant correlation between genetic differences and geographical distances across the nine geographic populations (Fig. 6), therefore there is no sign of isolation by distance.

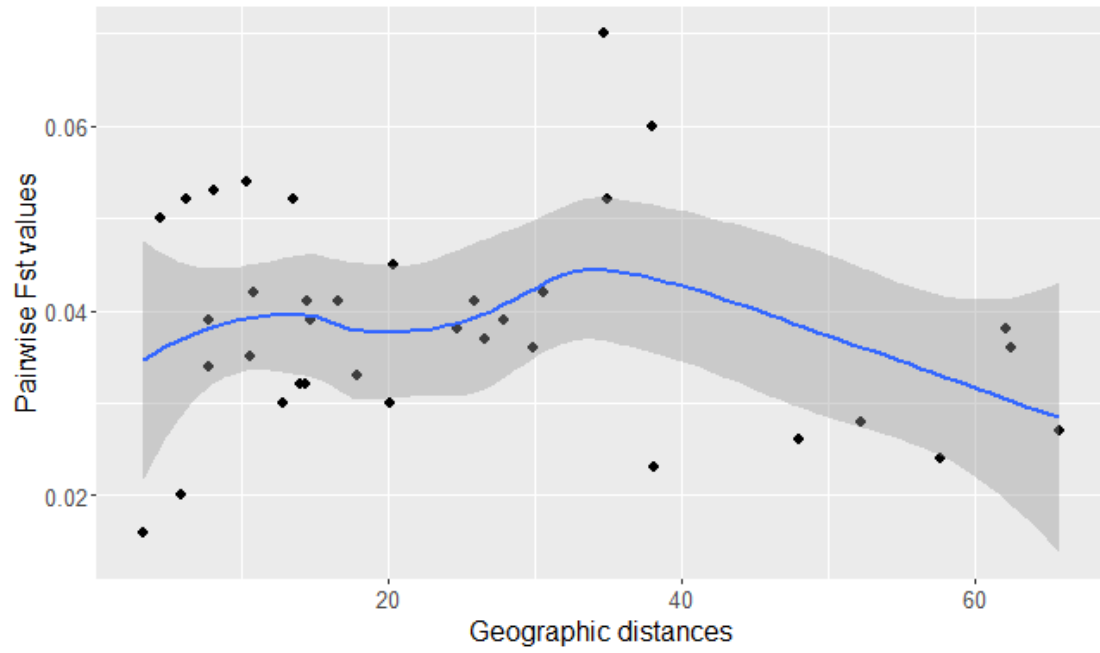


Fig.6 Scatterplot of geographic distances between centroid points and pairwise F_{st} values of all geographic populations. A local polynomial regression is fitted with a 95% confidence interval.

According to the analysis in GENECLASS2, only four individuals were detected as first-generation migrants with probability of at least 0.95. Two of them are migrants from the eastern part to the western part of the Carpathian Basin ($P=0.9981, 0.9922$), one has migrated from Western Carpathian Basin to Thrace ($P=0.9962$), and one from the Altai Mountains to Thrace ($P=0.9948$).

Discussion

My results, based on the data of 15 microsatellite markers analysed with several methods, suggest the division of sampled geographic EIE populations into four main clades: 1. Carpathian Basin, 2. Northern Macedonia, 3. Thrace-Anatolia, and 4. the unit of eastern populations including South Caucasus, Crimea-Ukraine, Volga-Ural, and the Altai Mountains. This may be surprising in two respects: 1. the three western clades, which are located in relatively close proximity, are quite differentiated, and 2. despite of the long geographical distances, each of them shows higher relatedness to the easternmost Altai Mountains population, than to any other population in the western clades.

Regarding the Carpathian Basin, a slight divergence was detected between the western and the eastern parts, confirming the difference found by (Vili et al. 2009) in the Slovakian part of the population, but still showing high similarity. Thrace and Anatolia also share very similar gene pools, but they substantially differ from the population of South Caucasus to the east. Northern Macedonia proved to be the most genetically distinct, even though it is geographically located between the other two clades found in Europe. Although vast majority of the global population breeds in the eastern part of the EIE's distribution area (Karyakin, 2018), it forms one uniform clade, contrary to the divided western populations. South Caucasus and the Altai Mountains showed the highest genetic diversity of all populations, while Crimea-Ukraine and Volga-Ural are highly similar to them but bear less reliable results due to small sample sizes and geographically less focused sampling. The nonmigrating breeding group in the Crimean Peninsula consists of only 5-10 pairs and is substantially isolated from the population in Eastern Ukraine (Karyakin, 2020; Vetrov & Milobog, 2008), which makes it a potential victim of inbreeding depression, although current warfare actions in the area pose presumably the largest threat to these two populations and their habitats. Even though the study did not include the populations in the Transbaikal Region and Northern Russia, they presumably possess reduced diversity compared to other eastern populations due to their already decreasing numbers.

Out of 204 individuals (with 182 adults), only four were identified as first-generation migrants, suggesting a very low recent migration rate. Yet, the results suggest a high genetic overlap between the sampled populations, which could be the result of a high gene flow, explaining the relatively high heterozygosity values in all geographical populations. Other raptor species that are considered somewhat philopatric can occasionally breed as far as 400 km away from their natal area (Cadahía et al., 2009; Nemesházi et al., 2016). Nonetheless, the low level of recent

migration despite of the long-distance wandering behaviour of juvenile and immature individuals serves as genetic evidence for strong philopatry. Only Thrace and Anatolia deviated significantly from Hardy-Weinberg equilibrium, which could be caused by recent, undetected migration between them. However, the two together still showed deviation and it seems unlikely that the reason is higher inbreeding than in the other populations, thus there is possibly another, unknown reason behind it.

Even though the declines of western populations in the second half of the 20th century (Bagyura et al., 2002; Haraszthy et al., 1996) most likely caused bottlenecks and fragmentation, their genetic diversity is less reduced than one would expect it to be. Hailer et al. (2006) proposed that white-tailed eagles can retain most of their heterozygosity due to their long generation time, which seems to be the case for EIEs as well. However, the non-migratory behaviour and strong philopatry which is characteristic of the western EIE populations (Horváth et al., 2018b; Horvát, 2022) can result in inbreeding over a long period of time in isolated populations. This may have happened mostly in Northern Macedonia where the population consists of only a small number of breeding pairs and had presumably recovered only from a handful of individuals (Karyakin, 2020)

The relatively close relationship of the remote Altai population to the western ones implies the shared ancestry of EIEs across all its distribution range and that both the Altai and the South Caucasus have preserved most of their genetic diversity since their divergence from the western populations. This poses two main hypotheses: 1. the western areas were separately colonised by EIEs in the past from the East and their genetic differences can be attributed to different founder effect events. 2. the once integrated western clades have undergone fragmentation and bottlenecks, resulting in a reduced diversity and significant differentiation, as suggested above. Other large raptor species have been proved to have different genetic lineages due to remaining at different refugia during the Last Glacial Maximum (Hailer et al., 2007; Nebel et al., 2019; Nemesházi et al., 2016), although my results do not directly suggest such an event. The eastern populations mainly differ from the western ones in that they are migratory and are part of a widespread group of populations in direct connection with each other (Karyakin, 2020). Here, the lack of fragmentation most likely prevents genetic drift and isolation, while migration might also induce diversity. Both hypotheses and even a combination of the two is possible, but the study of more eastern populations and further analysis on migration patterns and colonization scenarios would provide more insight regarding the population structure forming events of the EIE.

This study proves that geographically isolated western populations of the EIE possess distinct genetic profile and reduced diversity compared to the populations in South Caucasus and the Altai Mountains. It also serves as strong evidence that high level of philopatry and lack of migration causes further isolation of already fragmented populations, despite of high dispersion capabilities. Consequently, inbreeding and deterioration of genetic diversity poses a serious threat in isolated EIE populations, especially in Northern Macedonia with only a low number of individuals, therefore the close monitoring and, if necessary, conservation actions of such populations is strongly suggested.

Acknowledgements

I want to thank my supervisors, Dr. Szilvia Pásztory-Kovács and Dr. Krisztián Szabó for all the help and guidance they provided, and Bernadett Zsinka for helping with the organization of the stored samples. I am also grateful to Dr. Márton Horváth, Imre Fatér, Tibor Juhász, and Attila Bereczky for collection of the Hungarian samples under the projects “HELICON - Conservation of imperial eagles by managing human-eagle conflicts in Hungary (LIFE10NAT/HU/019)” and “Pannoneagle LIFE Project - Conservation of imperial eagles in the Pannonian region (LIFE15/NAT/HU/000902)“. Foreign samples are thanks to the collaboration of the following organizations; Raptor Protection of Slovakia; Ministry of the Environment of the Czech Republic; BirdLife Österreich; Bird Protection and Study Society of Serbia; Bulgarian Society for the Protection of Birds; Department of Wildlife Management Ministry of Forestry and Water Affairs Ankara, Turkey; Russian Raptor Research and Conservation Network; and to Dr. Márton Horváth (Northern Macedonia and South Caucasus).

Author contribution

- My laboratory work for this study consisted of performing PCR on 204 samples for eight loci, 10 samples for seven loci, and checking the success of amplification by gel electrophoresis.
- I scored and identified the alleles of 204 samples for eight loci and 10 samples for seven loci.
- All figures and abovementioned analyses were done by myself, therefore this study contained only my own results.

Hereby I declare that I have been awarded with special prize for my work titled “A rákosi vipera (*Vipera ursinii rakosiensis*) és potenciális madár predátorainak napszakos aktivitás mintázata” at the TDK conference of the University of Veterinary Medicine Budapest in 2021. I submit the award-winning essay as part of my application for the current conference.

References

- Abdul-Muneer, P. M. (2014). Application of Microsatellite Markers in Conservation Genetics and Fisheries Management: Recent Advances in Population Structure Analysis and Conservation Strategies. *Genetics Research International*, 2014. <https://doi.org/10.1155/2014/691759>
- Abuladze, A. (2013). *Materials towards a Fauna of Georgia: Birds of Prey of Georgia* (Vol. 4).
- Allendorf, F. W., Hohenlohe, P. A., & Luikart, G. (2010). Genomics and the future of conservation genetics. In *Nature Reviews Genetics* (Vol. 11, Issue 10). <https://doi.org/10.1038/nrg2844>
- Bagyura, J., Szitta, T., Haraszthy, L., Firmánszky, G., Viszló, L., Kovács, A., Demeter, I., & Horváth, M. (2002). Population increase of Imperial Eagle (*Aquila heliaca*) in Hungary between 1980 and 2000. *Aquila*, 107, 133–144.
- BirdLife International. (2019). *Aquila heliaca* (amended version of 2017 assessment). The IUCN Red List of Threatened Species 2019. <https://dx.doi.org/10.2305/IUCN.UK.2019-3.RLTS.T22696048A155464885.en>.
- BirdLife International. (2021). *Aquila heliaca* (Europe assessment). *The IUCN Red List of Threatened Species 2021*. <https://dx.doi.org/10.2305/IUCN.UK.2021-3.RLTS.T22696048A166304029.en>.
- Busch, J. D., Katzner, T. E., Bragin, E., & Keim, P. (2005). Tetranucleotide microsatellites for aquila and haliaeetus eagles. *Molecular Ecology Notes*, 5(1). <https://doi.org/10.1111/j.1471-8286.2004.00823.x>
- Cadahía, L., López-López, P., Urios, V., Soutullo, Á., & Negro, J. J. (2009). Natal dispersal and recruitment of two Bonelli's eagles *aquila fasciata*: A four-year satellite tracking study. *Acta Ornithologica*, 44(2). <https://doi.org/10.3161/000164509X482777>
- Danko, Š., & Mihók, J. (2022). *A parlagi sas (Aquila heliaca) kelet-szlovákiai állományának alakulása 1967 és 2018 között* (J. Bagyura, Ed.; Vol. 16). Magyar Madártani és Természetvédelmi Egyesület.
- Demerdzhiev, D., Horváth, M., Kovács, A., Stoychev, S., & Karyakin, I. (2011). Status and population trend of the eastern imperial eagle (*Aquila heliaca*) in Europe in the period 2000-2010. *Acta Zoologica Bulgarica*, 63(SUPPL.3).
- Demerdzhiev, D., Angelov, I., & Dobrev, D. (2022). Foraging Patterns of Non-Territorial Eastern Imperial Eagle (*Aquila heliaca*): A Case of Successful Adaptation. *Diversity*, 14(12), 1060. <https://doi.org/10.3390/d14121060>
- Dray, S., & Dufour, A. B. (2007). The ade4 package: Implementing the duality diagram for ecologists. *Journal of Statistical Software*, 22(4). <https://doi.org/10.18637/jss.v022.i04>
- Falush, D., Stephens, M., & Pritchard, J. K. (2003). Inference of population structure using multilocus genotype data: Linked loci and correlated allele frequencies. *Genetics*, 164(4). <https://doi.org/10.1093/genetics/164.4.1567>
- Formenti, G., Theissinger, K., Fernandes, C., Bista, I., Bombarely, A., Bleidorn, C., Ciofi, C., Crottini, A., Godoy, J. A., Höglund, J., Malukiewicz, J., Mouton, A., Oomen, R. A., Paez, S., Palsbøll, P. J., Pampoulie, C., Ruiz-López, M. J., Svardal, H., Theofanopoulou, C., ... Zammit, G. (2022). The era of reference genomes in conservation genomics. *Trends in Ecology & Evolution*, 37(3), 197–202. <https://doi.org/10.1016/j.tree.2021.11.008>
- Frankham, R. (1995). CONSERVATION GENETICS. *Annual Review of Genetics*, 29(1), 305–327. <https://doi.org/10.1146/annurev.ge.29.120195.001513>
- Goossens, B. (2015). Microsatellite records for volume 7, issue 4. *Conservation Genetics Resources*, 7(4). <https://doi.org/10.1007/s12686-015-0493-8>

- Hailer, F., Gautschi, B., & Helander, B. (2005). Development and multiplex PCR amplification of novel microsatellite markers in the White-tailed Sea Eagle, *Haliaeetus albicilla* (Aves: Falconiformes, Accipitridae). *Molecular Ecology Notes*, 5(4). <https://doi.org/10.1111/j.1471-8286.2005.01122.x>
- Hailer, F., Helander, B., Folkestad, A. O., Ganusevich, S. A., Garstad, S., Hauff, P., Koren, C., Nygård, T., Volke, V., Vilà, C., & Ellegren, H. (2006). Bottlenecked but long-lived: High genetic diversity retained in white-tailed eagles upon recovery from population decline. *Biology Letters*, 2(2). <https://doi.org/10.1098/rsbl.2006.0453>
- Hailer, F., Helander, B., Folkestad, A. O., Ganusevich, S. A., Garstad, S., Hauff, P., Koren, C., Masterov, V. B., Nygård, T., Rudnick, J. A., Shiraki, S., Skarphedinsson, K., Volke, V., Wille, F., & Vilà, C. (2007). Phylogeography of the white-tailed eagle, a generalist with large dispersal capacity. *Journal of Biogeography*, 34(7). <https://doi.org/10.1111/j.1365-2699.2007.01697.x>
- Hám, I. (2022). *Parlagi sasok (Aquila heliaca) első felmérése és eltűnésének okai a Delibláti homokpusztán 1976 és 1986 között* (J. Bagyura, Ed.; Vol. 16). Magyar Madártani és Természetvédelmi Egyesület.
- Haraszthy, L., Bagyura, J., Szitta, T., Petrovits, Z., & Viszló, L. (1996). *Biology, Status and Conservation of the Imperial Eagle Aquila heliaca in Hungary in: Eagle Studies* (B.-U. Meyburg & R. D. Chancellor, Eds.). World Working Group on Birds of Prey (WWGBP).
- Hauser, S. S., Athrey, G., & Leberg, P. L. (2021). Waste not, want not: Microsatellites remain an economical and informative technology for conservation genetics. *Ecology and Evolution*, 11(22). <https://doi.org/10.1002/ece3.8250>
- Hedrick, P. W. (2000). Inbreeding depression in conservation biology. *Annual Review of Ecology and Systematics*, 31. <https://doi.org/10.1146/annurev.ecolsys.31.1.139>
- Horal, D. (2011). Eastern Imperial Eagle (*Aquila heliaca*) in the Czech Republic. *Acta Zoologica Bulgarica*, 63(SUPPL.3).
- Horváth, M. B., Martínez-Cruz, B., Negro, J. J., Kalmár, L., & Godoy, J. A. (2005). An overlooked DNA source for non-invasive genetic analysis in birds. *Journal of Avian Biology*, 36(1). <https://doi.org/10.1111/j.0908-8857.2005.03370.x>
- Horváth, M., Demeter, I., Fatér, I., Firmánszky, G., Kleszó, A., Kovács, A., Szitta, T., Tóth, I., Zalai, T., & Bagyura, J. (2011). Population dynamics of the eastern Imperial Eagle (*Aquila heliaca*) in Hungary between 2001 and 2009. *Acta Zoologica Bulgarica*, 63(SUPPL.3).
- Horváth, M., Béres, I., Özcan, C., Juhász, T., Kovács, A., Tatar, B., Karyakin, I., Schmidt, M., & Tavares, J. (2018). Breeding Population Surveys of Eastern Imperial Eagles and Steppe Eagles in Central Anatolia. *II International Scientific and Practical Conference 'Eagles of Palearctic: Study and Conservation'*.
- Horváth, M., Juhász, T., Fatér, I., Schmidt, M., Dvorak, M., Wendelin, B., Wichmann, G., Gamauf, A., McGrady, M., Raab, R., Spakovszky, P., Stoychev, S., Demerdzhiev, D. A., Spasov, S. D., Dobrev, D. D., Meyburg, B.-U., Horal, D., Literak, I., Chavko, J., ... Prommer, M. (2018). Juvenile Dispersal Movements of Eastern Imperial Eagles in the Resident Populations. *LLC Sibecocenter*. <http://rusraptors.ru/index.php/RC/article/view/178>
- Horváth, M. (2022). *Parlagi sas Aquila heliaca Savigny 1809. In Magyarország ragadozó madarai és baglyai I. kötet. Vágómadár-alakúak* (L. Haraszthy & J. Bagyura, Eds.). Magyar Madártani és Természetvédelmi Egyesület.
- Horváth, M., Juhász, T., Fatér, I., Deák, G., Árvay, G., & Pásztory-Kovács, S. (2023). Status of the Eastern Imperial Eagle (*Aquila heliaca*) in Hungary between 2019 and 2023. *Eagles of the Palearctic: Study and Conservation: Third International Scientific and Practical Conference*.

- Jakab, S. (2015). *A nem-invazív mintavételi módszer megbízhatóságának elemzése és betolakodó egyedek vizsgálata egy parlagi sas (Aquila heliaca) populációban*. University of Veterinary Medicine Budapest.
- Jánossy, D. (1981). Plio-Pleistocene bird remains from the Carpathian Basin VI. *Sysematical and Geographical Catalogue*, 87, 9–22.
- Jombart, T. (2008). Adegnet: A R package for the multivariate analysis of genetic markers. *Bioinformatics*, 24(11). <https://doi.org/10.1093/bioinformatics/btn129>
- Karyakin, I. V., Nikolenko, E. N., Levin, A. S., & Kovalenko, A. V. (2011). Eastern Imperial Eagle in Russia and Kazakhstan: Population status and trends. *Acta Zoologica Bulgarica*, 63(SUPPL.3).
- Karyakin, I. V. (2018). Distribution, population number and status of the Eastern Imperial Eagle in Russia and Kazakhstan. *II International Scientific and Practical Conference "Eagles of Palearctic: Study and Conservation"*.
- Karyakin, I. V. (2020). Breeding Population Structure of the Eastern Imperial Eagle. *Raptors Conservation*, 41, 64–332. <https://doi.org/10.19074/1814-8654-2020-41-64-332>
- Kotrošan, D., & Hatibović, E. (2012). Raptors in Bosnia and Herzegovina - their status and perspectives for monitoring development. *Acrocephalus*, 33(154–155). <https://doi.org/10.2478/v10100-012-0004-x>
- Kretzmann, M. B., Capote, N., Gautschi, B., Godoy, J. A., Donázar, J. A., & Negro, J. J. (2003). Genetically distinct island populations of the Egyptian vulture (*Neophron percnopterus*). *Conservation Genetics*, 4(6). <https://doi.org/10.1023/B:COGE.00000006123.67128.86>
- Lazarova, I., Dobrev, D., Gradev, G., Petrov, R., Stoychev, S., Klisurov, I., & Demerdzhiev, D. (2020). Main mortality factors for the Eastern Imperial Eagle (*Aquila heliaca* Savigny, 1809) in Bulgaria. In *Ornis Hungarica* (Vol. 28, Issue 2). <https://doi.org/10.2478/orhu-2020-0021>
- Lemopoulos, A., Prokkola, J. M., Uusi-Heikkilä, S., Vasemägi, A., Huusko, A., Hyvärinen, P., Koljonen, M. L., Koskineniemi, J., & Vainikka, A. (2019). Comparing RADseq and microsatellites for estimating genetic diversity and relatedness — Implications for brown trout conservation. *Ecology and Evolution*, 9(4). <https://doi.org/10.1002/ece3.4905>
- Lisichanets, E. (2014). *Conservation of Imperial Eagles (Aquila heliaca) in Macedonia*. The Rufford Foundation. <https://www.rufford.org/projects/emanuel-lisichanets/conservation-of-imperial-eagles-aquila-heliaca-in-macedonia/>
- Lisichanets, E. (2017). Long-Term Conservation Measures for Imperial Eagles in Macedonia. *The Rufford Foundation*. <https://www.rufford.org/projects/emanuel-lisichanets/long-term-conservation-measures-for-imperial-eagles-in-macedonia/>
- Martínez-Cruz, B., David, V. A., Godoy, J. A., Negro, J. J., O'Brien, S. J., & Johnson, W. E. (2002). Eighteen polymorphic microsatellite markers for the highly endangered Spanish imperial eagle (*Aquila adalberti*) and related species. In *Molecular Ecology Notes* (Vol. 2, Issue 3). <https://doi.org/10.1046/j.1471-8286.2002.00231.x>
- Martínez-Cruz, B., Godoy, J. A., & Negro, J. J. (2007). Population fragmentation leads to spatial and temporal genetic structure in the endangered Spanish imperial eagle. *Molecular Ecology*, 16(3). <https://doi.org/10.1111/j.1365-294X.2007.03147.x>
- Milvus group. (2016). *Event: after 45 years, Imperial Eagle was found nesting in Romania, by Milvus Group experts!* <https://milvus.ro/en/event-after-45-years-imperial-eagle-was-found-nesting-in-romania-by-milvus-group-experts/>

- Nebel, C., Gamauf, A., Haring, E., Segelbacher, G., Väli, Ü., Villers, A., & Zachos, F. E. (2019). New insights into population structure of the European golden eagle (*Aquila chrysaetos*) revealed by microsatellite analysis. *Biological Journal of the Linnean Society*, 128(3). <https://doi.org/10.1093/biolinnean/blz130>
- Nemesházi, E., Kövér, S., Zachos, F. E., Horváth, Z., Tihanyi, G., Mórocz, A., Mikuska, T., Hám, I., Literák, I., Ponnikas, S., Mizera, T., & Szabó, K. (2016). Natural and anthropogenic influences on the population structure of white-tailed eagles in the Carpathian Basin and central Europe. *Journal of Avian Biology*, 47(6). <https://doi.org/10.1111/jav.00938>
- Ouborg, N. J., Pertoldi, C., Loeschcke, V., Bijlsma, R. K., & Hedrick, P. W. (2010). Conservation genetics in transition to conservation genomics. In *Trends in Genetics* (Vol. 26, Issue 4). <https://doi.org/10.1016/j.tig.2010.01.001>
- Peakall, R., & Smouse, P. E. (2006). GENALEX 6: Genetic analysis in Excel. Population genetic software for teaching and research. *Molecular Ecology Notes*, 6(1). <https://doi.org/10.1111/j.1471-8286.2005.01155.x>
- Pertoldi, C., & Randi, E. (2018). The ongoing transition at an exponential speed from Conservation genetics to Conservation genomics. *GABJ*, 2(2). <https://doi.org/10.46325/gabj.v2i2.124>
- Piry, S., Alapetite, A., Comuet, J. M., Paetkau, D., Baudouin, L., & Estoup, A. (2004). GENECLASS2: A software for genetic assignment and first-generation migrant detection. *Journal of Heredity*, 95(6). <https://doi.org/10.1093/jhered/esh074>
- Ralls, K., Ballou, J. D., Dudash, M. R., Eldridge, M. D. B., Fenster, C. B., Lacy, R. C., Sunnucks, P., & Frankham, R. (2018). Call for a Paradigm Shift in the Genetic Management of Fragmented Populations. *Conservation Letters*, 11(2). <https://doi.org/10.1111/conl.12412>
- Rannala, B., & Mountain, J. L. (1997). Detecting immigration by using multilocus genotypes. *Proceedings of the National Academy of Sciences of the United States of America*, 94(17). <https://doi.org/10.1073/pnas.94.17.9197>
- Rousset, F., Lopez, L., & Belkhir, K. (2017). Package “genepop.” In *Cran*.
- Rudnick, J. A., Katzner, T. E., Bragin, E. A., & Dewoody, J. A. (2008). A non-invasive genetic evaluation of population size, natal philopatry, and roosting behavior of non-breeding eastern imperial eagles (*Aquila heliaca*) in central Asia. *Conservation Genetics*, 9(3). <https://doi.org/10.1007/s10592-007-9397-9>
- Rudnick, J. A., Katzner, T. E., Bragin, E. A., Rhodes, O. E., & Dewoody, J. A. (2005). Using naturally shed feathers for individual identification, genetic parentage analyses, and population monitoring in an endangered Eastern imperial eagle (*Aquila heliaca*) population from Kazakhstan. *Molecular Ecology*, 14(10), 2959–2967. <https://doi.org/10.1111/j.1365-294X.2005.02641.x>
- Ryabtsev, V. V., & Katzner, T. E. (2007). Severe declines of Eastern Imperial Eagle *Aquila heliaca* populations in the Baikal region, Russia: A modern and historical perspective. *Bird Conservation International*, 17(3). <https://doi.org/10.1017/S0959270907000755>
- Sato, Y., Ito, H., Onuma, M., Maeda, T., Ogden, R., & Inoue-Murayama, M. (2015). Development of 20 microsatellite markers for the endangered Japanese golden eagle (*Aquila chrysaetos japonica*) using next generation sequencing. *Conservation Genetics Resources*, 7(917–944).
- Schmidt, M., & Horal, D. (2018). Eastern Imperial Eagle (*Aquila heliaca*) on its most north-west distribution edge. *Eagles of Palearctic: 2nd International Scientific and Practical Conference*.
- Škorpíková, V., Bělka, T., Štěpánek, P., & Horal, D. (2014). Data on birds recorded in Republic of Moldova in 2013 and 2014. *Ciconia*, 22–23, 43–47.

- Sultanov, E. H., Karimov, T., Mammadov, A. F., Samadov, A., Isayev, S., & Jabraili, G. (2011). Imperial Eagle, Egyptian Vulture and Lesser Kestrel in Azerbaijan. *Red Data Book Species of Birds of Azerbaijan*.
- Sunde, J., Yıldırım, Y., Tibblin, P., & Forsman, A. (2020). Comparing the Performance of Microsatellites and RADseq in Population Genetic Studies: Analysis of Data for Pike (*Esox lucius*) and a Synthesis of Previous Studies. *Frontiers in Genetics*, 11. <https://doi.org/10.3389/fgene.2020.00218>
- R Core Team. (2023). R Core Team 2023 R: A language and environment for statistical computing. R foundation for statistical computing. <https://www.R-project.org/>. *R Foundation for Statistical Computing*.
- Ukolov, I. I., Nastachenko, A. S., & Abbasov, A. (2018). Ornithological findings in Azerbaijan based on observations in 2017–2018. *The Russian Journal of Ornithology*, 27.
- Van Oosterhout, C., Hutchinson, W. F., Wills, D. P. M., & Shipley, P. (2004). MICRO-CHECKER: Software for identifying and correcting genotyping errors in microsatellite data. *Molecular Ecology Notes*, 4(3). <https://doi.org/10.1111/j.1471-8286.2004.00684.x>
- Vasvári, M. (1938). Verbreitung und Oekologie des Kaiseradlers (*Aquila heliaca* Sav.). *Festschrift Für Prof. Dr. Embrik Strand.*, 5, 290–317.
- Vetrov V, & Milobog YU. (2008). Distribution of the Imperial Eagle in a Steppe Zone of Ukraine. *Raptors Conservation*, 14, 28–30.
- Vili, N., Chavko, J., Szabó, K., Kovács, S., Hornung, E., Kalmár, L., & Horváth, M. (2009). Genetic structure of the Imperial Eagle (*Aquila heliaca*) population in Slovakia. *Slovak Raptor Journal*, 3(1). <https://doi.org/10.2478/v10262-012-0029-z>
- Vili, N., Nemesházi, E., Kovács, S., Horváth, M., Kalmár, L., & Szabó, K. (2013). Factors affecting DNA quality in feathers used for non-invasive sampling. *Journal of Ornithology*, 154(2). <https://doi.org/10.1007/s10336-013-0932-9>
- Weigmann, H.-D. (1968). Reduction of disulfide bonds in keratin with 1,4-dithiothreitol. I. Kinetic investigation. *Journal of Polymer Science Part A-1: Polymer Chemistry*, 6(8), 2237–2253. <https://doi.org/10.1002/pol.1968.150060819>