

Why conserving the Balkan lynx

A molecular-genetic point of view

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1. Introduction
2. Units
3. Origin of released animals
4. Small populations





Breitenmoser & Breitenmoser-Würsten 2008

mtDNA

Hellborg et al. 2002. Differentiation and levels of genetic variation in northern European lynx (*Lynx lynx*) populations revealed by microsatellites and mitochondrial DNA analysis. *Conservation Genetics* 3, 97-111.

Gugolz et al. 2008. Historical DNA reveals the phylogenetic position of the extinct Alpine lynx. *Journal of Zoology* 275, 201-208.

Sindičić M, T Gomerčić, A Galov, P Polanc, Đ Huber, A Slavica: Repetitive sequences in Eurasian lynx (*Lynx lynx* L.) mitochondrial DNA control region (review in process)

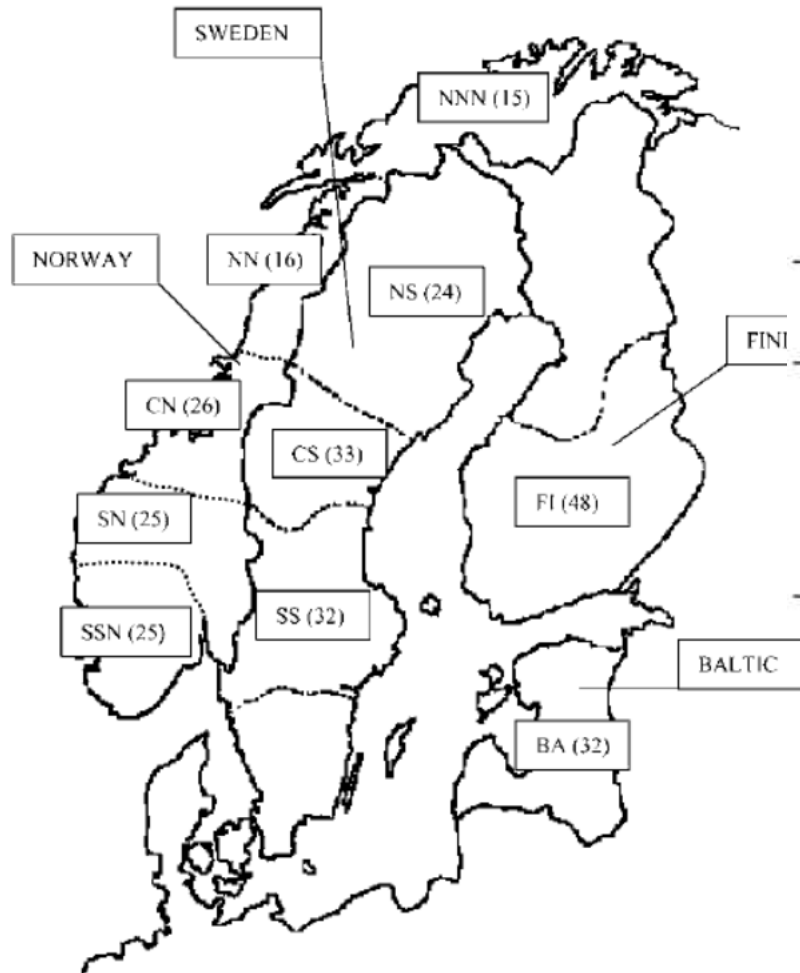
Schmidt et al. *unpubl. data*. Genetic diversity of lynx in Eastern Europe.

nuclear markers

Hellborg et al. 2002.

Rueness et al. 2003. Cryptic population structure in a large, mobile mammalian predator: the Scandinavian lynx. *Molecular Ecology* 12: 2623-2633.

Breitenmoser-Würsten et al. Lynx in Europe.

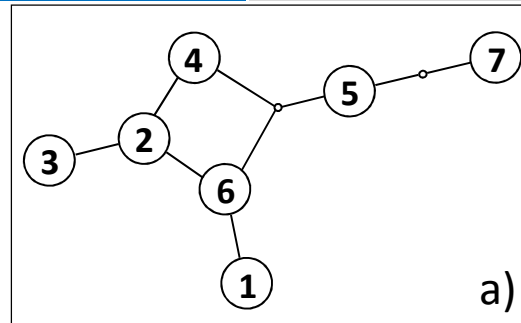


mtDNA Haplotypes

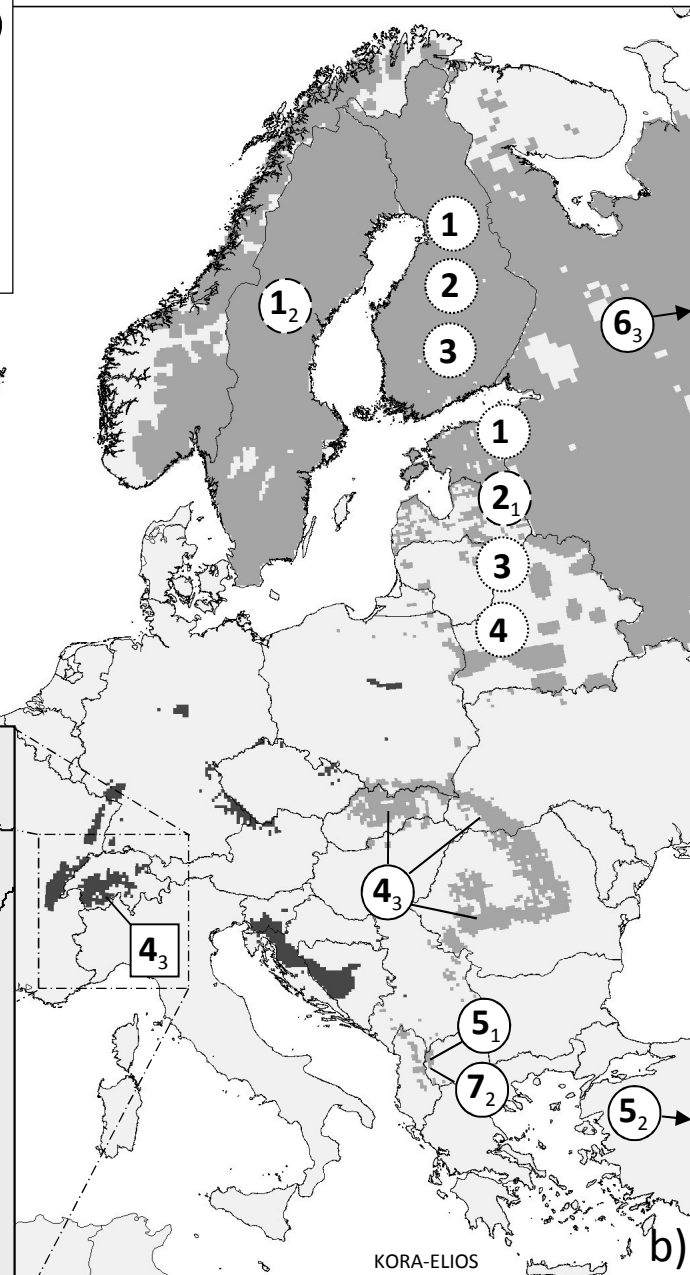
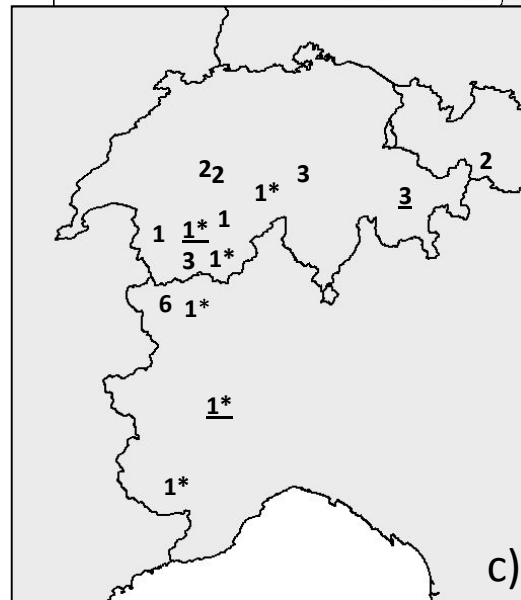
	Type 1	Type 2	Type 3	Type 4
Scandinavia	100	—	—	—
Finland	89.6	8.3	2.1	—
Baltic	65.6	15.6	12.5	6.3

low nucleotide diversity in the Fenno-Baltic population:
0.00066 and 0.00215

Minimum spanning tree



Distribution of analysed historic lynx samples, digit referring to haplotypes



Sindičić et al.

A single mtDNA haplotype was found among samples from Dinarics (N=29) and Carpathians (N=6), matching to GenBank sequence AY034816, found by Hellborg *et al.* (2002) in Estonia and Latvia. Gugolz *et al.* (2008) confirmed the presence of this haplotype in Carpathian Mountains and reintroduced Alpine population.

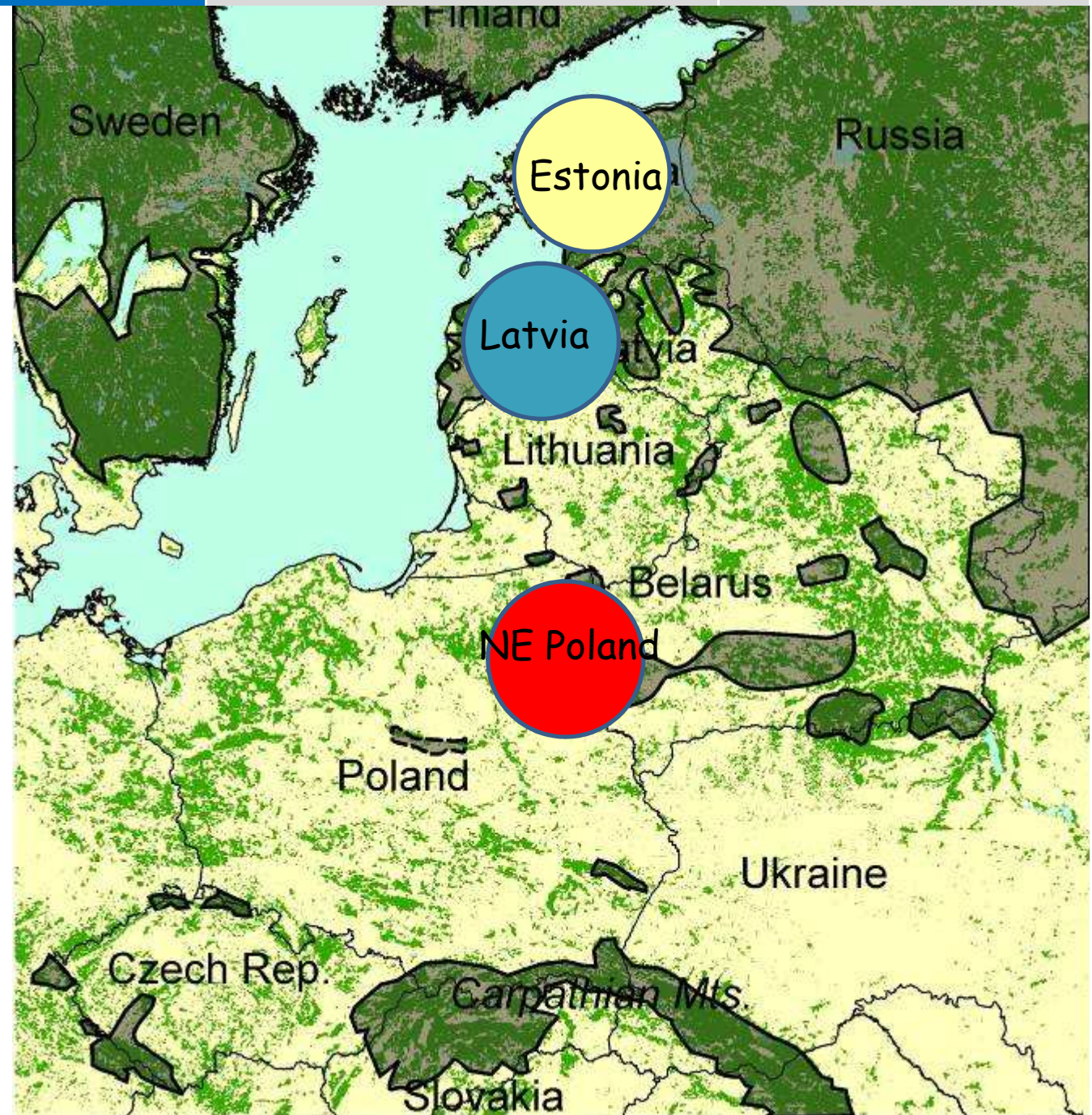
→ H4

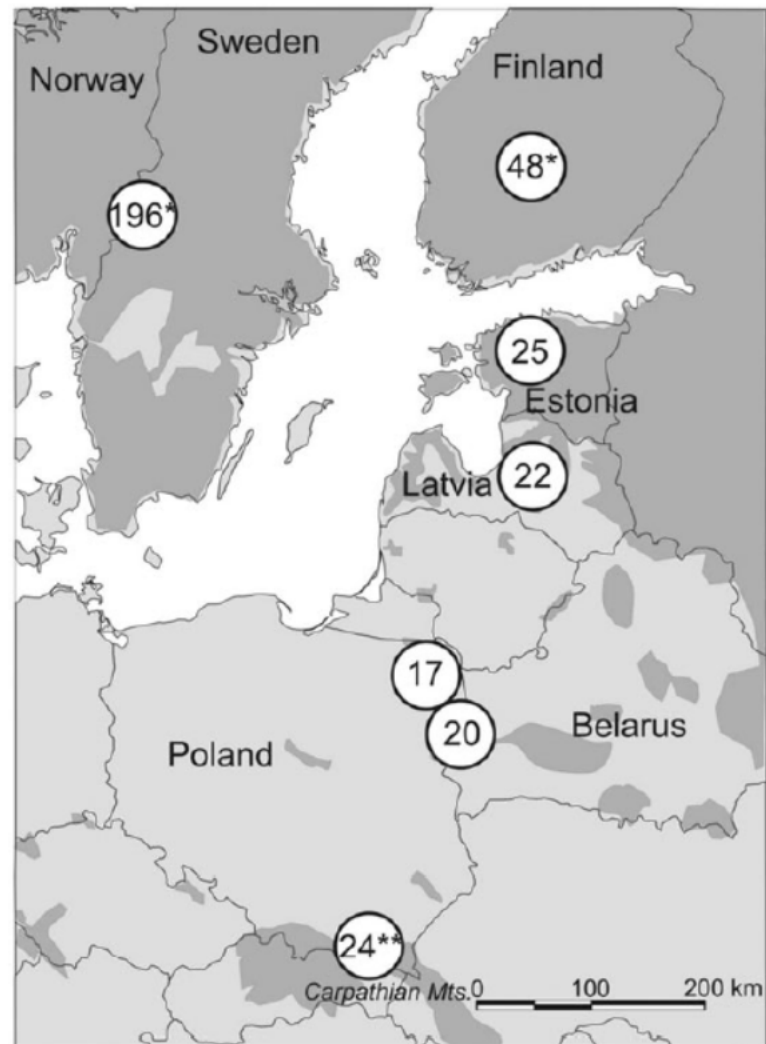
Table 3 Variable sites of two non-overlapping fragments (157 and 143 bp) of the control region in the Eurasian lynx *Lynx lynx*

Haplotype	1	1	1	1	0	0	0
	0	5	5	5	0	1	8
	4	0	6	7	9	6	9
H1	A	T	G	T	C	A	C
H2	.	C	.	C	.	.	.
H3	.	C	.	C	.	G	.
H4	.	C	.	C	.	.	T
H5	.	.	.	C	T	.	T
H6	.	.	.	C	.	.	.
H7	C	.	A	C	T	.	T

Haplotypes H1–H4 have been described previously (Hellborg *et al.*, 2002).

Distribution of
forests
and lynx
in central Europe
and
sampling sites for
microsatellite
study





Schmidt et al. 2009, 2011

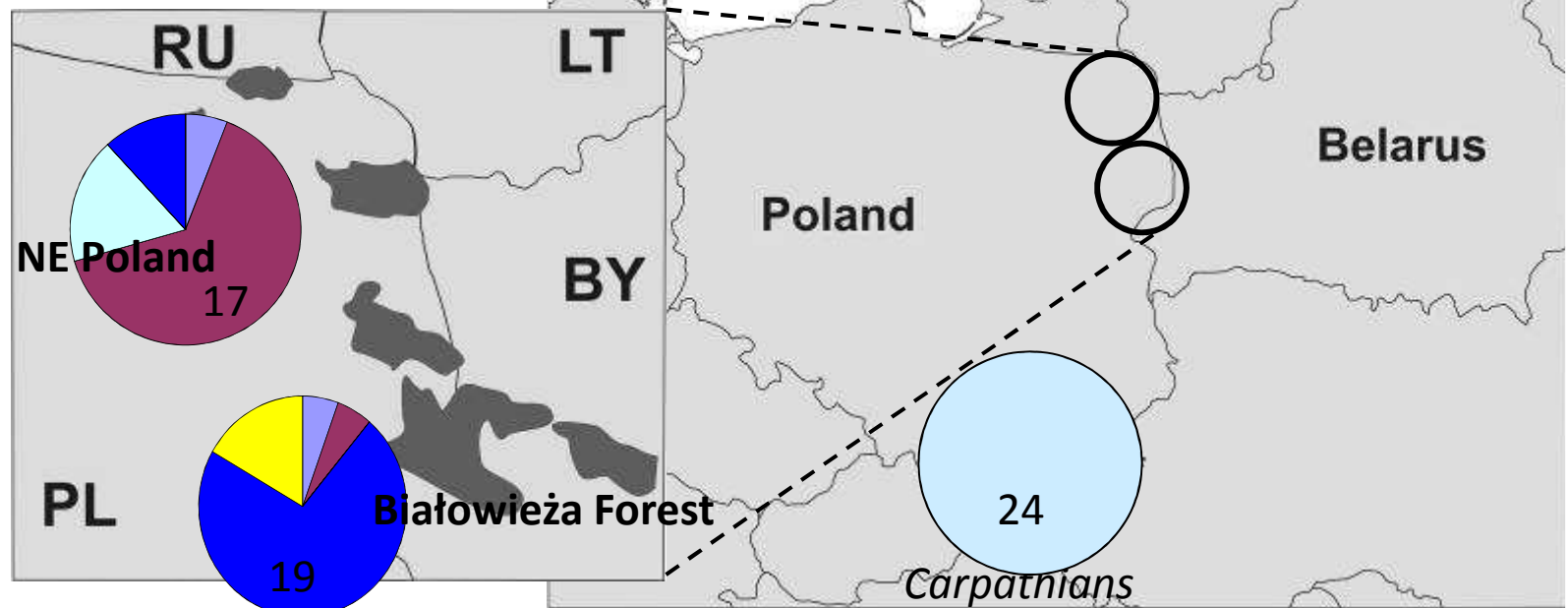
Genetic diversity of lynx in Eastern Europe (CR mtDNA)

Haplotypes:

- H 1
- H 2
- H 3
- H 4
- H 8
- H 9
- H 10
- H 11

$F_{ST} = 0.464, P < 0.001$
 $\Phi_{ST} = 0.570, P < 0.001$

Source: Ratkiewicz et al. unpubl.
 * Hellborg et al. 2002



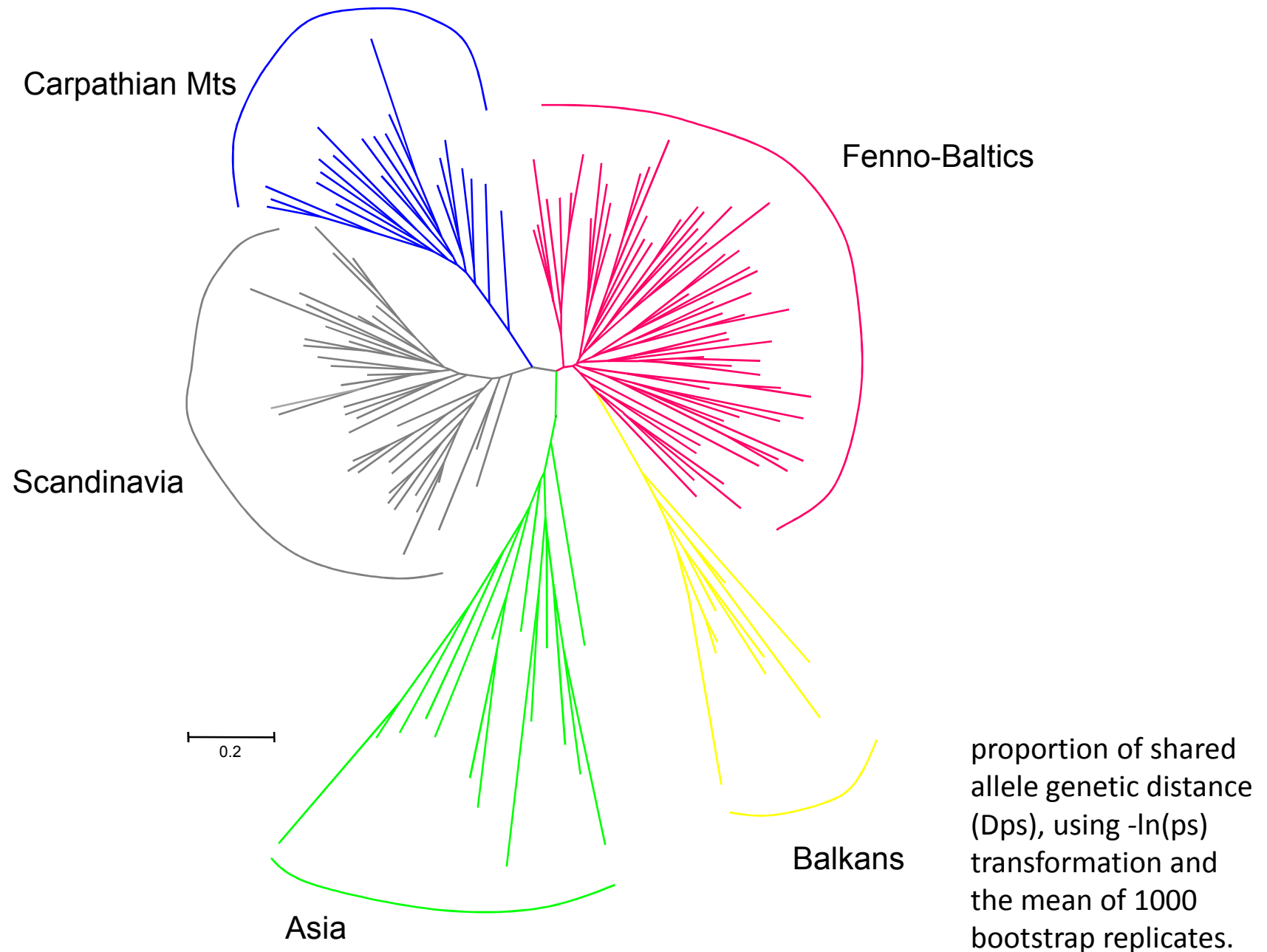
Haplotype	Scandinavia			Finland			Baltics			NE Poland		Carpathians			Balkans	
	H	S	G	H	S		H	S	G		S	Si	S	G		G
H1	X	X	X	X			X				X					
H2				X			X	X	X		X					
H3				X			X	X								
H4							X	X			X	X	X	X		
H5																X
H6																
H7																X
H8								X								
H9								X								
H10								X			X					
H11											X					

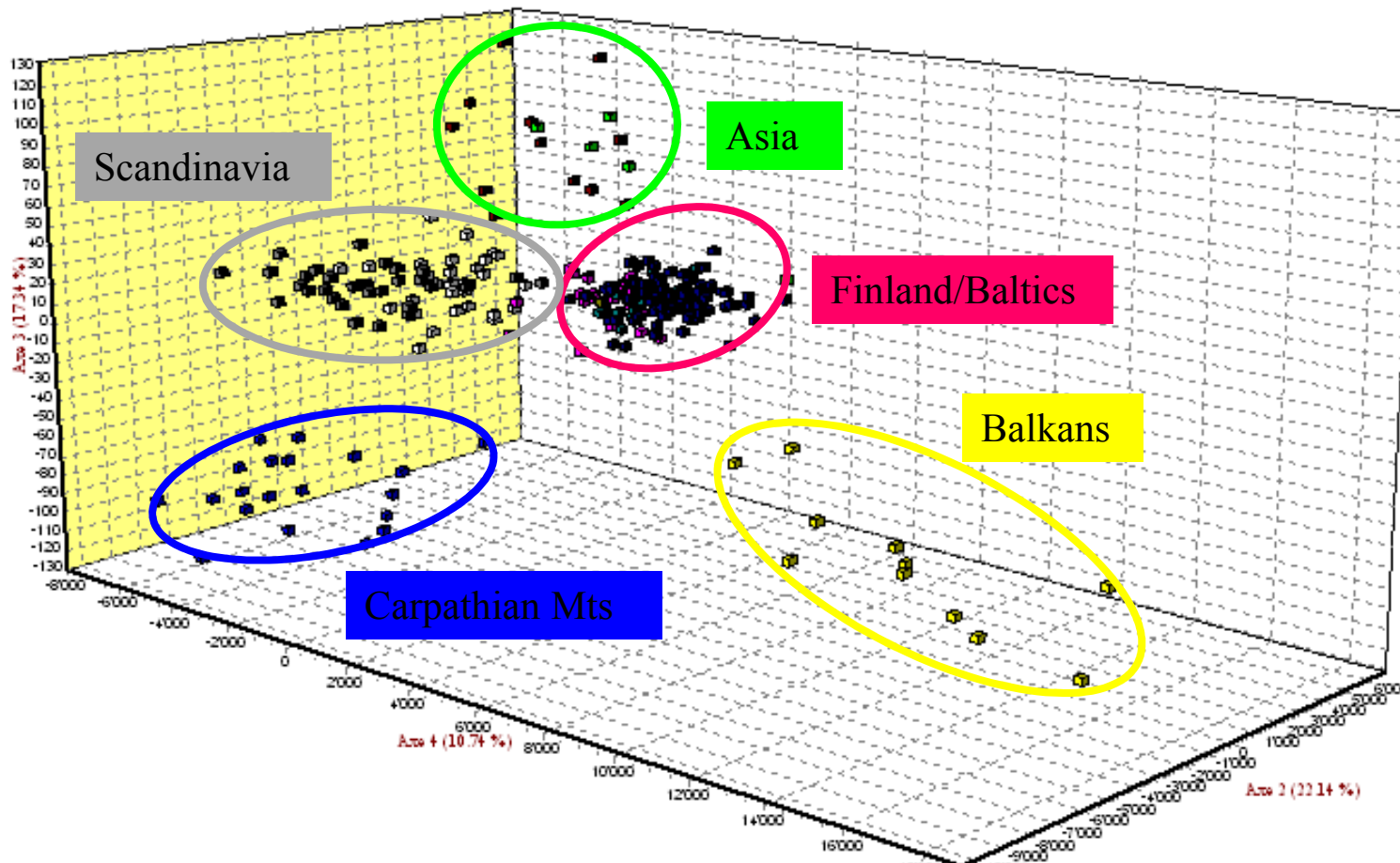
H Hellborg et al.

S Schmidt et al.

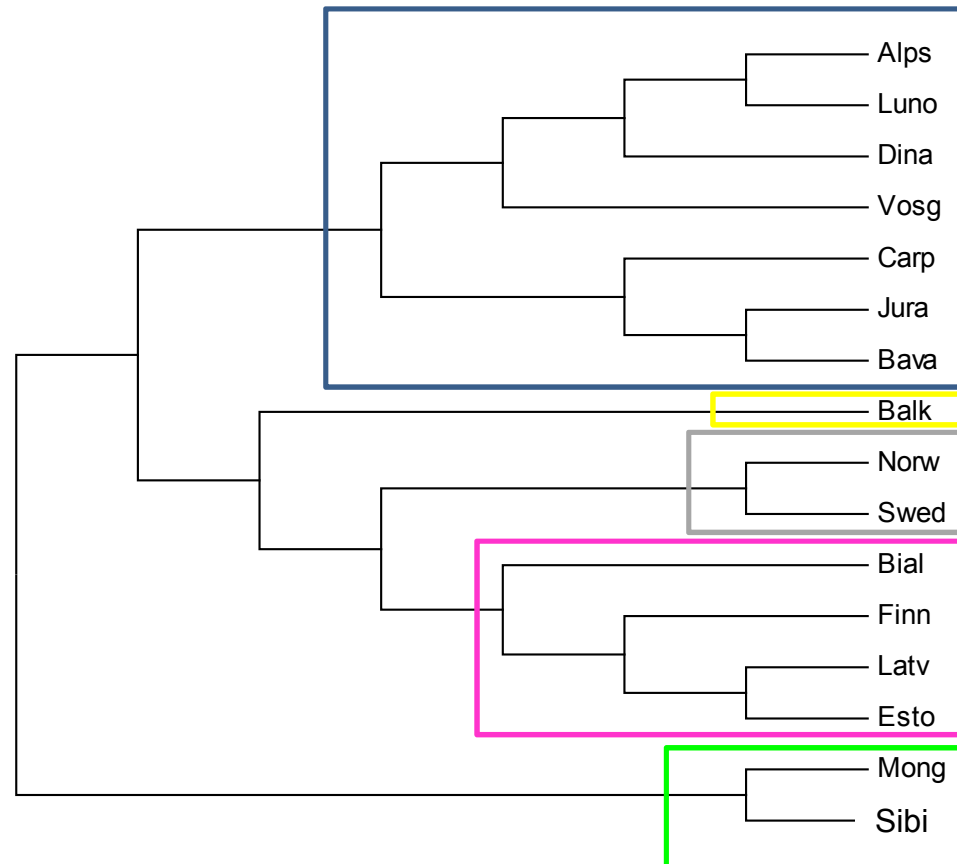
Si Sindicic et al.

G Gugolz et al.

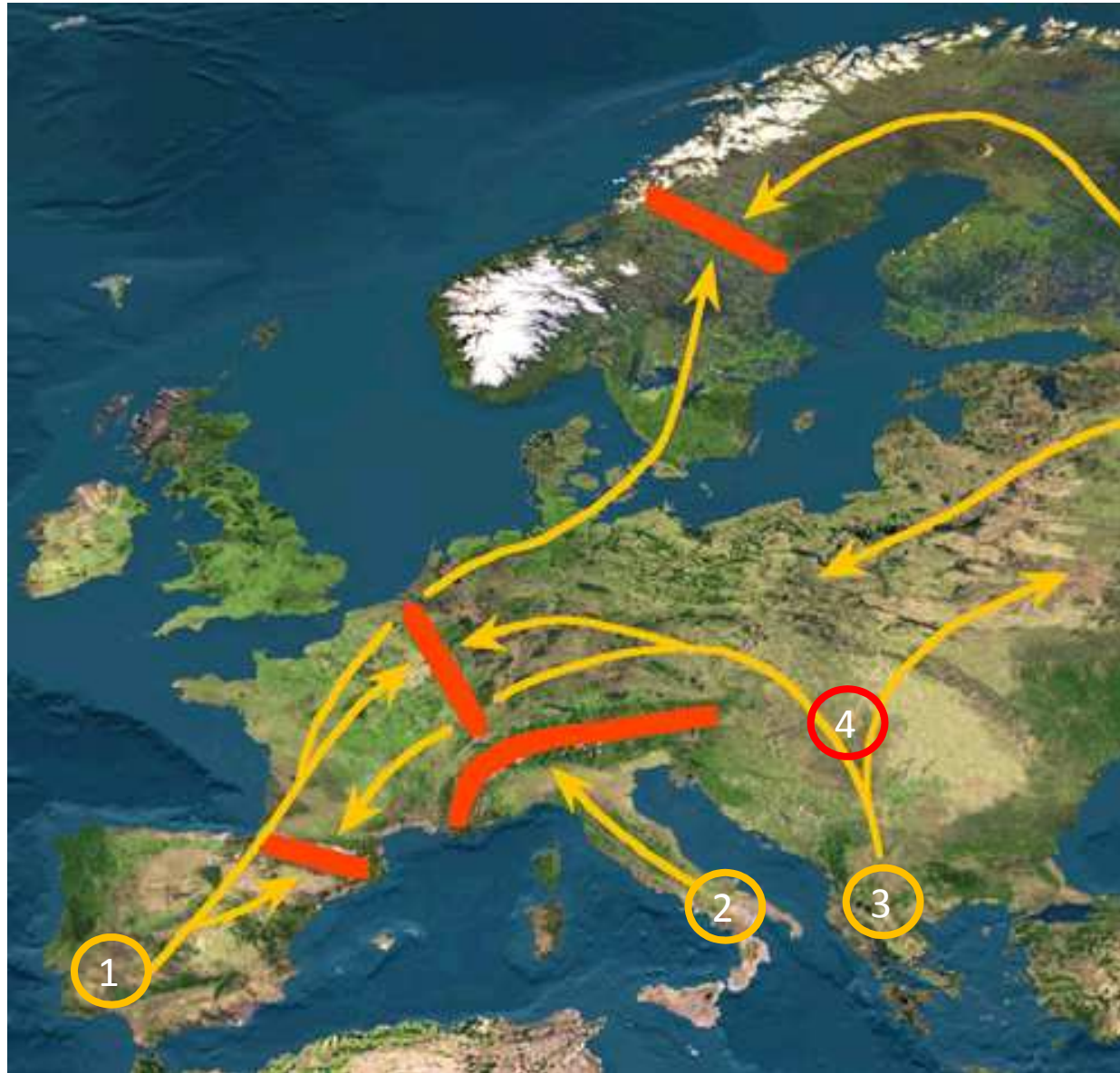




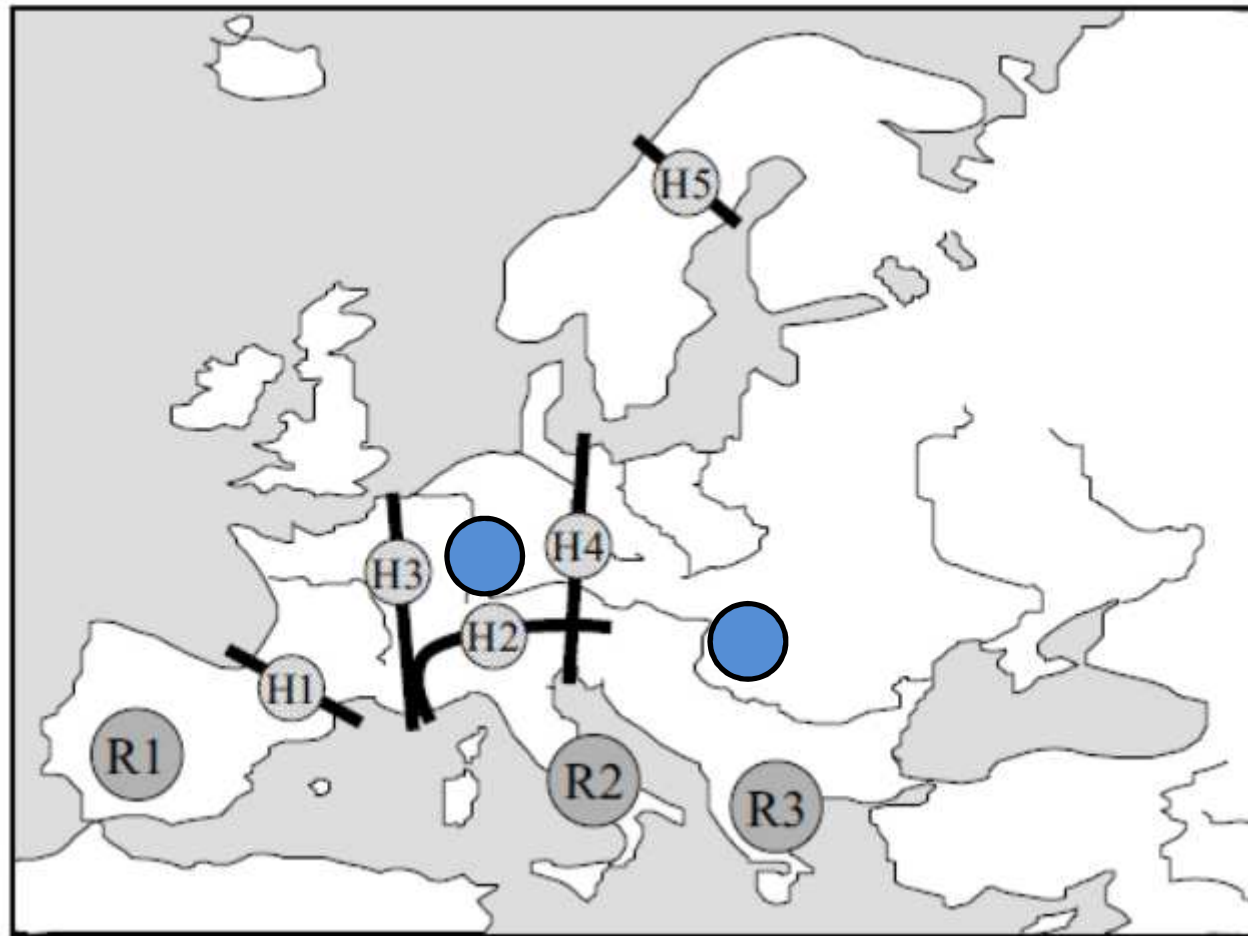
FCA



Neighbour-joining tree based on kinship coefficient $1-(k_f)$
(mean of 1000 bootstrap replicates)

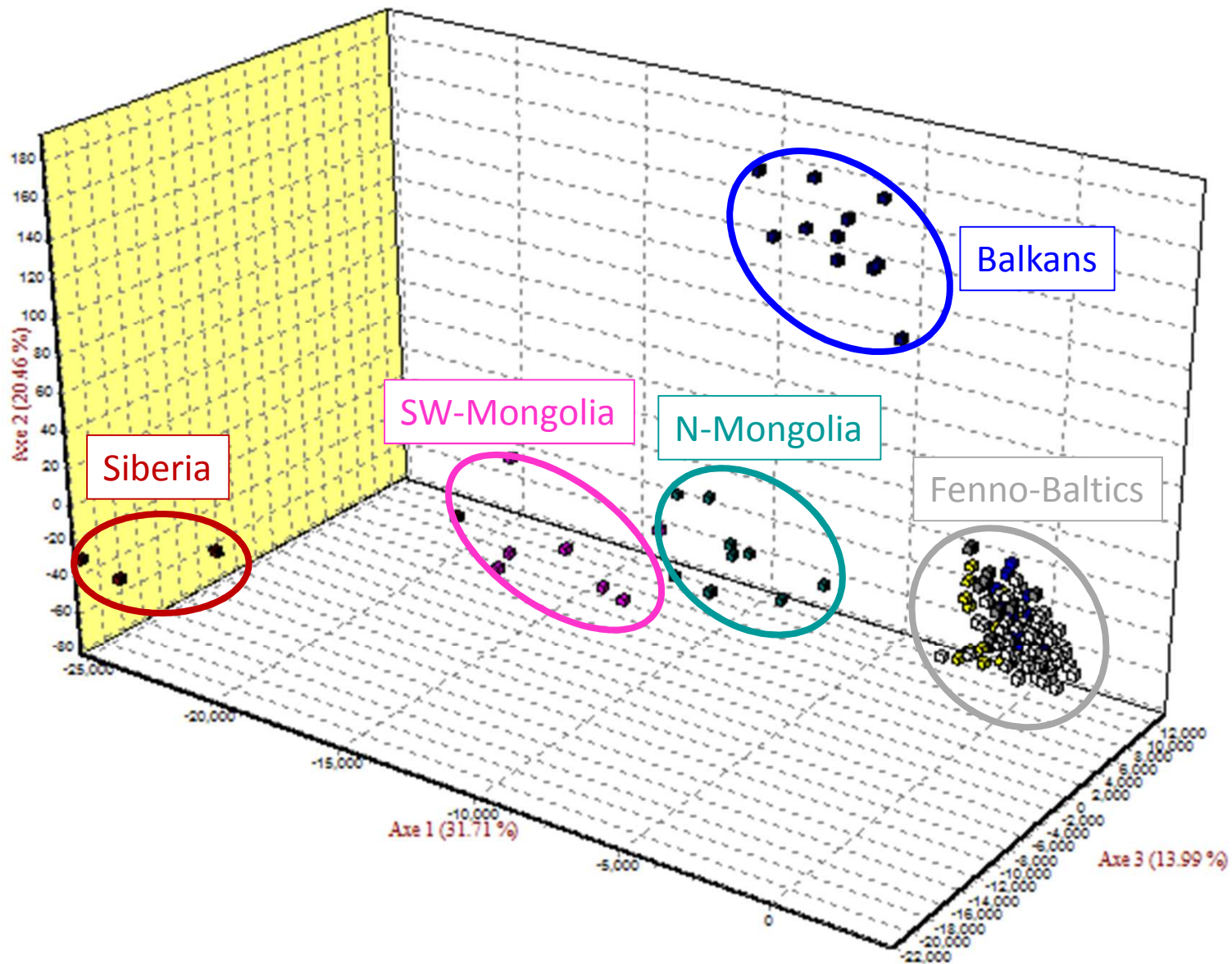


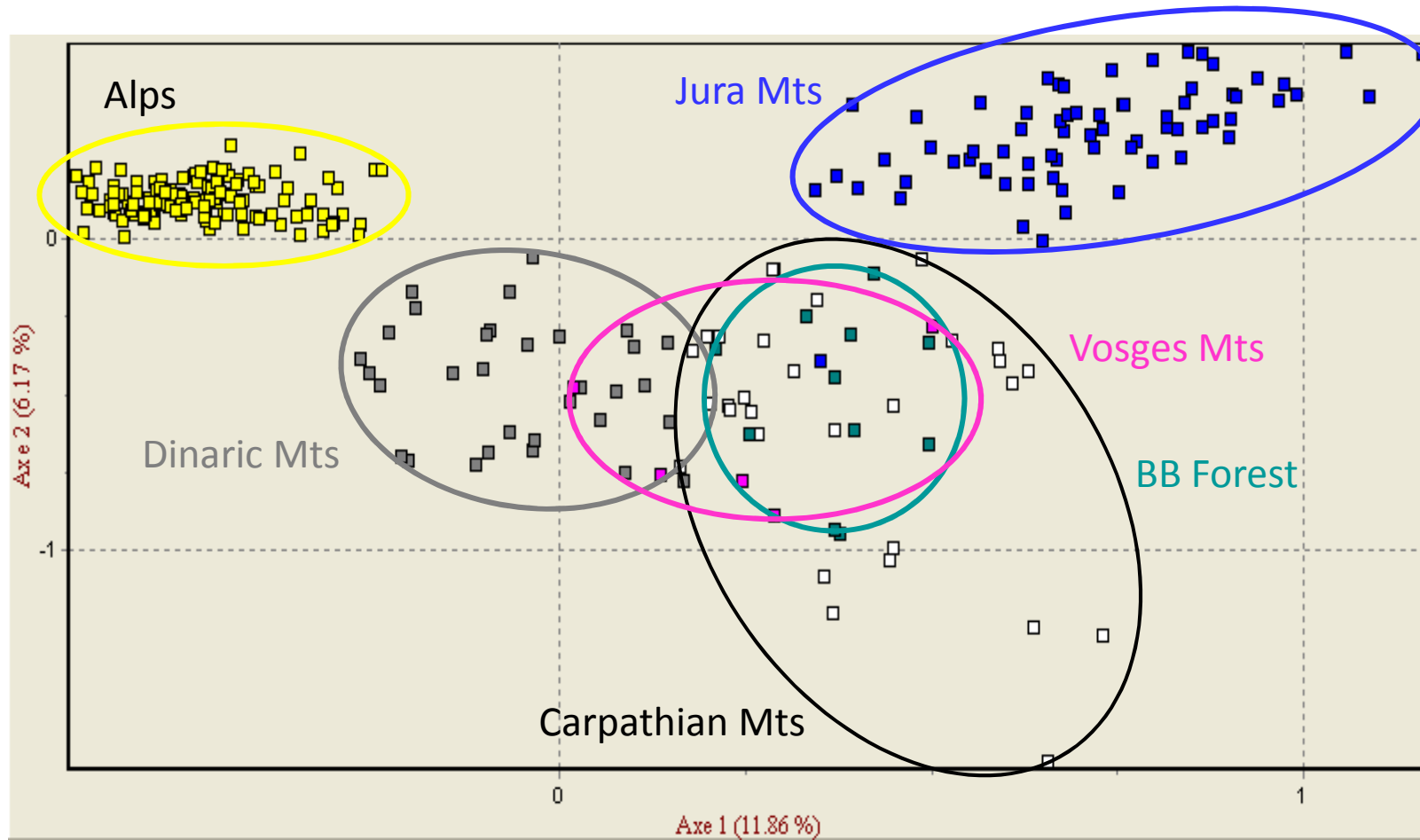
Taberlet et al. 1998



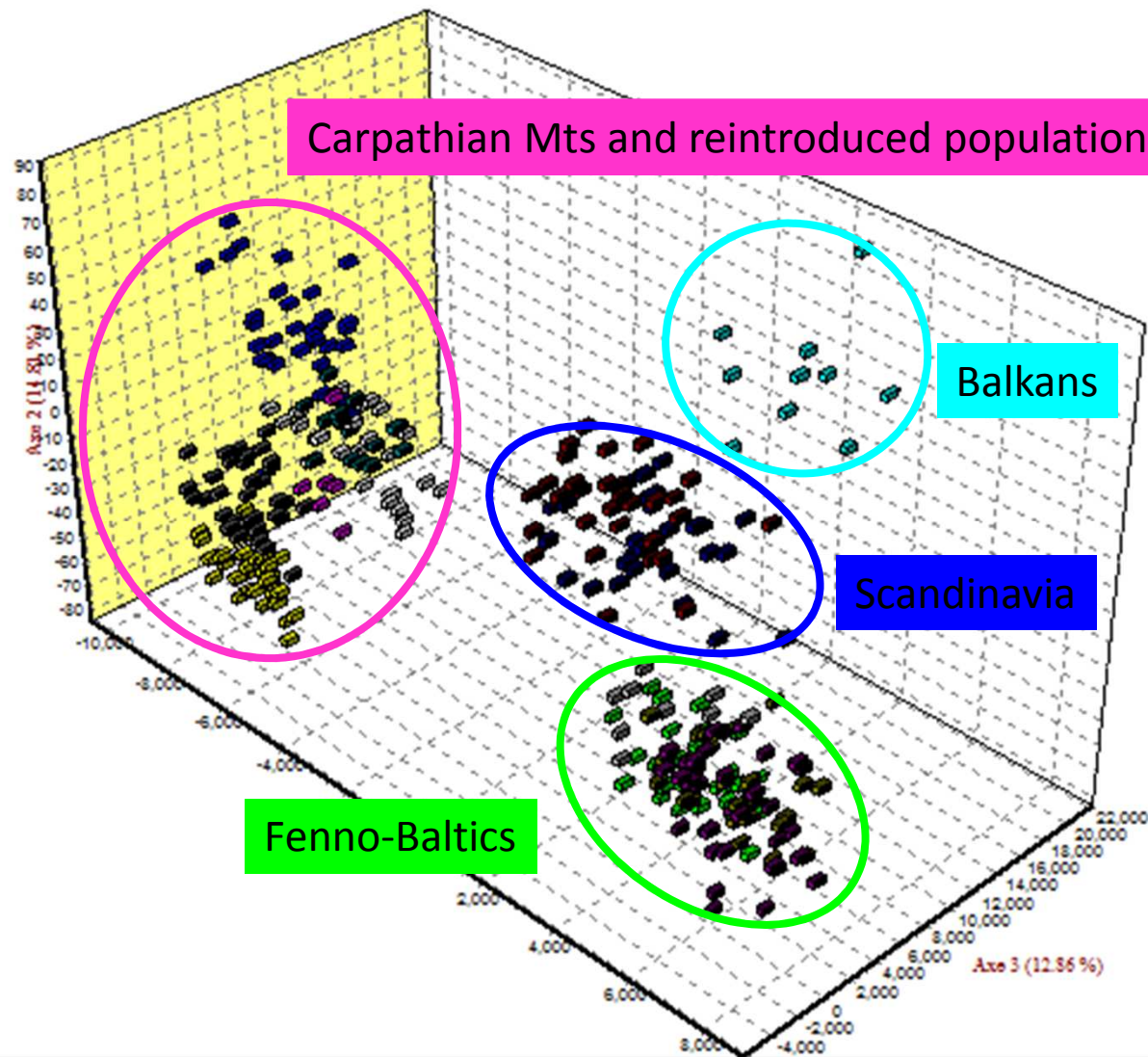
Schmitt 2007

→ increasing evidence for extra-Mediterranean ice-age refugia in Europe





FCA Allele frequencies



- only few mtDNA haplotypes for Eurasian lynx in Europe
- units (consistent results from mtDNA and nuclear DNA)
 - Carpathian Mts
 - Balkans
- ?? N Europe
- discrepancy for NE Europe
- need to analyse more samples in Europe and expand to outside of Europe to complete the phylogeography
- look at other segments of the mtDNA
- all reintroduced populations descend from animals from the Carpathian Mts (except Harz and Kampinos)

Genetics for conservation

Mean reasons for the loss of biological diversity

Destruction/loss of habitat → fragmentation
over exploitation
hybridisation (e.g. wildcat)

Consequences

- **small isolated populations**
- loss of genetic diversity (rare alleles)
- inbreeding (loss of adaptability)
- risk of local extinctions

Small isolated populations

Loss of genetic diversity

Bottleneck

strong decline of population size (natural & anthropogenic reasons)

Consequences

Loss of rare alleles

Change in allele frequencies

Occurrence

immigration through corridor

re-introduction (with few animals)

re-expansion of a relict population

(population can recover numerically, but not genetically)

Small isolated populations

Loss of genetic diversity

Founder effect

Founding of a population with a random selection of alleles
(limited selection from a gene pool of a large population)

Consequences

Loss of alleles

Allele frequencies of the new population differ from the source population

Occurrence

Re-introduction of a locally extinct species

Immigration through a corridor (geographic limitation)

Small isolated populations

Loss of genetic diversity

Genetic drift

Fast and strong change in allele frequencies

Consequences

Loss of (rare) alleles

Random change in allele frequencies

Differentiation of populations that origin from the same source

Influence stronger in small populations

Occurrence

Re-introduction of locally extinct species

Small isolated populations

Loss of genetic diversity

Inbreeding

Mating of partners who are closer related than expected

Consequences

Loss of alleles

increased in homozygosity

can lead to inbreeding depression (e.g. Florida panther)

Occurrence

In small populations

Re-introduction in locally extinct species

Signs of inbreeding depression

	Florida panther	Iberian lynx	Eurasian lynx in Switzerland	Wolf in Sweden
Increase in susceptibility to infectious diseases	FeLV No ?	FeLV Yes ?	No	No
Malformations	kinked tail cow lick cryptorchidism atrial septum defect YES		No	No?
Reproductive problems	sperm counts ↓ sperm anomalies Yes	litter size ↓ sperm quality ↓ Yes	No?	litter size ↓ Yes
Histological lesions		glomerulonephritis lymphoid depletion Yes?	cardiomyopathy Yes?	

When do we need to worry about inbreeding?

- F_{it} is approaching 0.25 (everyone is a sibling of each other). This value needs to be compared to outbred populations as it is relative.
- N_e (effective population size) is below 50. This value can be for a single population or a metapopulation.

Solution: Genetic management

Inbreeding: mating among relatives

$$(1-F_{it}) = (1-F_{st})(1-F_{is})$$

F_{it} = total inbreeding – pedigree data

F_{st} = IB due to finite population size – (molecular markers)

F_{is} = inbreeding due to non-random mating
(individual references) – molecular markers

All measures of IB are relative!

Genetic variability of lynx populations in Europe based on 22 microsatellites.

H_{exp} = expected heterozygosity, H_{obs} = observed heterozygosity.

Population	N	Typ	H_{exp}	H_{obs}	Alleles/Locus
<i>Autochthonous populations</i>					
Norway	30	a	0.606	0.575	4.32
Sweden	30	a	0.558	0.528	4.00
Finland	30	a	0.671	0.667	5.05
Latvia	29	a	0.686	0.713	5.41
Estonia	32	a	0.679	0.713	5.00
NE Poland	8	a	0.567	0.601	3.59
Balkans	10	a	0.481	0.420	2.68
Carpathian Mountains	31	a	0.633	0.592	4.59
<i>Reintroduced populations</i>					
Swiss Alps	35	r	0.448	0.460	2.45
Jura Mountains	32	r	0.517	0.517	2.91
Vosges Mountains	5	r	0.581	0.573	2.77
Dinaric Range (SLO, HR)	32	r	0.494	0.518	3.09
Bavarian-/Bohemian Forest	14	r	0.529	0.501	3.23

Signs of a bottleneck

Population	One-tailed-Wilcoxon-Test	L-Shape
Carpathian Mts	0.50	normal L-shaped distribution
Alps	< 0.001	shifted mode
Jura Mts	< 0.001	shifted mode
Dinaric range	0.09	normal L-shaped distribution
Vosges Mts	-	too few samples
Bohemian-Bavarian Forest	<0.001	shifted mode
Norway	0.251	normal L-shaped distribution
Sweden	0.500	normal L-shaped distribution
Finland	0.194	normal L-shaped distribution
Estonia	0.131	normal L-shaped distribution
Latvia	0.316	normal L-shaped distribution
Bialowiezia		too few samples
Balkans	<0.001	shifted mode

Material

- DNA of high quality and quantity



Material

- DNA of low quality and quantity





—————> Whole blood in tube with EDTA



—————> Tube with 95% alcohol or deep freeze



—————> Tube with 95% alcohol
or dry in container
or deep freeze it



—————> Paper envelop, not plastic!