"SeedNet - the Way Ahead"

Genetic Diversity of Native vs. Cultivated/Naturalized Populations of Dalmatian Sage

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Ljubljana, Slovenia, Nov. 5-6,2014

Dalmatian sage

Distribution:

- northern coastal region of the Mediterranean (N Spain, S France, S Italy and the western part of the Balkan Peninsula)

Cultivation:

- troughout the Mediterranean region, in Australia, Germany, USA etc.

Naturalized populations:

- plants that have escaped from earlier cultivation (throughout the Mediterranean region)

Gathering from the wild

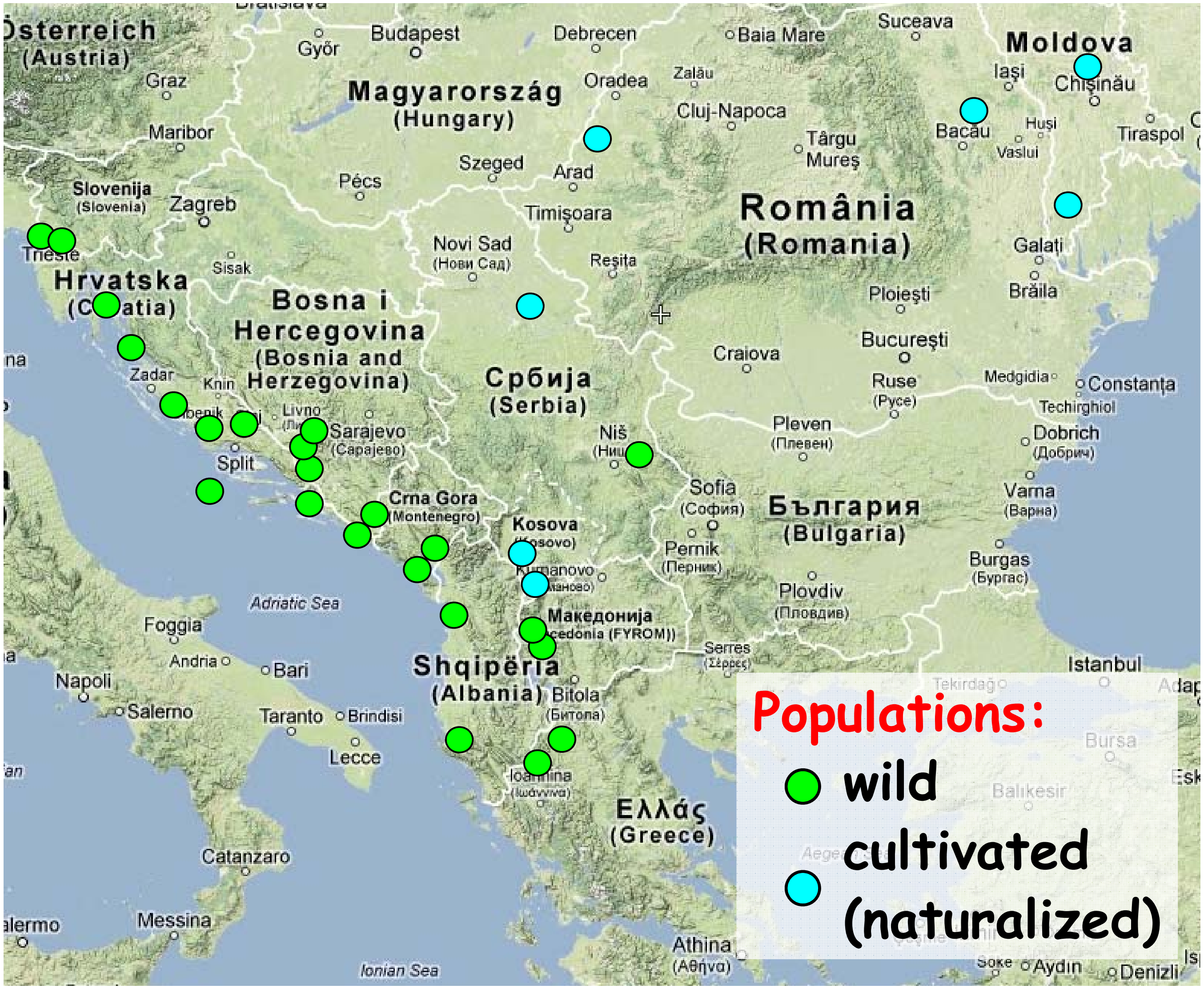
- negative impact on biodiversity

(1) Collecting

30 populations:

Slovenia (2)
Croatia (8)
BiH (4)
Montenegro (2)
Albania (2)
Macedonia (2)
Greece (2)
Kosovo (2)
Serbia (2)
Romania (2)
Moldova (2)

cca. 25 plants per population



Populations:
🟢 wild
🔵 cultivated (naturalized)

(2) Microsatellite analysis

Samples:

30 populations
709 plants

Molecular markers:

8 microsatellite loci
181 alleles (average: 20.63 alleles per locus)

Data analyses:

Genetic distance / Cluster analysis
Allelic richness
Hardy-Weinberg equilibrium: H_O, H_E, F_{IS}
Bottleneck
Structure

(A) Tree

Distance matrix:

- Cavalli-Sforza and Edwards' chord distance

Clustering method:

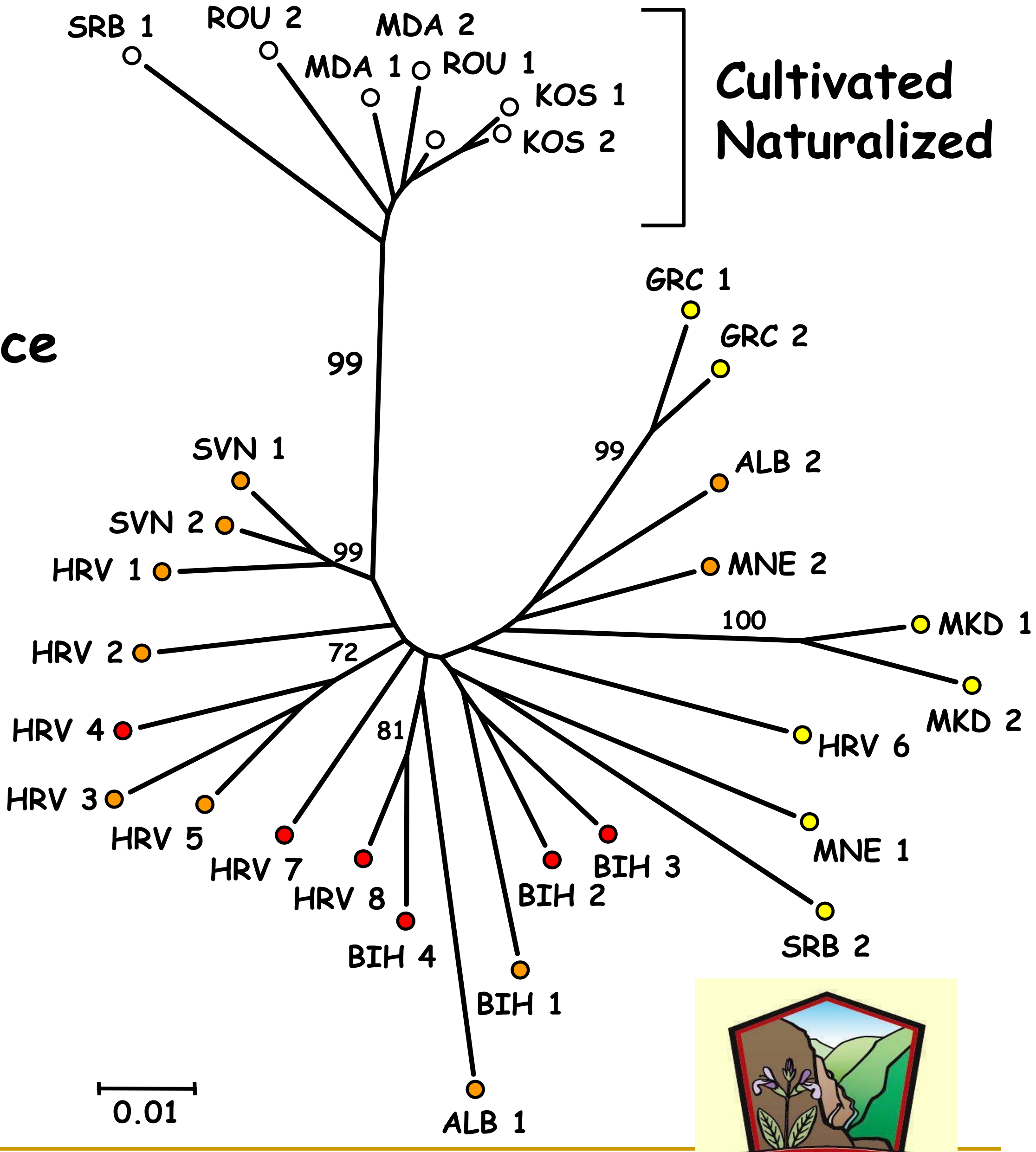
- Neighbour-Joining

Reliability:

- Boostraping

Allelic richness (N_{ar}):

○ < 5
🟡 5 - 7
🟠 7 - 9
🔴 > 9



(B) Allelic Richness

Wild

7.96

(5.26-10.41)

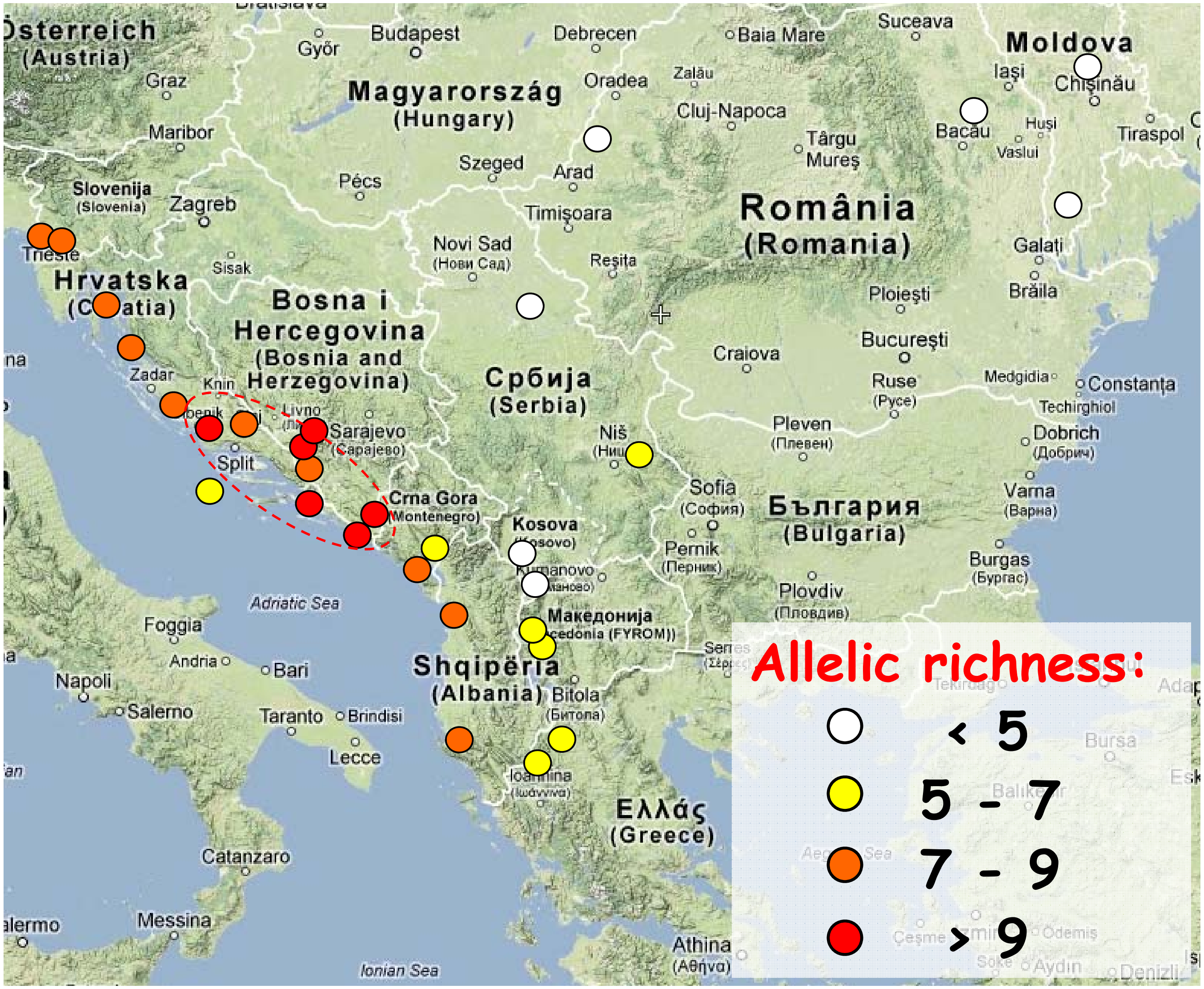
Cultivated

3.67

(2.71-4.19)

> 9

Southern
Dalmatia
(HRV)
Herzegovina
(BiH)



(C) Hardy-Weinberg Equilibrium

Parameter	Wild	Cultivated	P
	Average (Range)	Average (Range)	
Observed heterozygosity (H_O)	0.733 (0.54-0.85)	0.590 (0.31-0.79)	< 0.001
Expected heterozygosity (H_E)	0.751 (0.61-0.85)	0.545 (0.38-0.61)	< 0.001
Inbreeding coefficient (F_{IS})	0.025 (-0.08-0.12)	-0.067 (-0.30-0.17)	< 0.01

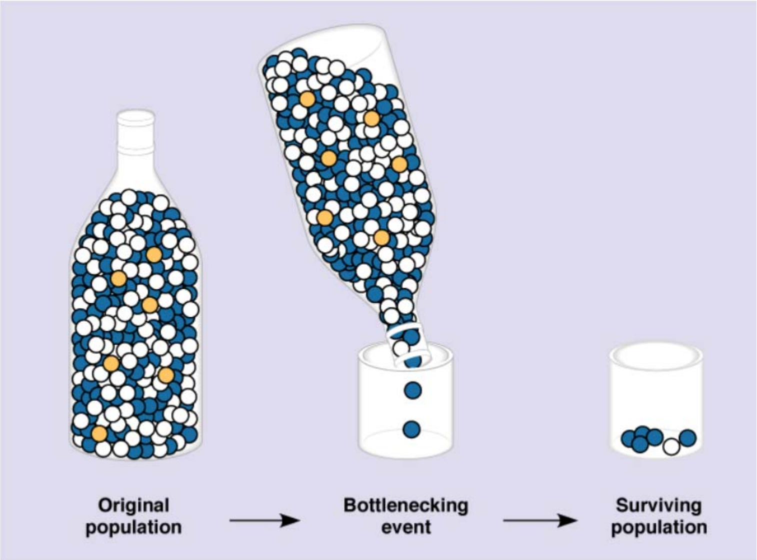
- 3 populations exhibited significant deviations from HWE:
    - SRB-2 (Sićevo): heterozygote deficiency
    - ROU-1, ROU-2: heterozygote excess

(D) AMOVA

- Analysis of Molecular Variance (AMOVA)
- AMOVA estimates and partitions total molecular marker variance and tests the significance of population genetic structure

Source of variation	% of total variation	ϕ-statistics
Among groups: wild vs. cultivated	11.94	0.119***
Among populations within groups	9.78	0.111***
Within populations	78.28	0.217***
Including only wild populations:		
Among populations	10.82	0.108***
Within populations	89.18	

(E) Bottleneck



- reduced number of alleles
- rare alleles are preferentially lost, but these do not affect expected heterozyogosity / gene diversity (H_E) much
- bottleneck event yields gene diversity excess when taking number of alleles into account

- gene diversity observed (H_E) was compared to the gene diversity expected at mutation-drift equilibrium (H_{EQ}) and calculated from the observed number of alleles:

If	Gene diversity	Sign of
$H_E > H_{EQ}$	excess	population bottleneck
$H_E < H_{EQ}$	deficiency	population expansion

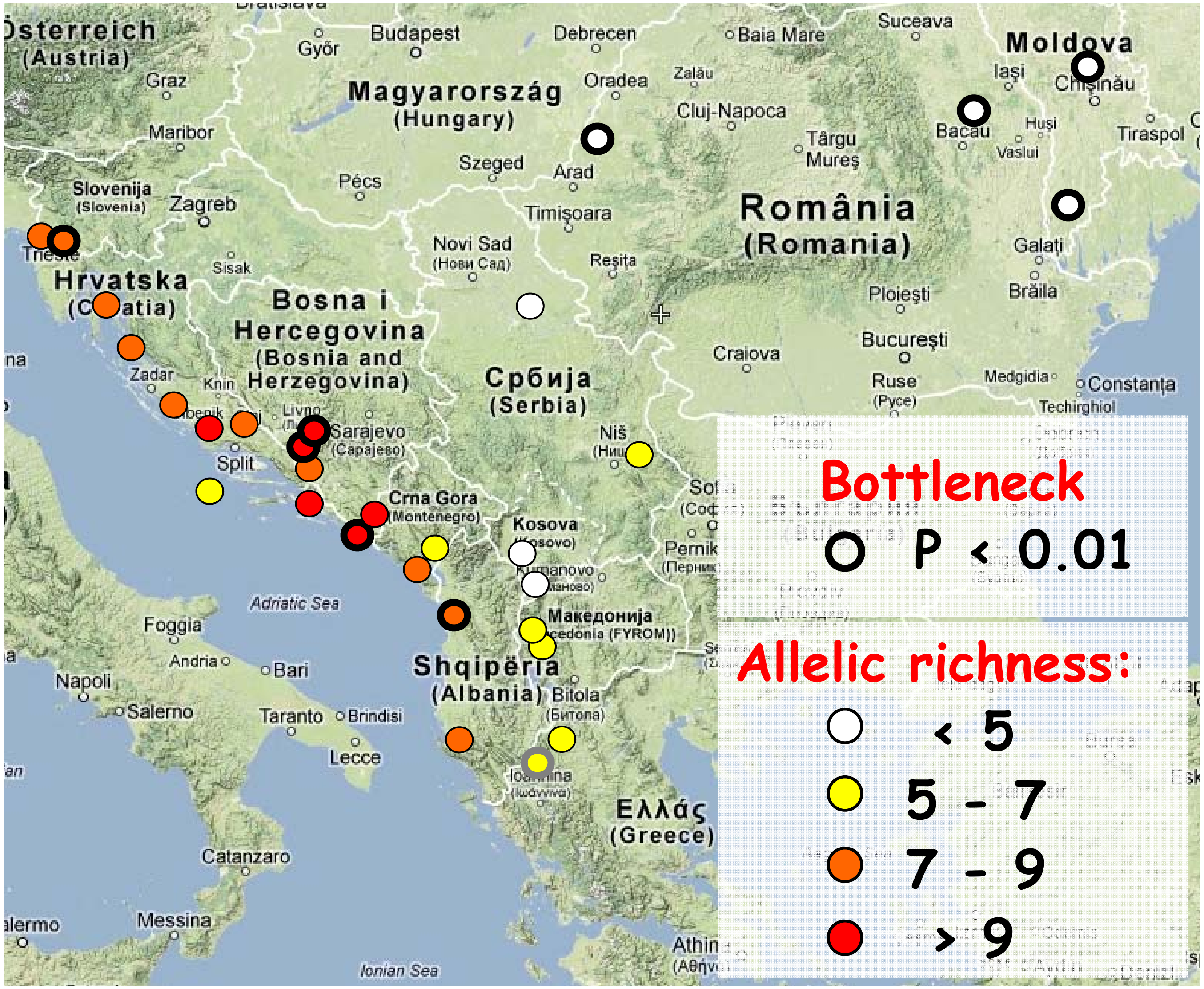
Evidence of Recent Bottleneck Events

Cultivated

4 populations
- consequence of selection (founder effect)

Wild

5 populations
- due to excessive collecting in the wild for commercial purposes



(F) Structure

- Bayesian model-based clustering method for inferring population structure

Assumption:

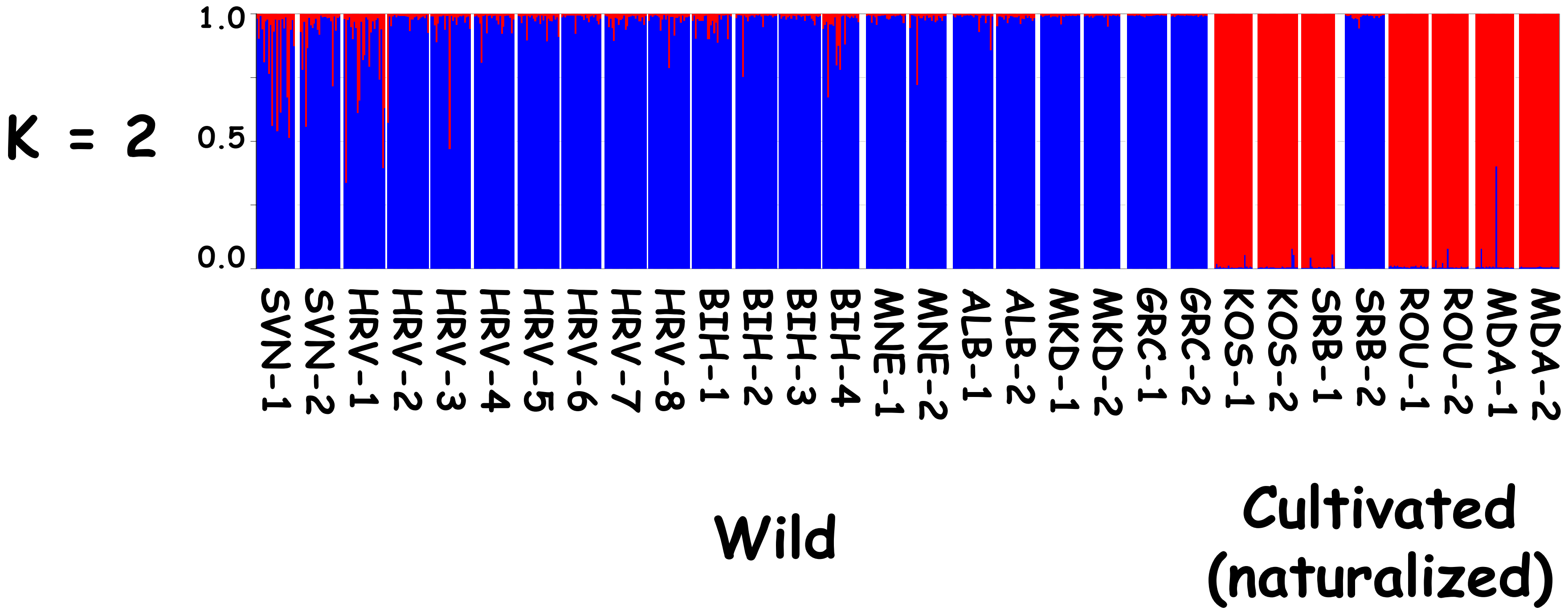
- there are K populations (ancestral populations; *gene pools*), each of which is characterized by a set of allele frequencies at each locus

Goal:

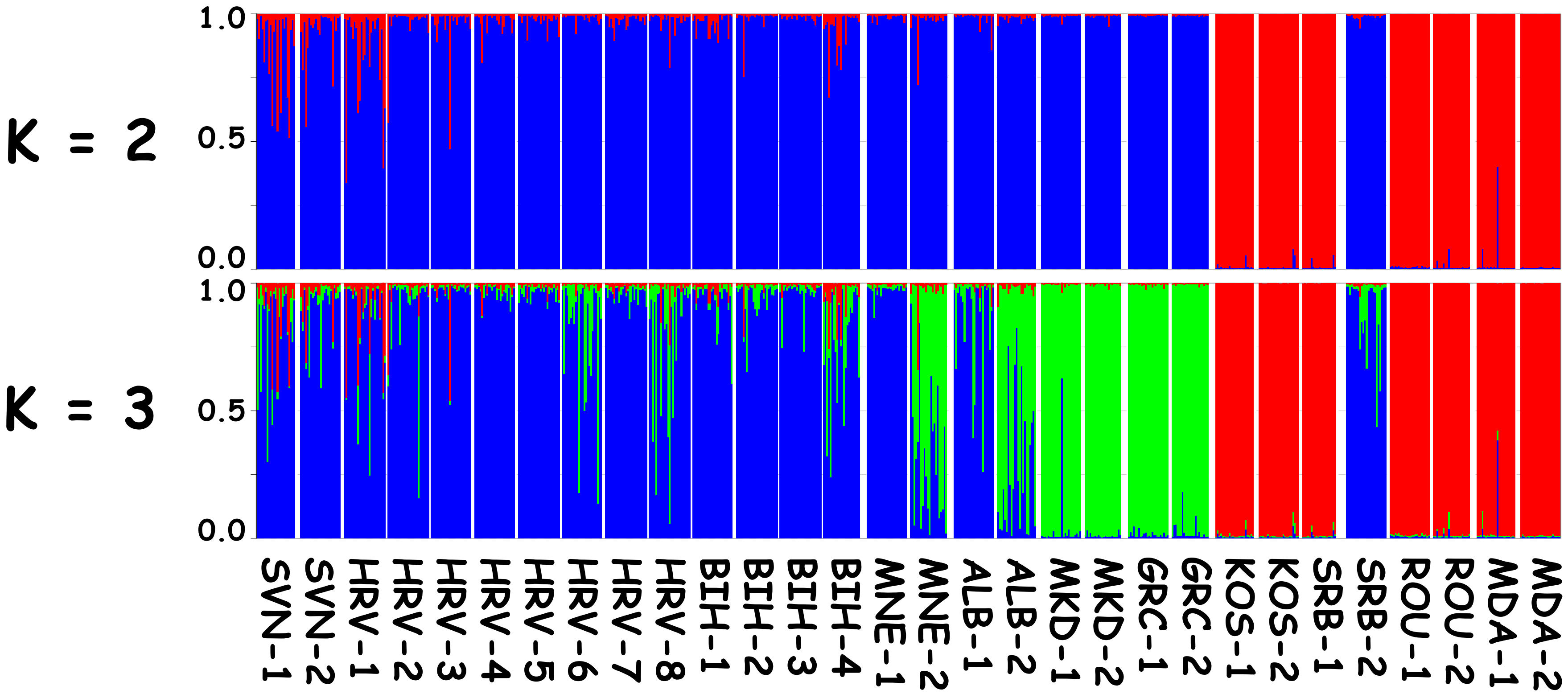
- assign individuals to *gene pools* in such a way that within each *gene pool* the departures from:

  (1) Hardy-Weinberg equilibrium (HWE) and
  (2) linkage equilibrium (LE)
  are minimized

Proportion of Membership (Q)



Proportion of Membership (Q)



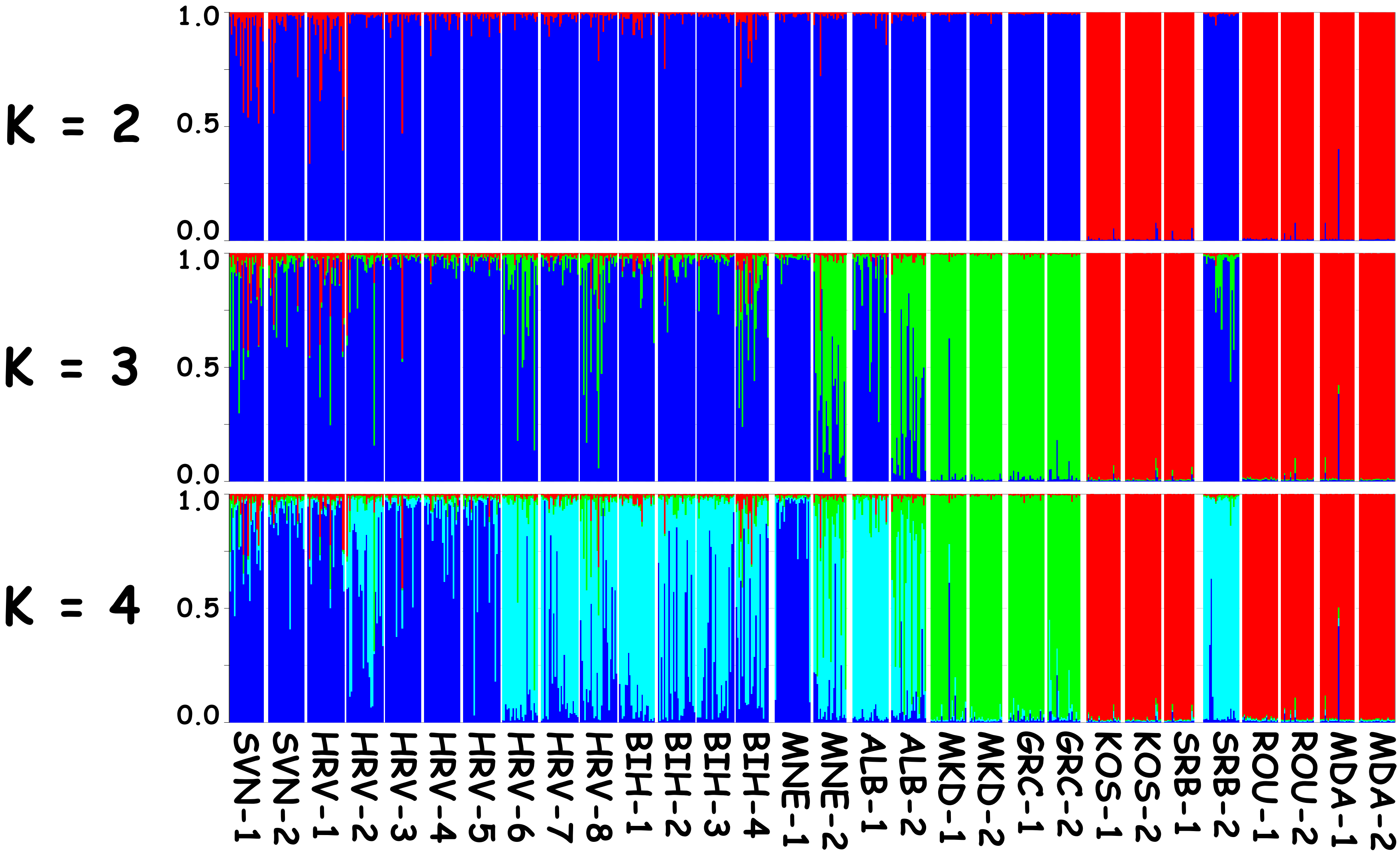
Wild

Cultivated
(naturalized)

Adriatic

MKD/GRC

Proportion of Membership (Q)



Nothern
Adriatic

Southern
Adriatic

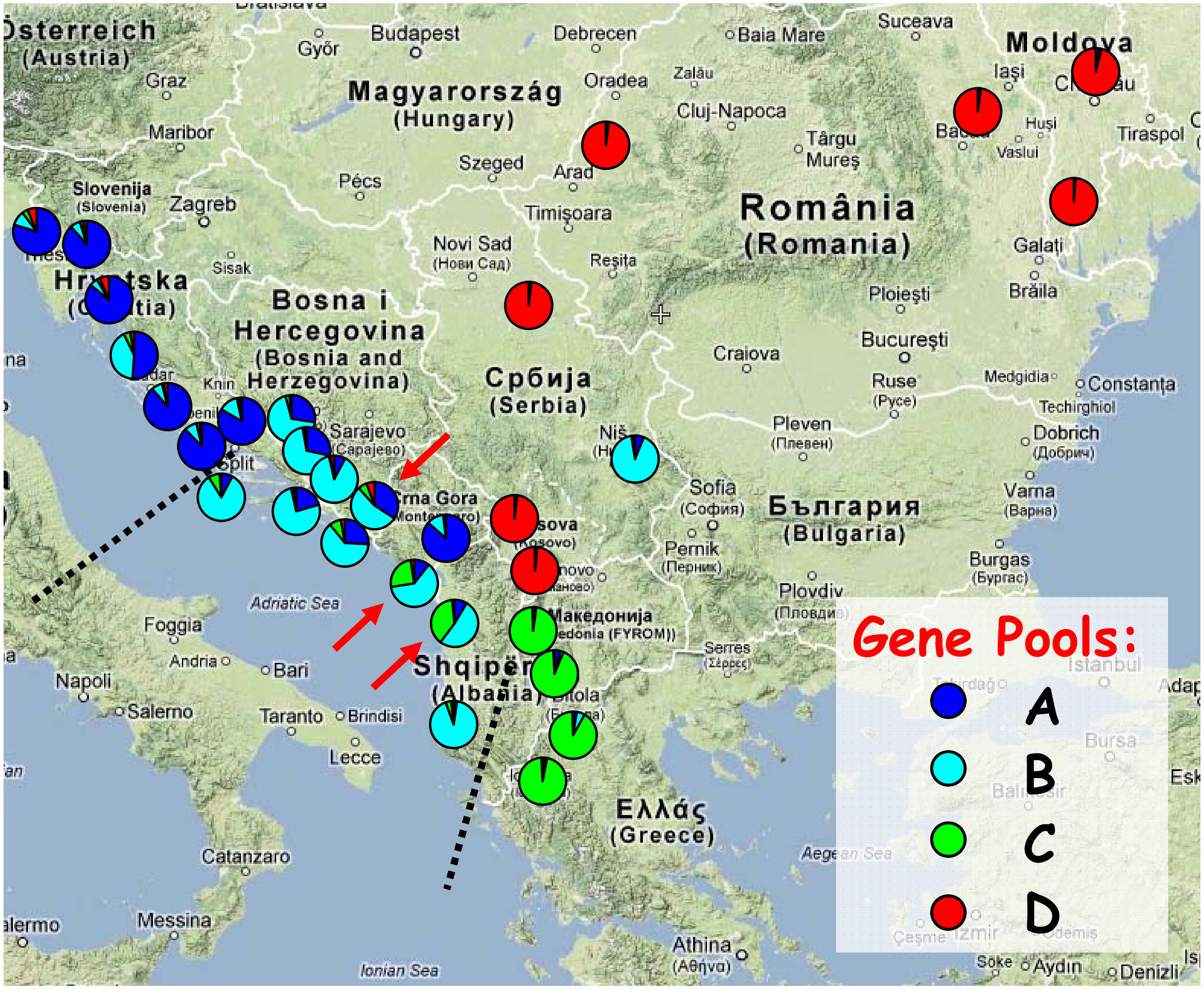
MKD/GRC Cultivated

Genetic Structure: 4 gene pools

K = 4

Geographical pattern

The most admixed populations:
BIH-4
ALB-2
MNE-2



Collaborators

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Projects

1. Genetic Structure of Dalmatian Sage
   (*Salvia officinalis* L.) Populations:
   Model for a Collaborative Research on MAP Genetic Resources
   South East European Development Network on Plant Genetic Resources (SEEDNET)

2. Biodiversity of Medicinal and Aromatic plants
   Ministry of Science, Education and Sports, Croatia

The way ahead

Current project:

Epigenetic vs. Genetic diversity in Natural Plant Populations: A case study of Croatian endemic *Salvia* species (EpiSalvia)

Financed by: Croatian Science Foundation

Aim:

- the assessment of the amount and structure of epigenetic and genetic diversity of *S. officinalis*, *S. brachyodon*, *S.* x *auriculata*
- the chosen species are evolutionary closely related but with contrasting range sizes and genetic diversity influenced by different phenomena: genetic bottleneck and interspecies hybridization

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