

STEPPE EAGLE POPULATION STRUCTURE GENETIC STUDY: IS THERE HOPE FOR THE ENDANGERED SPECIES?

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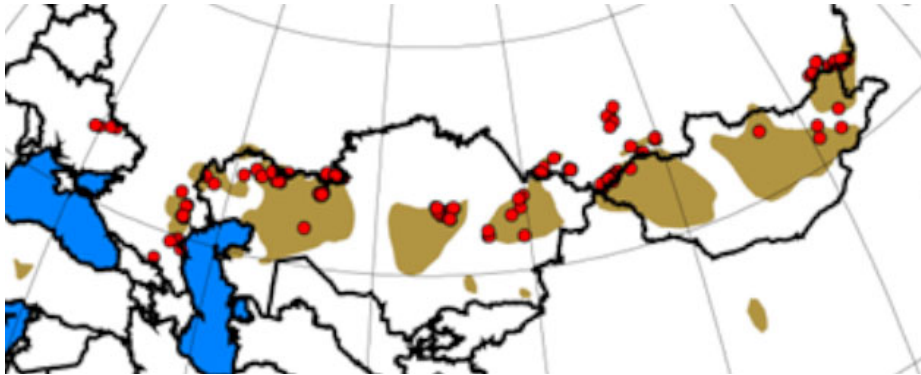
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Materials and methods

- * Comparative analysis of the Steppe eagle and Eastern Imperial eagle population structure;
- * Genetic markers and methods:
 - * Sanger sequencing of the mitochondrial control region (D-loop) full-length (1147 bp) and 420-450 bp polymorphic fragments;
 - * Fragment analysis of nuclear microsatellites (9 multispecies STR loci);
 - * Frequency and genetic distance statistical methods.
- * Sampling and research design:
 - * 262 *Aquila nipalensis* and 104 *Aquila heliaca* independent samples + 10 *Aquila chrysaetos* and 2 *Aquila rapax* samples for comparison;
 - * Genetic markers and methods sensitivity and accuracy check;
 - * Effective population number estimation;
 - * Paternity testing to study natal philopatry.

Population numbers and sampling

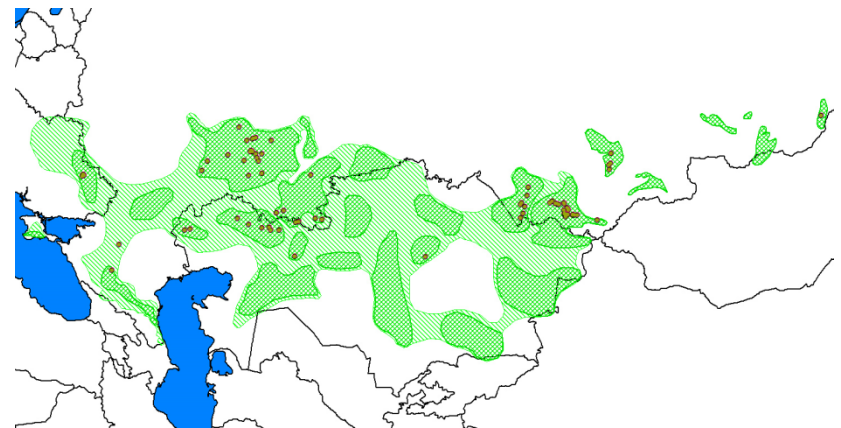
Aquila nipalensis: 50 000 – 75 000 ind. (IUCN)



- About 300 independent samples from about all the area;
- Sampling from nesting groups discriminated by GIS methods.

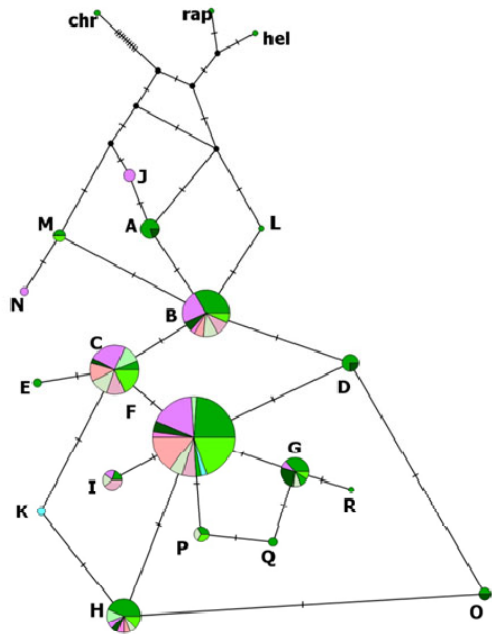
Aquila heliaca: 3 500 – 15 000 ind. (IUCN)

- About 120 independent samples from a fragment of the species area;
- Sampling from a continuous nesting area with some assemblies shown by GIS methods.



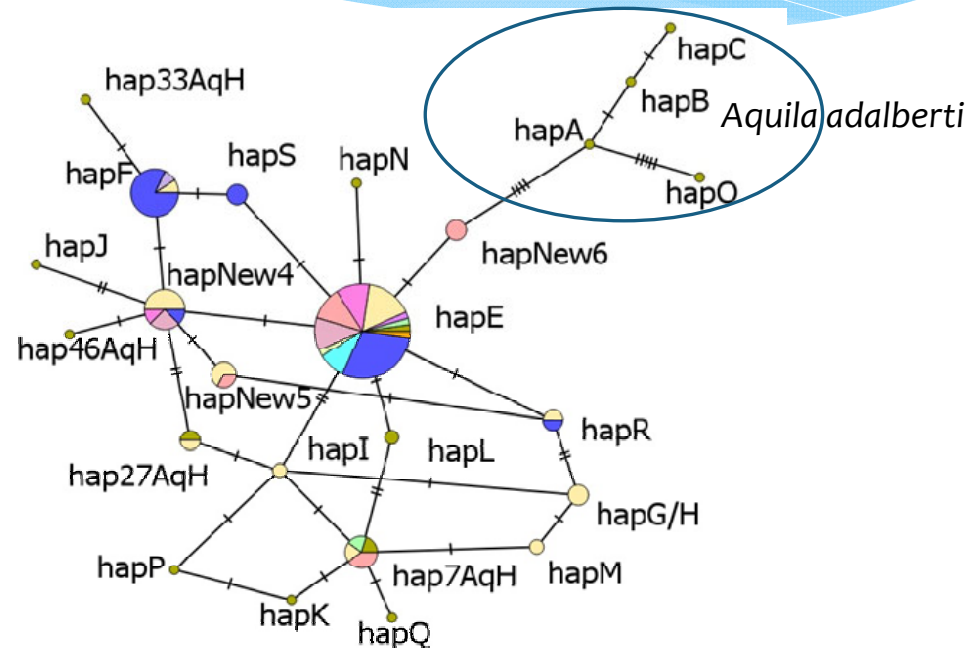
Mitochondrial D-loop polymorphic fragment haplotype TCS networks

Aquila nipalensis: about 220 samples



- 18 haplotypes;
- monotypic;
- “string-of-beads”-like topology

Aquila heliaca: about 85 samples and all known haplotypes



- about 20 haplotypes;
- clear haplogroup for the Spanish Imperial eagle;
- same topology;

Both species have traces of coming through population bottlenecks

Inbreeding and population fragmentation

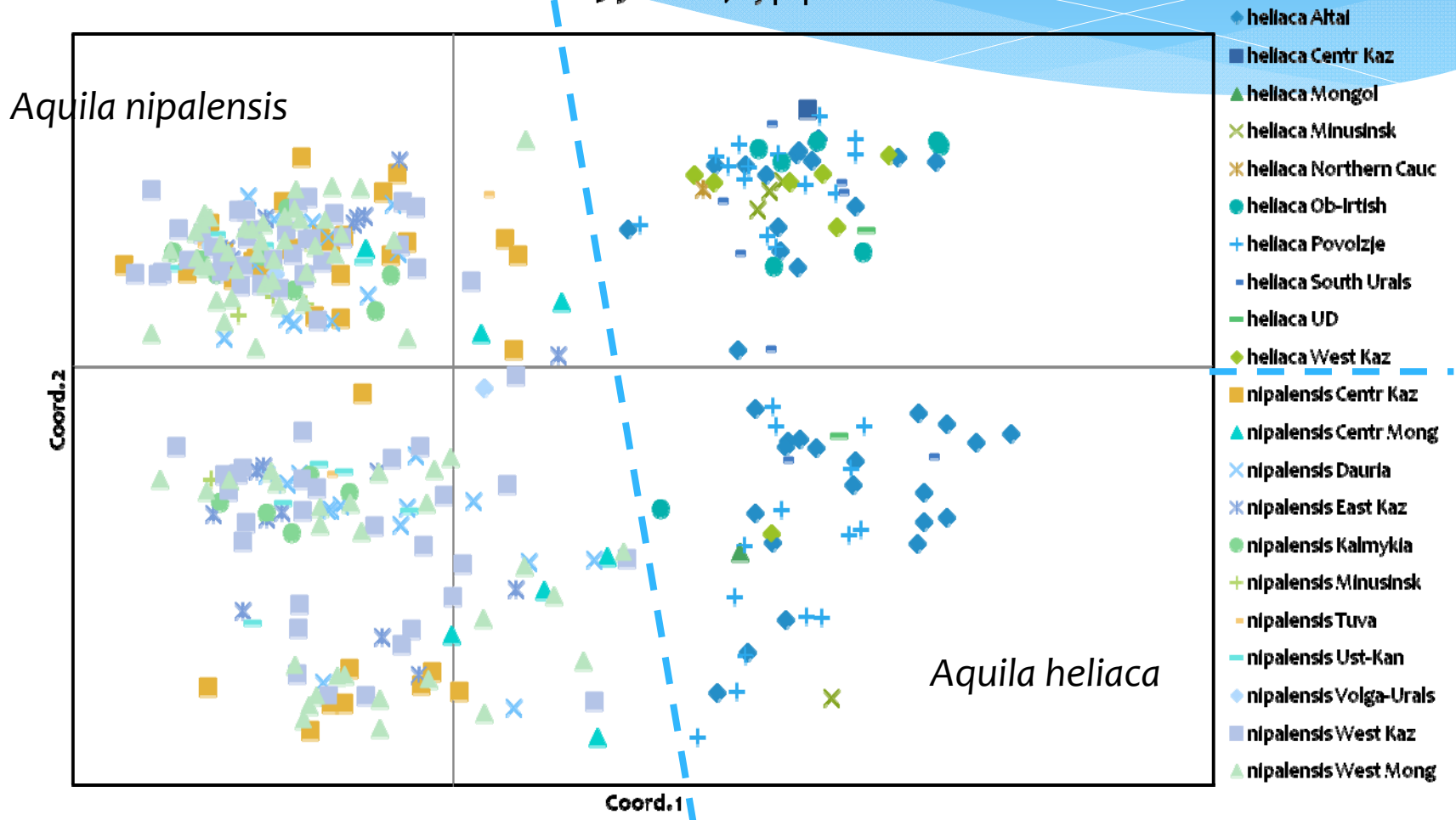
	N	Na	Ho	He	F
<i>Aquila heliaca</i>	89,444 ± 3,941	8,333 ± 1,155	0,622 ± 0,064	0,709 ± 0,037	0,136 ± 0,060
<i>Aquila nipalensis</i>	227,111 ± 9,367	12,444 ± 1,042	0,598 ± 0,046	0,744 ± 0,045	0,178 ± 0,064

N – number of samples; Na – number of alleles; Ho – observed heterozygosity; He – expected heterozygosity; F – Wright's fixation index (inbreeding coefficient)

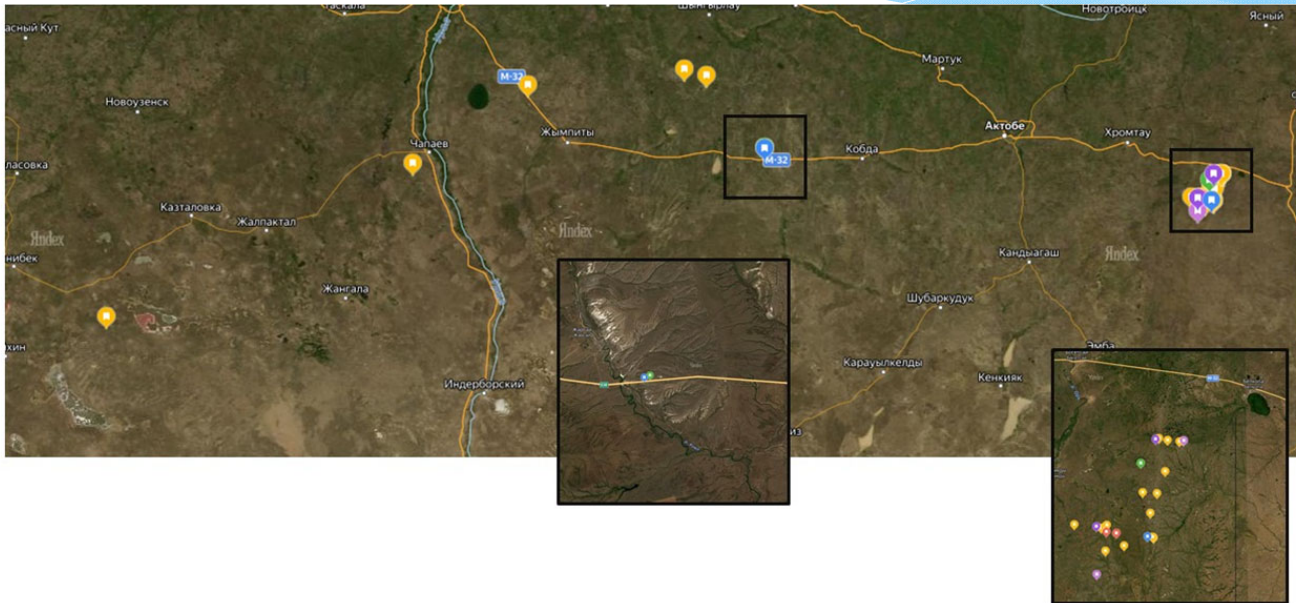
- * **Population fragmentation tests:** genetic distance between species is much higher than intraspecies population fragmentation which is by two orders of magnitude less (AMOVA for some nesting groups) or not significant (frequency G-statistics).

Population fragmentation: principal component analysis

Sample-to-sample genetic distance PCoA
5-9 STR loci, by population



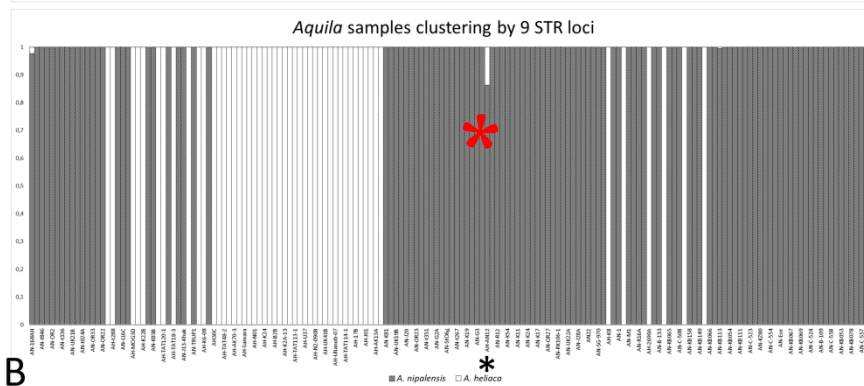
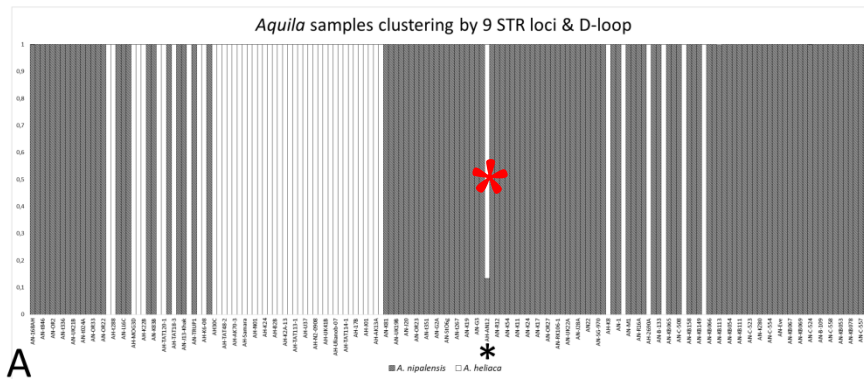
Steppe eagle genetic population structure and natal philopatry



- * Individual identification and paternity analysis of feather samples from 25 nests from the Western Qazaqstan in 2012 and 2013:
 - * 5 pairs of closely related birds recorded at different nests with distances from 1.2 km to 330 km.

Hybridization and mitochondrial DNA introgression in declining populations

114 Steppe, 51 Imperial eagle and 1 putative hybrid samples
Bayesian clustering



■ *Aquila nipalensis* □ *Aquila heliaca*

- mixed Imperial and Steppe eagle pair progeny can be fertile;
- hybrid bird turned out to be a female nesting on the ground;



The *Aquila heliaca* mitochondrial genome introgression can be a threat for the Steppe eagle but looks like a rare event

Effective population number estimation

	<i>Aquila nipalensis</i>	<i>Aquila heliaca</i>
Linkage disequilibrium method (lowest allele frequencies used 0.02)		
Estimated Ne	850.9	139.5
95% CIs for Ne (Parametric)	426.9 – 7825.1	89.1 – 278.4
Molecular coancestry method		
Estimated Ne	<u>7,1</u>	40.7
95% CIs for Ne (Jackknife on Loci)	<u>1.7 – 16.3</u>	1.0 – 150.1

Summary

- * Mitochondrial DNA analysis and molecular coancestry N_e estimation show traces of severe population and genetic diversity bottlenecks in the Steppe eagle evolutionary history;
- * Despite the population number decrease, no critical genetic threats were found for the Steppe eagle;
- * The Steppe eagle population is genetically monotypic and not fragmented;
- * Steppe eagles have some natal philopatry, so specific conservation measures taken at key nesting territories can help saving the species from the extinction;
- * In addition to genetic diversity, effective population number and inbreeding coefficient dynamics, the hybridization with the Eastern Imperial eagle should be monitored as a possible threat of loss of the species genetic uniqueness and stability.



Thank you for your attention!