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Genetic diversity of the West Carpathians golden eagle (*Aquila chrysaetos*)

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Abstract. The fragmentation analysis was chosen to examine the genetic diversity of golden eagles from the West Carpathians. Different types of tissues were sampled - blood, egg contents, eggshell and feathers - between May 1984 and July 2009. In total, 66 samples were compared. All loci in the tested samples showed F_{IS} values significantly greater than zero, which indicates an excess of homozygotes. There is a remarkable lower heterozygosity in Slovak, West Carpathian golden eagles than in eagles from Spain or Great Britain.

Key words: Aquila chrysaetos, fragmentation analysis, West Carpathians

Introduction

Aquila chrysaetos from Accipitridae is one of four genera of eagles living in the Slovak Republic. Besides the golden eagle; *A. heliaca*, *A. pomarina* and *Hieraaetus pennatus* are inhabitants of the Slovakian region of the West Carpathians. The golden eagle primarily inhabits mountainous areas above 800 m a.s.l. Typical habitats include old forests with mountain meadows and pastures. In Slovakia, *Aquila chrysaetos* is a protected animal under Act no. 543/2002 of the Law on nature and landscape protection and by regulation of the Ministry of the Environment. Additionally, 24/2003 is included in the category of species of European importance. The golden eagle is included in the Red list of threatened bird species in Slovakia as a "vulnerable species". The breeding population in Slovakia is estimated at 120-140 pairs (Nuhlíčková and Maderič 2010).

The eagle is an apex predator, residing at the top of ecological food webs and the species is very sensitive to environmental changes such as chemical contaminants, food shortages, sex ratio distortion, and habitat destruction. Golden eagles represent one of the most important ecological indicators of environmental health (Chang *et al.* 2008)

Microsatellite analysis is a good method for studying population genetics. In this study, fragmentation analysis was chosen because of the reliability of produced data. MSAT is also a good tool for the detection of genetic relationships and diversity, parentage and kinship, as well as in the study of recent population history due to a high mutation rate. In recent history, there are additional studies available regarding micrasatellite analysis of raptor species, including *Aquila chrysaetos* (e.g. Nesje *et al.* 2000; Martinez-Cruz *et al.* 2002; Busch *et al.* 2005; Bourke and Dawson 2006; Dawnay *et al.* 2009). The multiplex PCR assay has been established for the study of White-tailed eagle (Hailer *et al.* 2005) as well as for *Aquila chrysaetos* (Bieliková *et al.* 2010).

Material and Methods

Sample collection and DNA extraction

For analysis, different types of tissues were sampled - blood, egg contents, eggshell and feathers - between May 1984 and July 2009 (Table 1). The samples originate from the West Carpathians, Slovakia. DNA was extracted from blood, feather and eggshell inner membrane using the QIAGEN DN easy Blood and Tissue Kit according to standard protocol for blood and feathers and the protocol for isolation of total DNA from animal tissues with modifications as described Busch *et al.* (2005) for eggshell membrane.

Fragment analysis and genetic diversity

Microsatellite analyses of AA26 and AA36 loci was performed according to Martinez-Cruz *et al.* (2002) and loci AA04 and AA39 according to Dawnay *et al.* (2008). Forward primers were fluorescently labeled with Cy-5. Fragmentation analyses ran on GenomeLab GeXP (Beckman Coulter). Raw data were analysed by GenomLab software (Beckman Coulter). Scored alleles were analysed by Genepop4 (Rousset 2012). Genetic diversity and differentiation (allele frequency, genotype frequency, real and effective number of allele, observed and expected heterosygosity and Hardy-Weinberg equilibrium) were tested. F_{IS} were calculated as in Weir and Cockerham (1984).

Results and Discussion

All of the examined loci were polymorphic. The mean number of alleles per locus was 8.5. All loci in the tested samples showed F_{IS} values significantly greater than zero, which indicates an excess of homozygotes, possibly arising from inbreeding (Table 1). F_{IS} is a coefficient that indicates the overall deviation from the Hardy-Weinberg expectation for all loci within the populations sampled. Positive F_{IS} values indicate increasing homozygosity conditions in a population, while negative values indicate an excess of heterozygotes (Svoboda *et al.* 1985).

Conversely, positive values of FIS do not necessarily indicate inbreeding. These values can be inflated if genetic subpopulations exist, but are recognized when the samples are taken; this situation is known as the Wahlund effect (Wahlund 1928). Bieliková *et al.* (2010) mentioned similar findings for loci AA04 and AA26 in wild-living populations and loci AA04, AA26 and AA36 for captive-bred populations. The value of observed heterozygosity (H0) calculated for each locus is higher (AA26 loci, aa36, AA04, AA39) than the expected heterozygosity (HE). The resulting average value of H0 is clearly lower than HE. Therefore, it can be concluded that the overall genetic variability in Slovak populations of *Aquila chrysaetos* is not sufficient. Consequently, the population has likely exhibited the Wahlund effect.

More details about observed alleles, their frequencies and F_{IS}, as well as number of homozygotes and heterozygotes are shown in Table 2.

Locus	Primer sequences 5´ -3´	Repeat motif	Size range (bp)	n	A	Observed allele (bp)	H_0	H_E	F_{IS}
Aa04	F: Cy5-TGCAGCTCAAAAGCAAAGG R: CAACCCCAACTCTCACACCT	$(GT)_{12}$	122-170	45	12	122, 124, 126, 128, 142, 146, 150, 152, 156, 158, 162, 166	0.69**	0.89	0.207
Aa26	F: Cy5-TGCAGCTCAAAAGCAAAGG R: CAACCCCAACTCTCACACCT	$(AC)_{14}$	140-154	66	6	140, 142, 148, 150, 152, 154	0.58*	0.75	0.235
Aa36	F: Cy5-GCAAAGGTAAACTGCATCTGG R: ATGCACTATTGGTAAACAGGCA	$(AC)_{16}$	96-124	66	6	96, 98, 100, 102, 104, 124	0.38*	0.66	0.428
Aa39	F: Cy5-ACAGGCCAGCACCAAGAG R: TTTGGAGCCATTGTTACCGT	$(AC)_{13}$	187-229	51	10	187, 189, 191, 195, 197, 199, 205, 209, 211, 229	0.53*	0.84	0.391

Table 1. Characterization of 4 polymorphic microsatellite loci for the golden eagle *Aquila chrysaetos* (Accipitridae, Aves). n - number of unrelated tested Golden eagle individuals from a Slovak population; A - number of alleles per locus; H0 - observed heterozygosity; HE, - expected heterozygosity; * P = 0; ** P = 0,027.

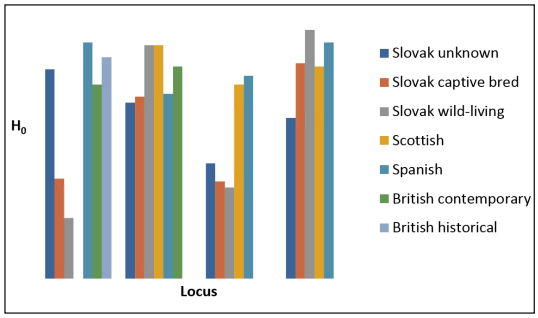

Fig. 1. Comparison of observed heterozygosity among Slovak population of *A. chrysaetos* (this study), captive-bred and wild living eagles (Bielikova *et al.* 2010), and golden eagle population from Scotland (Bourke and Dawson 2006), Spain (Martinez-Cruz *et al.* 2002) and Great Britain (Bourke *et al.* 2010) - loci AA04, AA26. AA36 and AA39, respectively.

In the past, analysis was completed on the populations of family Accipitridae. This includes golden eagle populations from Spain (Martinez-Cruz *et al.* 2002), Scotland (Bourke and Dawson 2006), both the Slovak wild population and individuals bred in captivity (Bieliková *et al.* 2010) and the British contemporary and historical populations (Bourke *et al.* 2010). A graphic comparison of observed heterozygosity among European eagle populations is shown in Fig. 1. There is a remarkable decrease of H0 in wild-living Slovak individuals (0.2) compared to those in captivity (0.33), as well as compared to those of an unknown population from the Slovak republic (0.69) and an additional Spanish individual (0.78).

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Locus	Allele size (bp)	Sample count	Freqency	FIS	Homs	Hets
AA04	122	3	0.0333	-0.0233	0	3
	124	3	0.0333	-0.0233	0	3
	126	21	0.2333	0.2033	4	13
	128	20	0.2222	0.3699	5	10
	142	5	0.0566	0.3744	1	3
	146	7	0.0778	0.2361	0	7
	150	3	0.0333	-0.0233	0	3
	152	11	0.1222	0.2854	0	11
	156	7	0.0778	-0.0732	0	7
	158	5	0.0556	-0.0476	0	5
	162	1	0.0111	0.0000	0	1
	166	4	0.0444	0.4854	1	2
	Total	90	0.2074	0.1429		
AA26	140	6	0.0455	0.3085	1	4
	142	48	0.3636	0.0255	9	30
	148	16	0.1212	0.7192	6	4
	150	40	0.3030	0.2182	9	22
	152	10	0.0758	0.3575	2	6
	154	12	0.0909	0.0909	1	10
	Total	132	0.2346	0.3050		
AA36	96	10	0.0758	0.3575	2	6
	98	72	0.5455	0.5167	28	16
	100	20	0.1515	0.6509	7	6
	102	4	0.0303	0.4902	1	3
	104	13	0.0985	0.2393	2	9
	124	12	0.0909	0.0909	1	10
	126	1	0.2074	0.1429	0	1
	Total	132	0.4278	0.3188		
AA39	187	17	0.1667	0.5132	5	7
	189	26	0.2549	0.5935	9	8
	191	25	0.2451	0.4253	7	11
	195	10	0.0980	-0.0989	0	8
	197	3	0.0294	0.6622	1	1
	199	5	0.0490	-0.0417	0	3
	205	5	0.0490	0.3776	0	5
	209	7	0.0686	0.2424	1	3
	211	3	0.0294	0.6622	1	2
	229	1	0.0098	0.0000	0	1
	Total	102	0.3914	0.3309		

Table 2. Observed allele frequencies for 4 polymorphic loci for Slovak population of *Aquila chrysaetos*; Hets – heterozygots; Homs – homozygotes.

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