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# Historical and Contemporary Demography of Dalmatian sage (*Salvia officinalis* L.)

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Republika Hrvatska

Cambridge, 2018

# OUTLINE

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- (1) Dalmatian sage (*Salvia officinalis* L.)
- (2) Genetic diversity
- (3) Genetic structure
- (4) Ecological niche modelling
- (5) Demographic history

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# (1) DALMATIAN SAGE

- *Salvia officinalis* L.
- outcrossing, insect-pollinated
- perennial subshrub
- widely used since ancient times for medicinal, culinary and ornamental purposes
- natural distribution:  
coastal region of the western Balkan and central and southern Apennine Peninsulas
- cultivation:  
throughout the Mediterranean region, in Australia, Germany, USA etc.
- naturalized (feral) populations:  
plants that have escaped from earlier cultivation



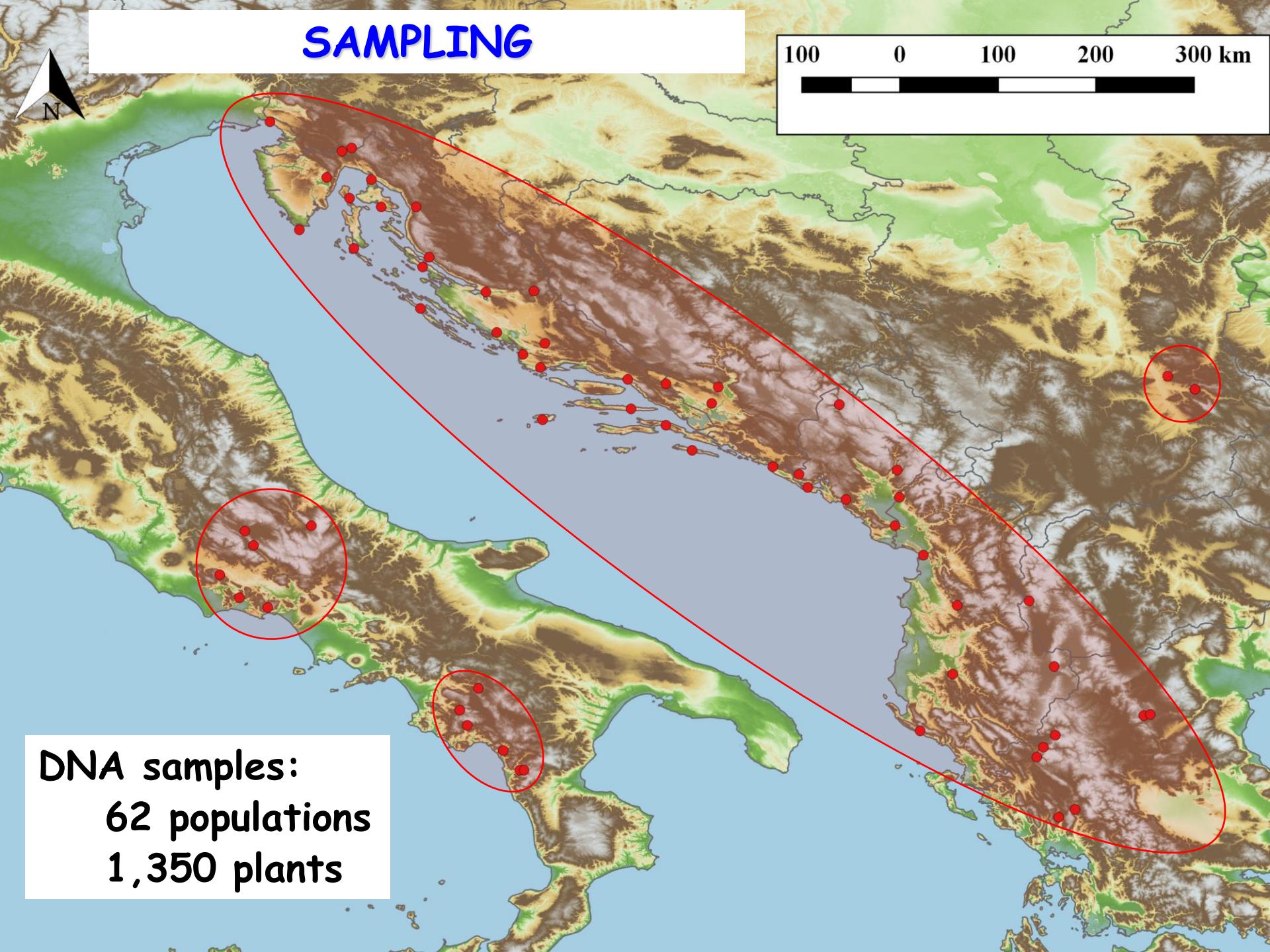
# DISTRIBUTION

100 0 100 200 300 km



# SAMPLING

100 0 100 200 300 km



DNA samples:  
62 populations  
1,350 plants

# SAMPLES / ANALYSES

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- samples:

62 populations / 1,350 plants

Italy (13), Slovenia (2), Croatia (23),  
Bosnia and Herzegovina (2), Montenegro (7), Serbia (2),  
Albania (5), Macedonia (1), Greece (7)

- molecular analysis:

8 microsatellite markers

191 alleles

# OUTLINE

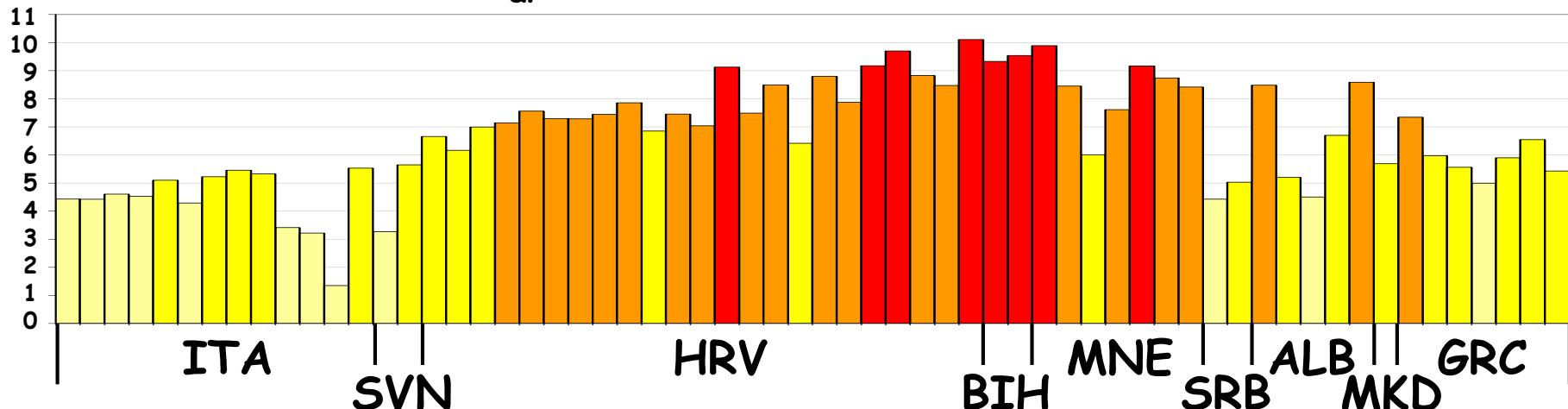
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- (1) Dalmatian sage (*Salvia officinalis* L.)
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- (3) Genetic structure
- (4) Ecological niche modelling
- (5) Demographic history

## (2) GENETIC DIVERSITY

Parameter	Mean	Range
Allelic richness ( $N_{ar}$ )	6.669	1.35-10.11
Observed heterozygosity ( $H_o$ )	0.665	0.081-0.832
Expected heterozygosity ( $H_e$ )	0.683	0.087-0.846

Allelic richness ( $N_{ar}$ )



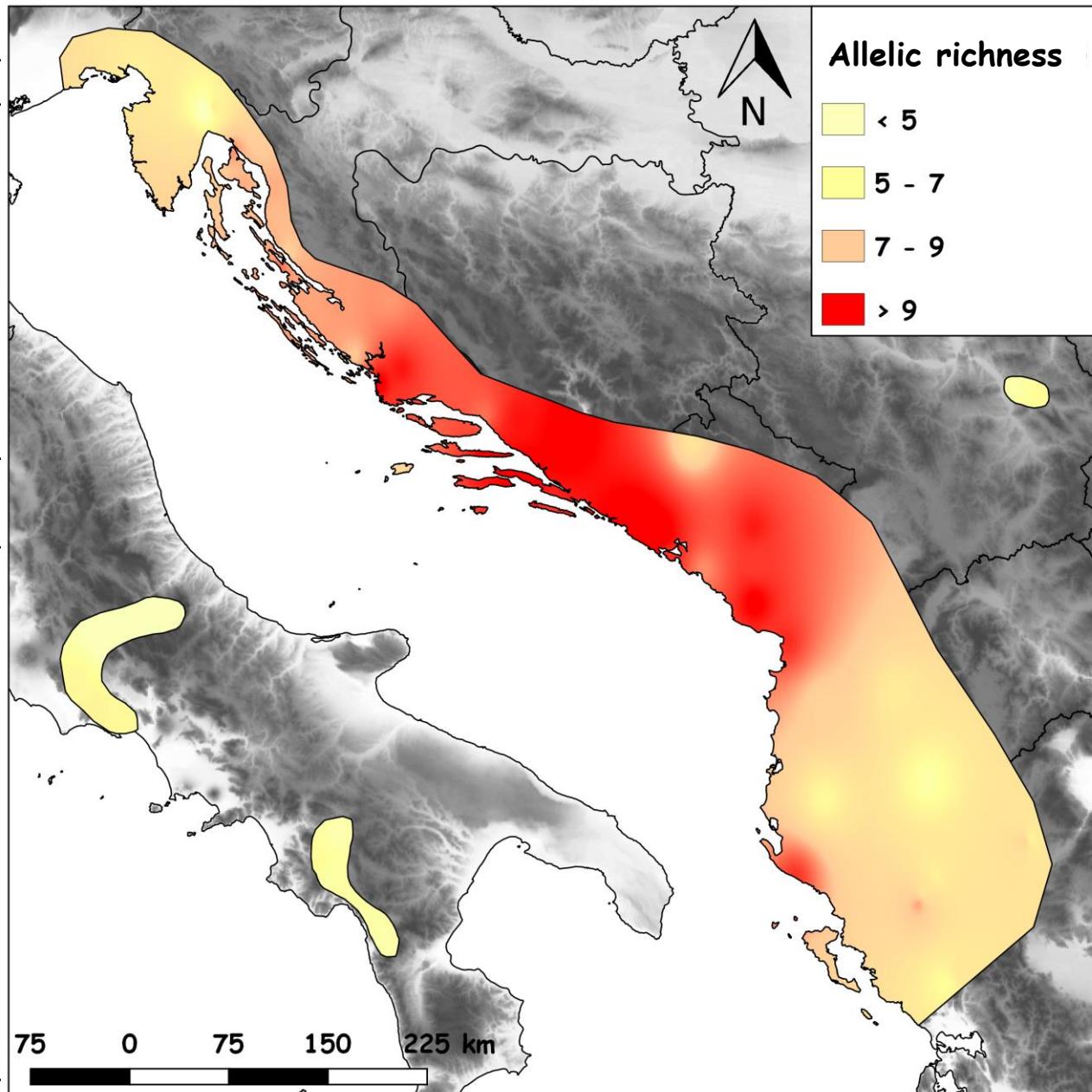
# ALLELIC RICHNESS

■  $N_{ar} > 9$

Br.	Population	Country
P38	Konavle	HRV
P41	Vrbanj	MNE
P35	Pelješac	HRV
P40	Mostar	BIH
P39	Međugorje	BIH
P34	Hvar	HRV
P45	Rumija	MNE
P28	Šparadići	HRV

■  $N_{ar} < 5$

P59	Arta	GRC
P03	Maratea	ITA
P04	Cilento	ITA
P52	Prespa	ALB
P01	Pollino 2	ITA
P48	Miljkovac	SRB
P02	Pollino 1	ITA
P06	Savoia	ITA
P10	d'Antino	ITA
P14	Novokračine	SVN
P11	M. Salviano	ITA
P12	Maiella	ITA



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### (3) GENETIC STRUCTURE

- Bayesian model-based clustering method for inferring population structure

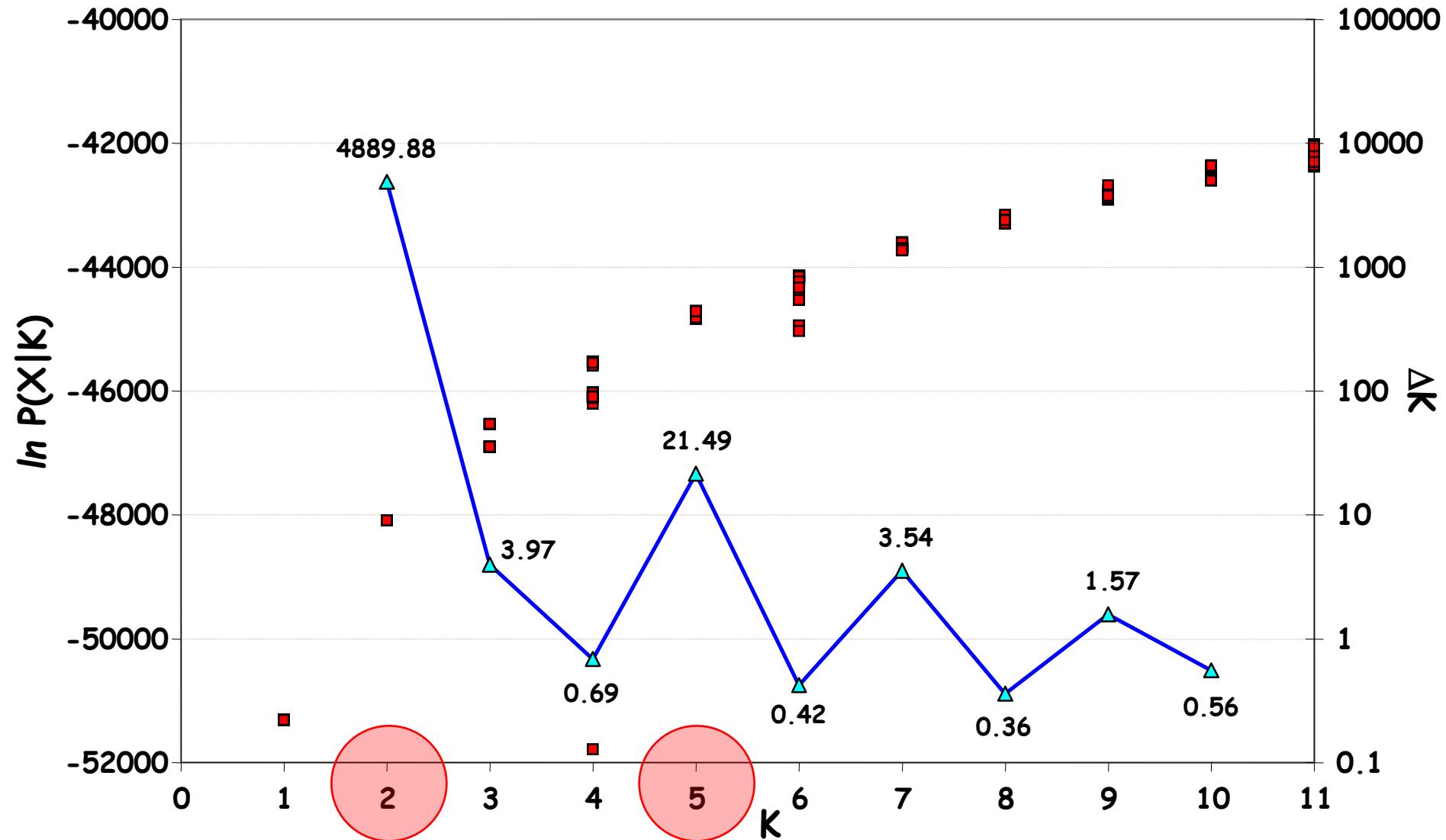
#### Assumption:

- there are K populations of origin each of which is characterized by a set of allele frequencies at each locus

#### Goal:

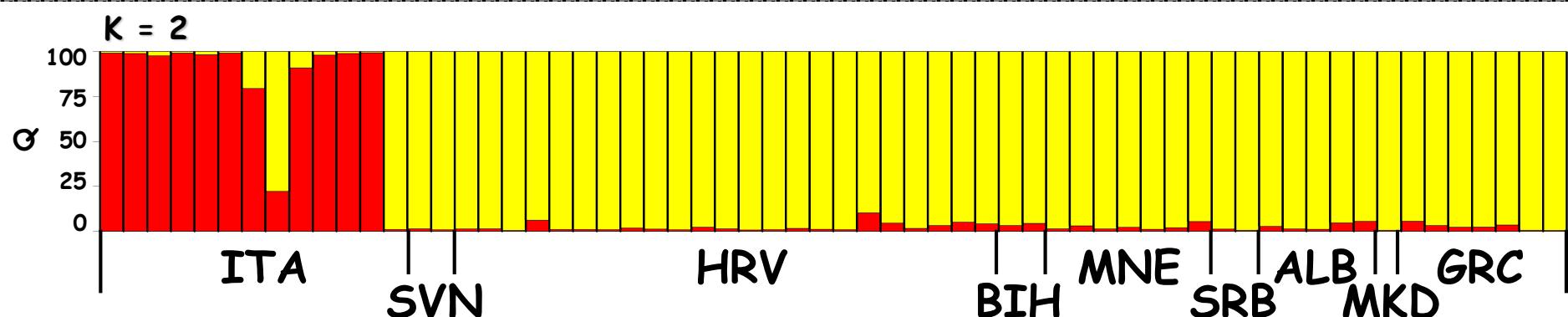
- assign individuals to populations of origin in such a way that within each population the departures from:
  - (1) Hardy-Weinberg equilibrium (HWE) and
  - (2) linkage equilibrium (LE)are minimized

# NUMBER OF CLUSTERS: $\ln P(X|K)$ and $\Delta K$



- $\ln P(X|K)$  values (20 runs per each  $K$ ; primary y axis - left)
- △  $\Delta K$  values for each  $K$  (secondary y axis - right)

$K = 2$



A

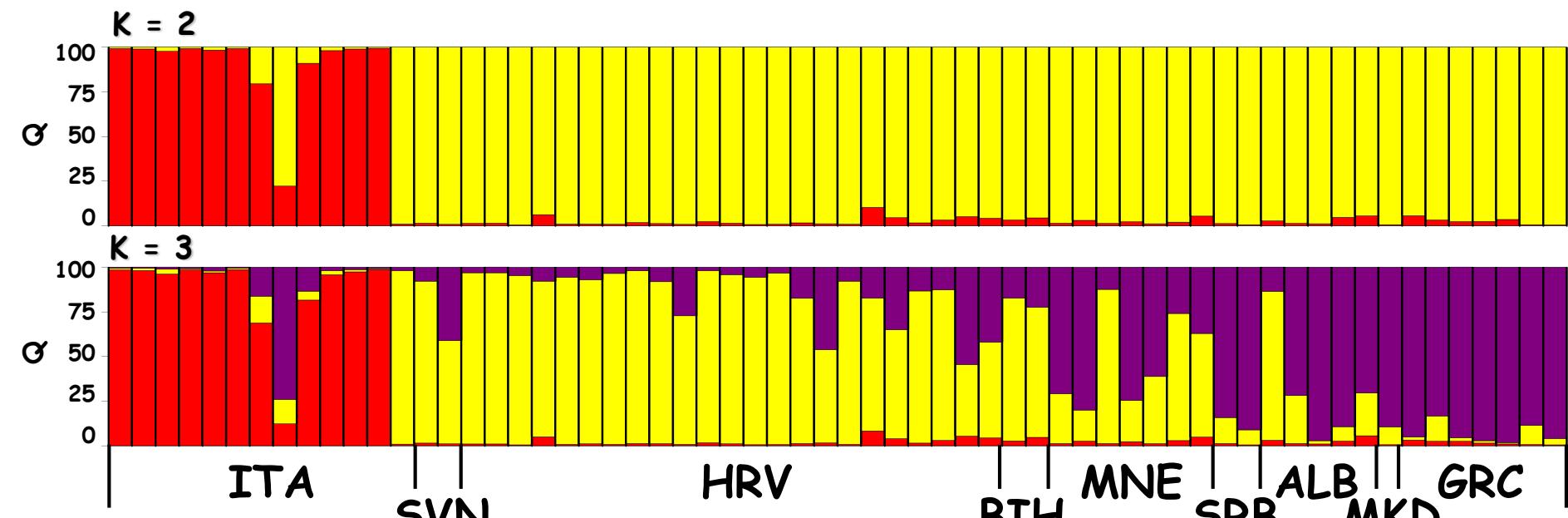
Apennine  
Peninsula

B

Balkan  
Peninsula

- mean proportion of membership ( $Q$ ) of each population in each of the two clusters (populations of origin)

$K = 3$



Apennines

Balkans

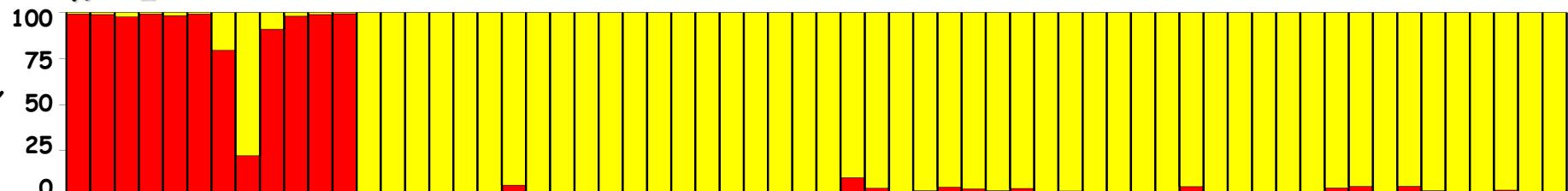
North-West  
populations

South-East  
populations

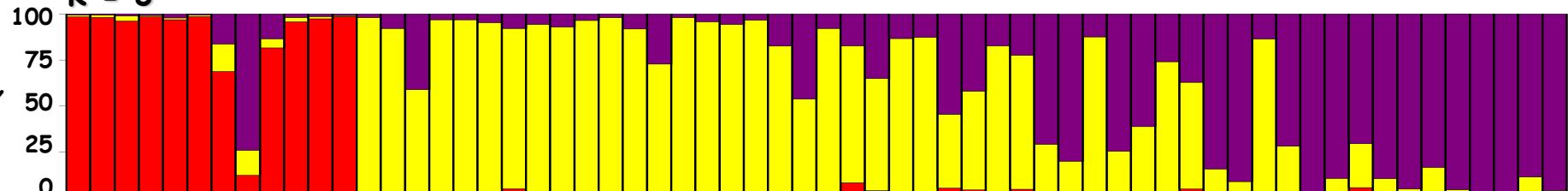
- the cluster B (Balkan) is at  $K = 3$  split into two clusters according to geographical locations of the sampling sites:  
North-West: Slovenia-Croatia-BiH  
South-East: Montenegro-Albania-Macedonia-Greece

**K = 4**

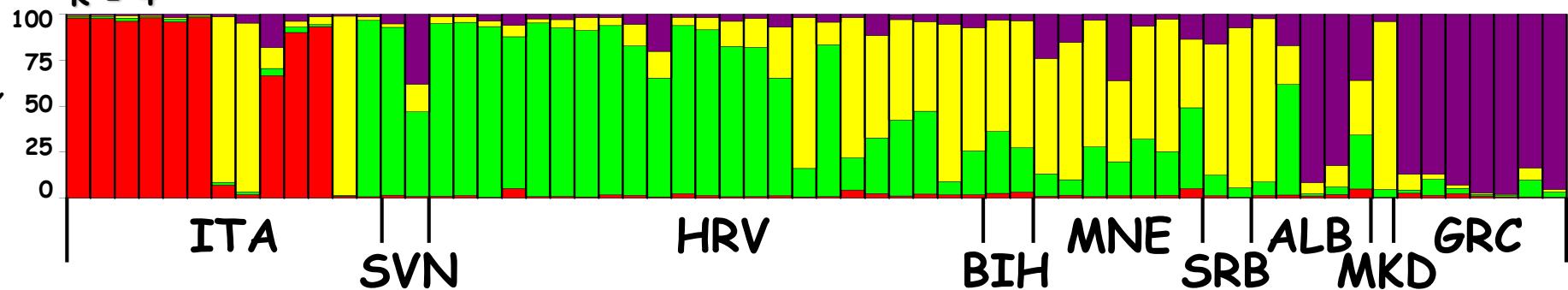
**K = 2**



**K = 3**



**K = 4**



ITA

SVN

HRV

BIH

MNE

ALB

MKD

GRC

Apennines

Northern  
Adriatic

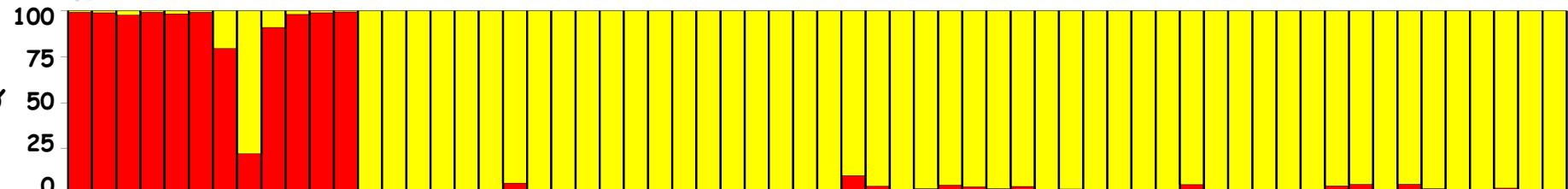
Balkans

Southern  
Adriatic

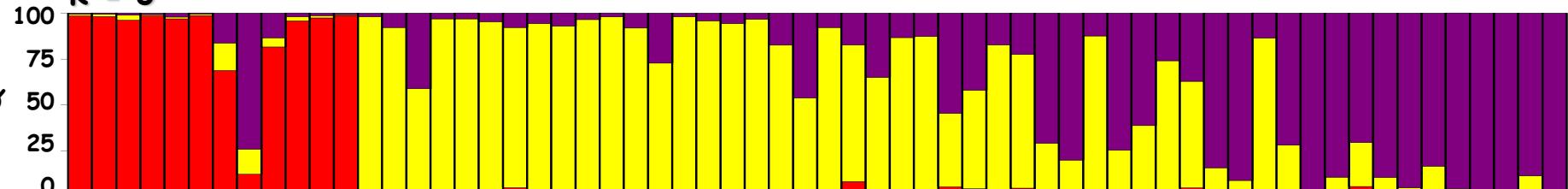
Albania  
Greece

$K = 5$

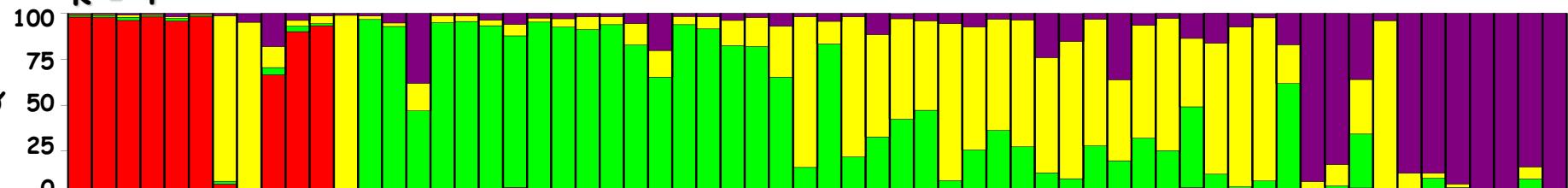
$K = 2$



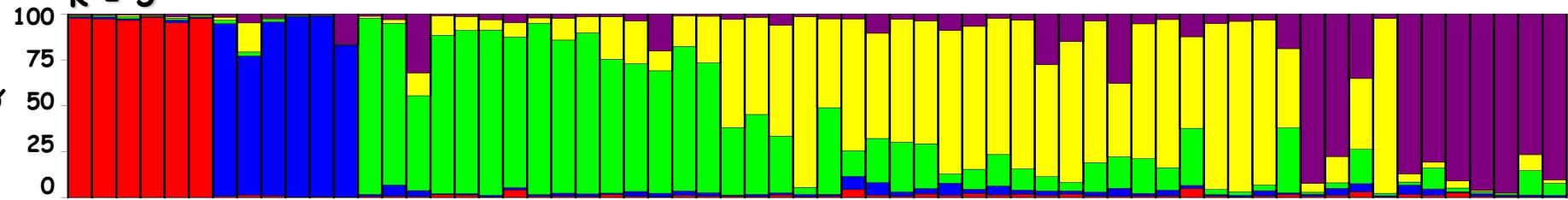
$K = 3$



$K = 4$



$K = 5$



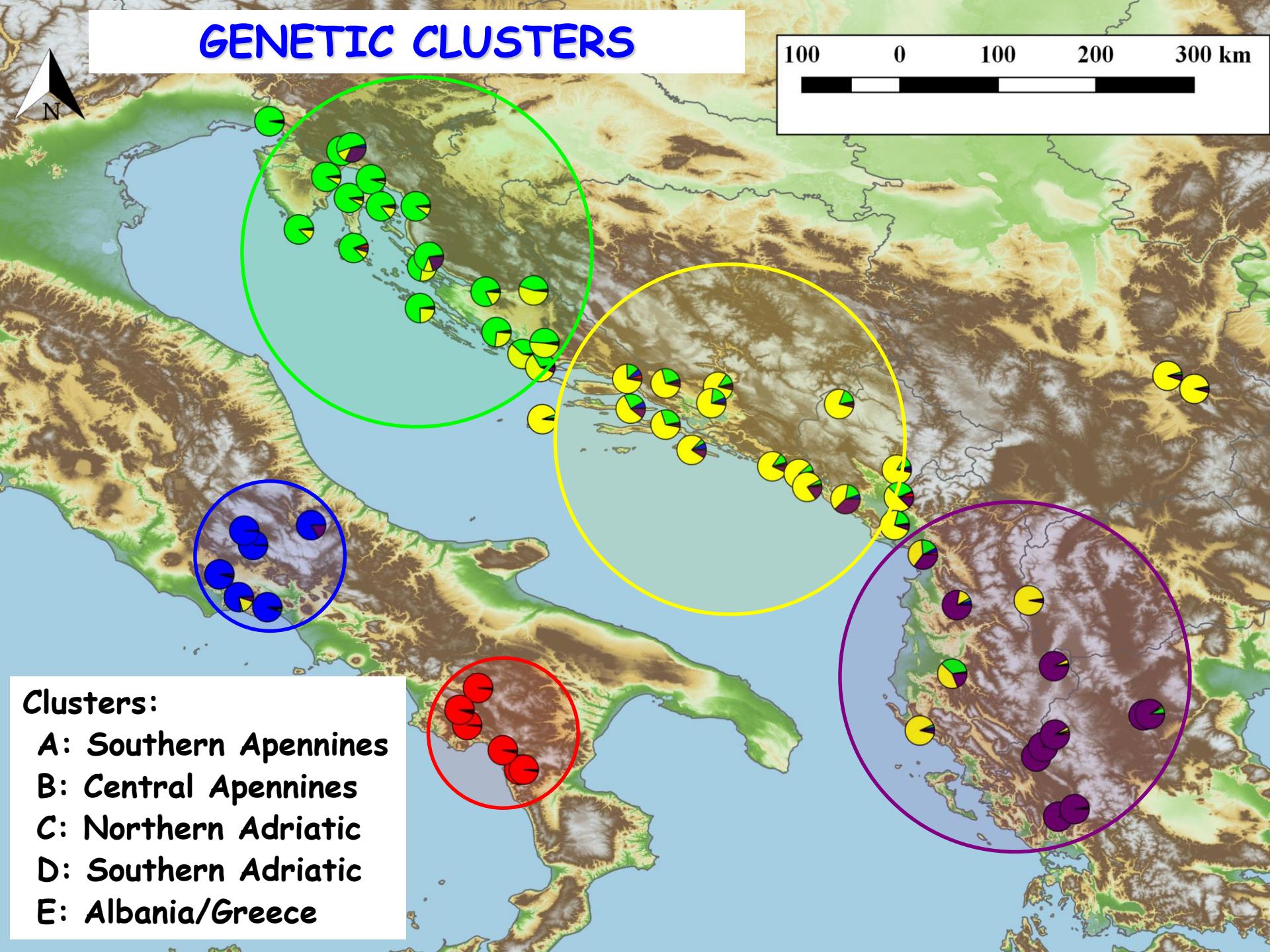
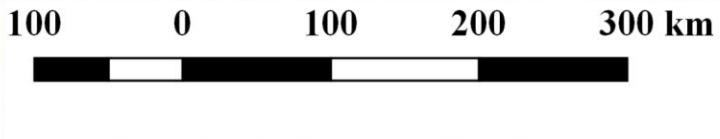
A B

C

D

E

# GENETIC CLUSTERS

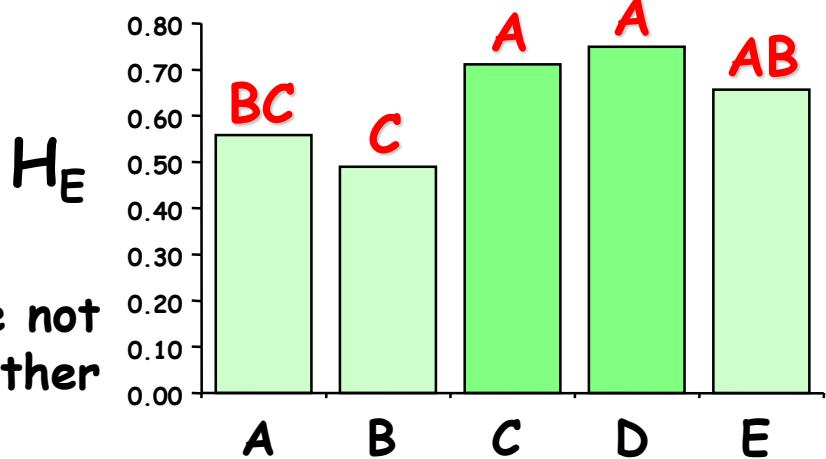
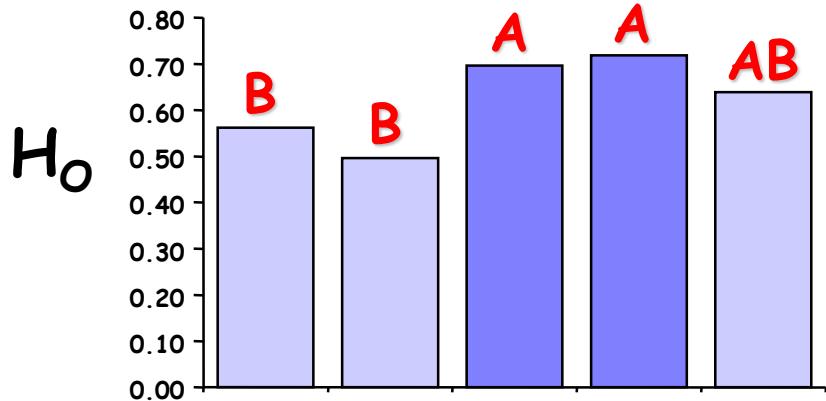
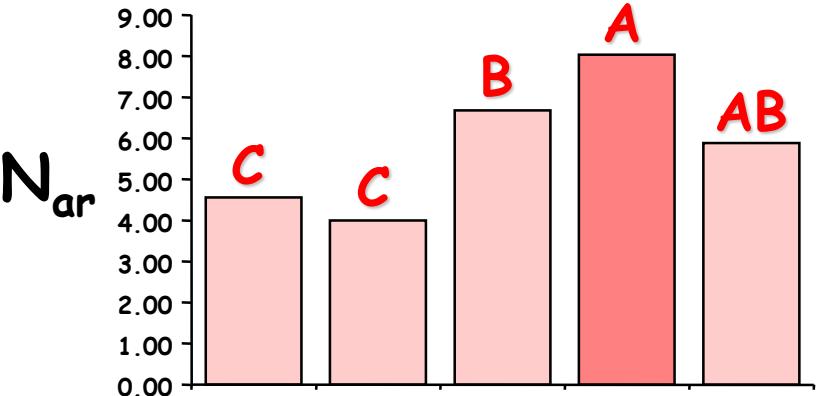


## Clusters:

- A: Southern Apennines
- B: Central Apennines
- C: Northern Adriatic
- D: Southern Adriatic
- E: Albania/Greece

# CLUSTER DIVERSITY

Cluster	No. populations	$N_{pr}$
A Southern Apennines	6	6
B Central Apennines	6	0
C Northern Adriatic	15	2
D Southern Adriatic	26	26
E Albania/Greece	9	9



Means with the same letter are not significantly different from each other

$N_{pr}$  - No. of private alleles

$N_{ar}$  - Allelic richness

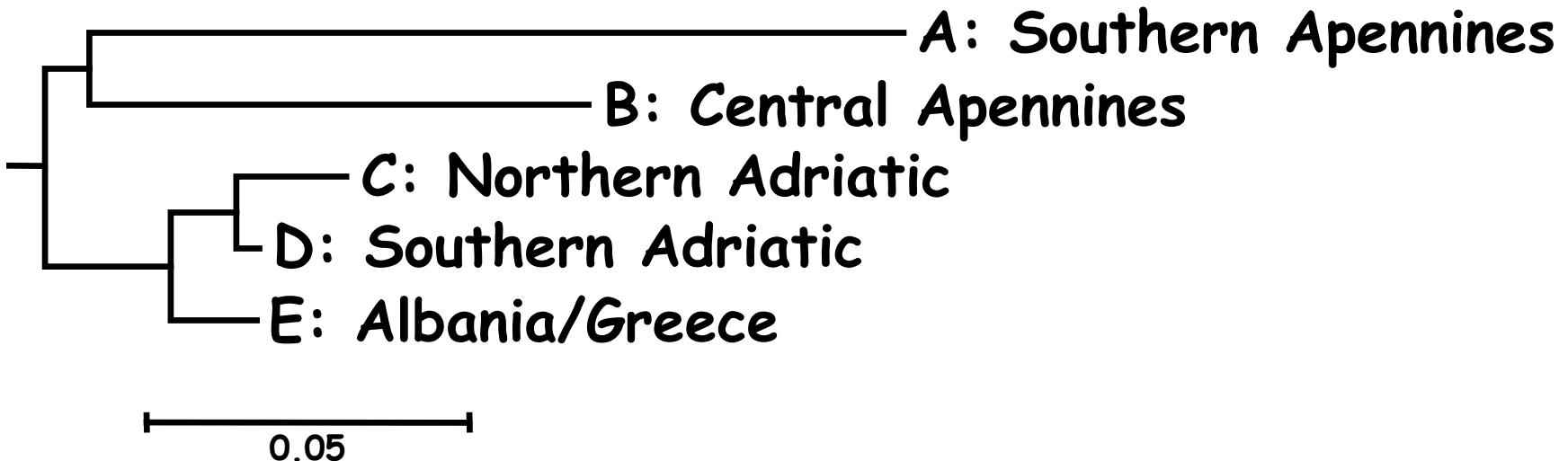
$H_O$  - Observed heterozygosity

$H_E$  - Expected heterozygosity

# RELATIONSHIPS AMONG CLUSTERS

Distance measure: net nucleotide distance

Tree algorithm: Neighbor-Joining



# OUTLINE

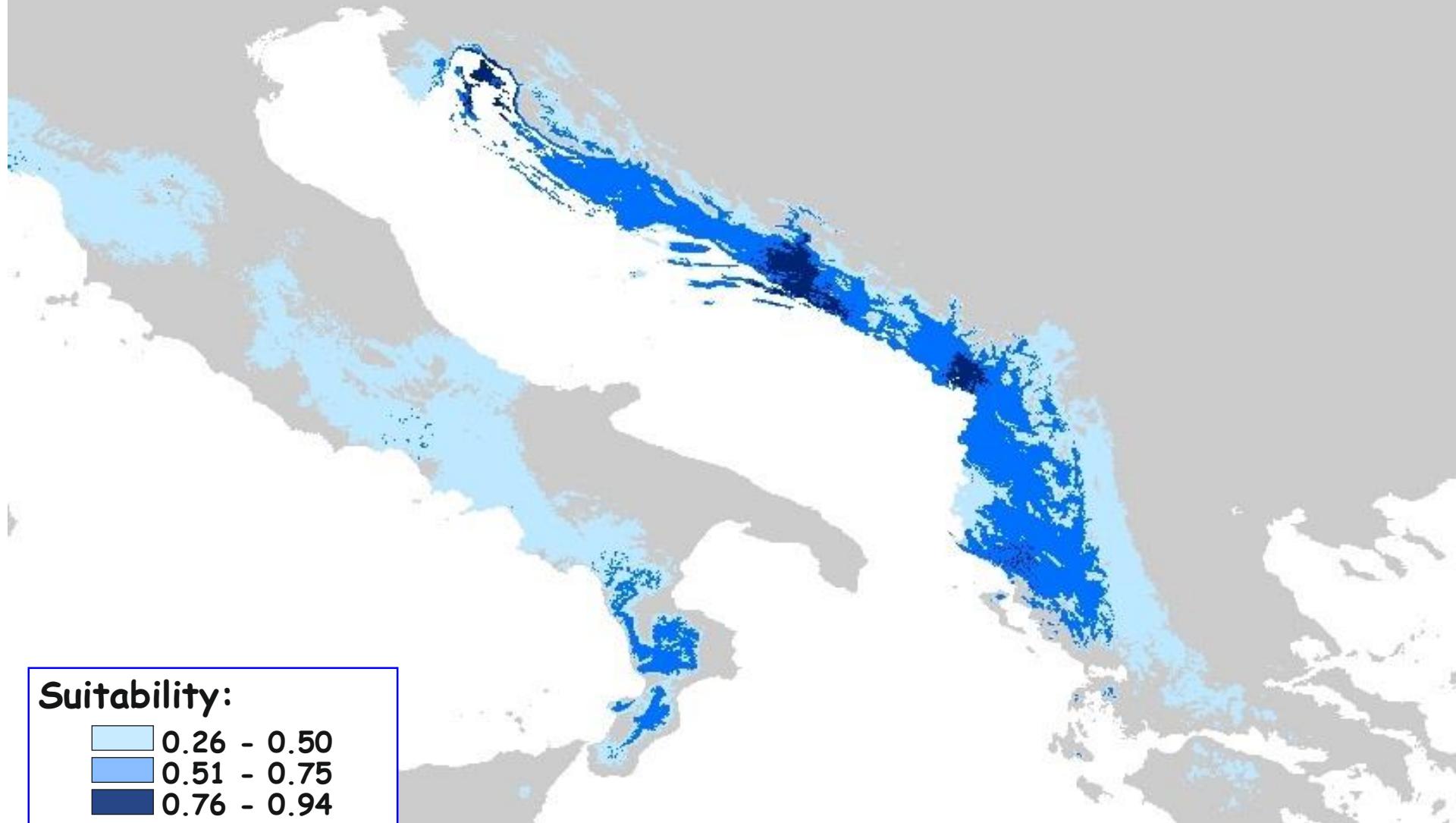
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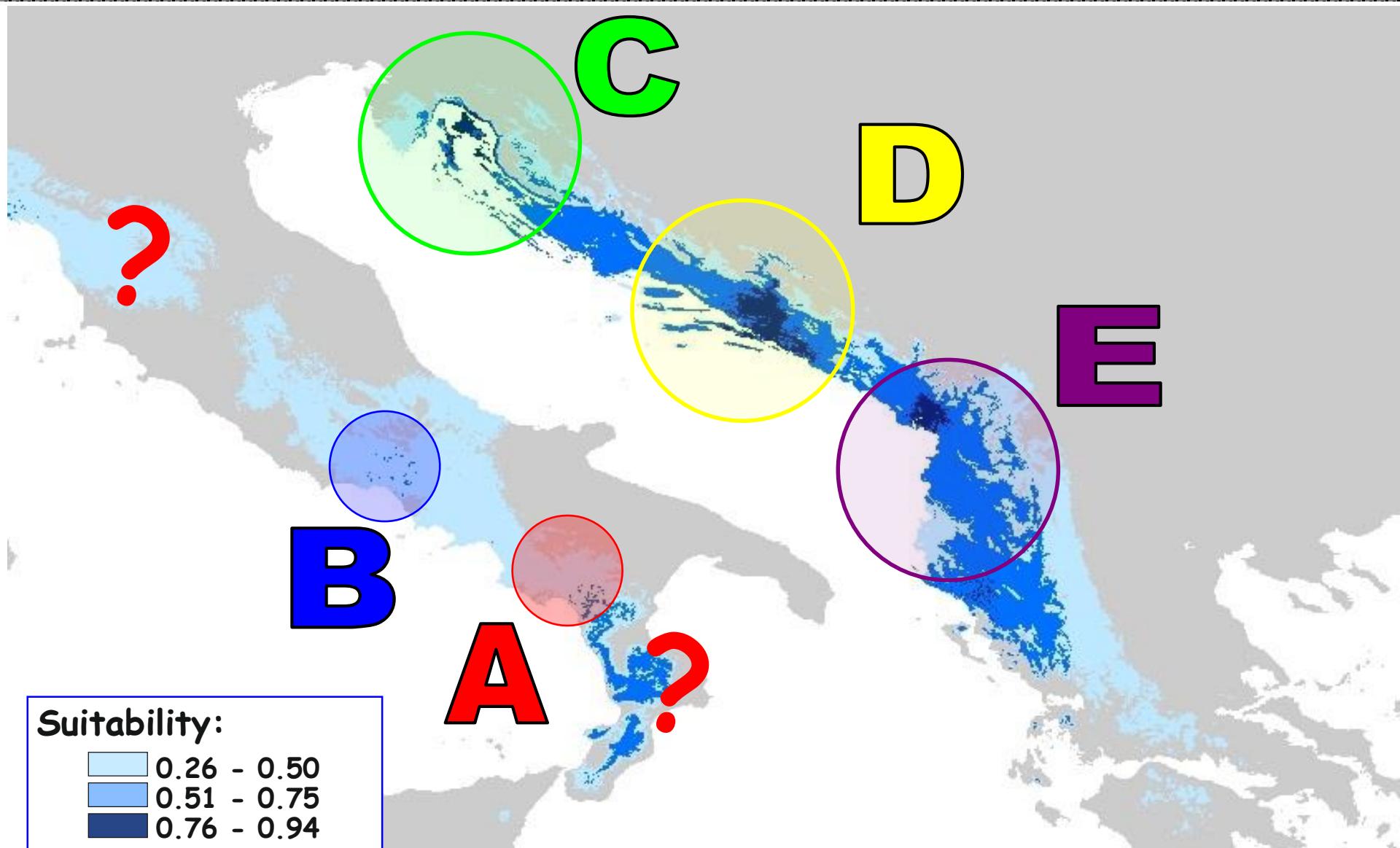
## (4) ECOLOGICAL NICHE MODELLING

- species distribution modelling (SDMs)
  - estimate the relationship between species records at sites and the environmental characteristics of those sites
  - predict the suitability of sites for occupation or persistence of the species
  - produce a modelled distribution of the species (= identify species' suitable environmental space)
- input data:
  - (1) geographic distribution  
68 data on occurrence (evenly distributed)
  - (2) environmental characteristics of the sites  
19 bioclimatic variables (WorldClim database)
    - 11 temperature- and 8 precipitation-related
    - representing the annual trends, seasonal variations and extremes in temperature and precipitation

# ENM: PRESENT DAY CONDITIONS



# ENM: PRESENT DAY CONDITIONS



# SUITABILITY: EDAPHIC FACTORS

## Soil Atlas of Europe

Classification: The World Reference Base for Soil Resources (WBR)

### (1) Balkans

Leptosols

Shallow soils  
over limestone

### (2) Apennines

Cambisols

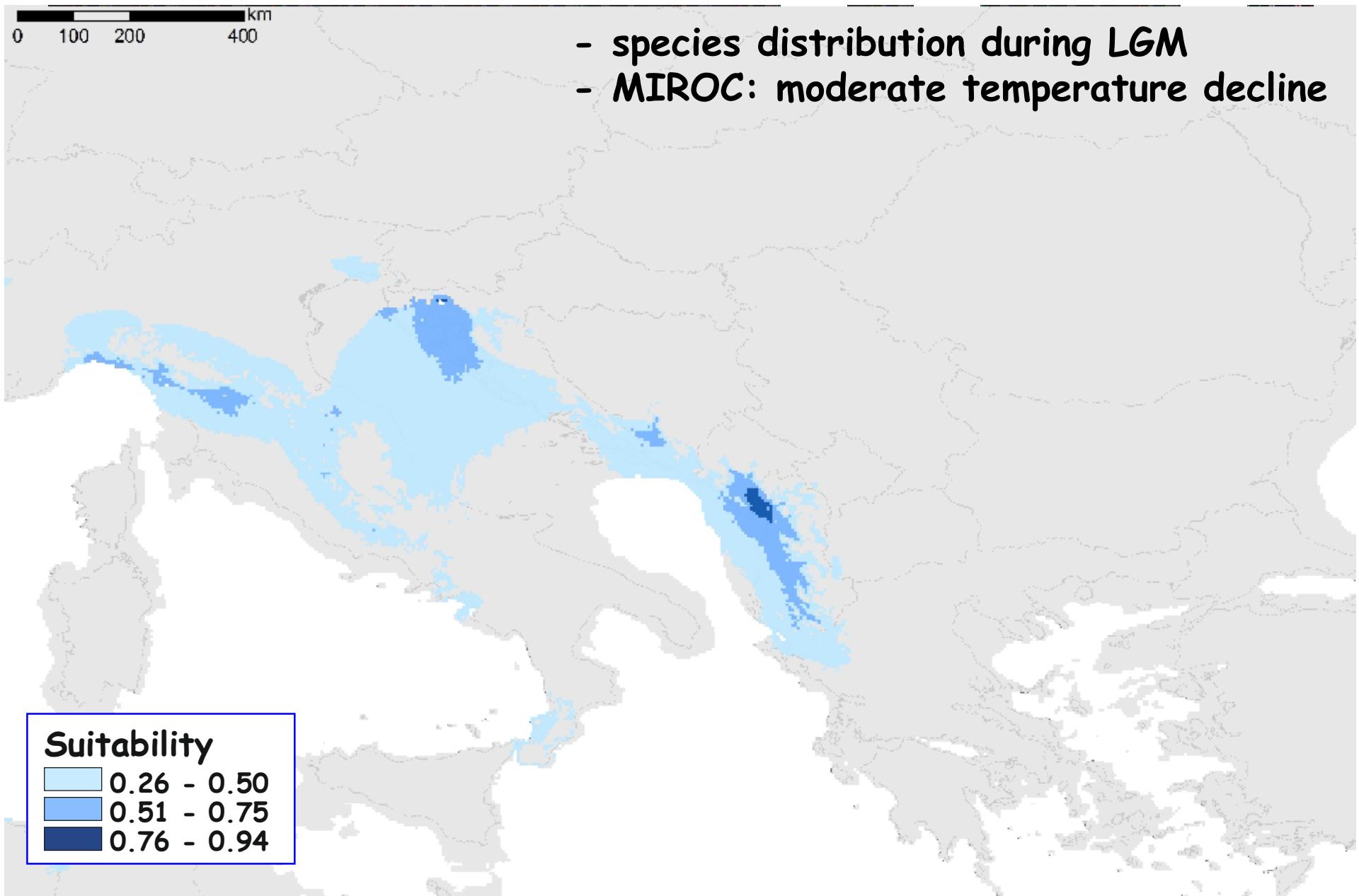
Moderately  
developed  
brown soils



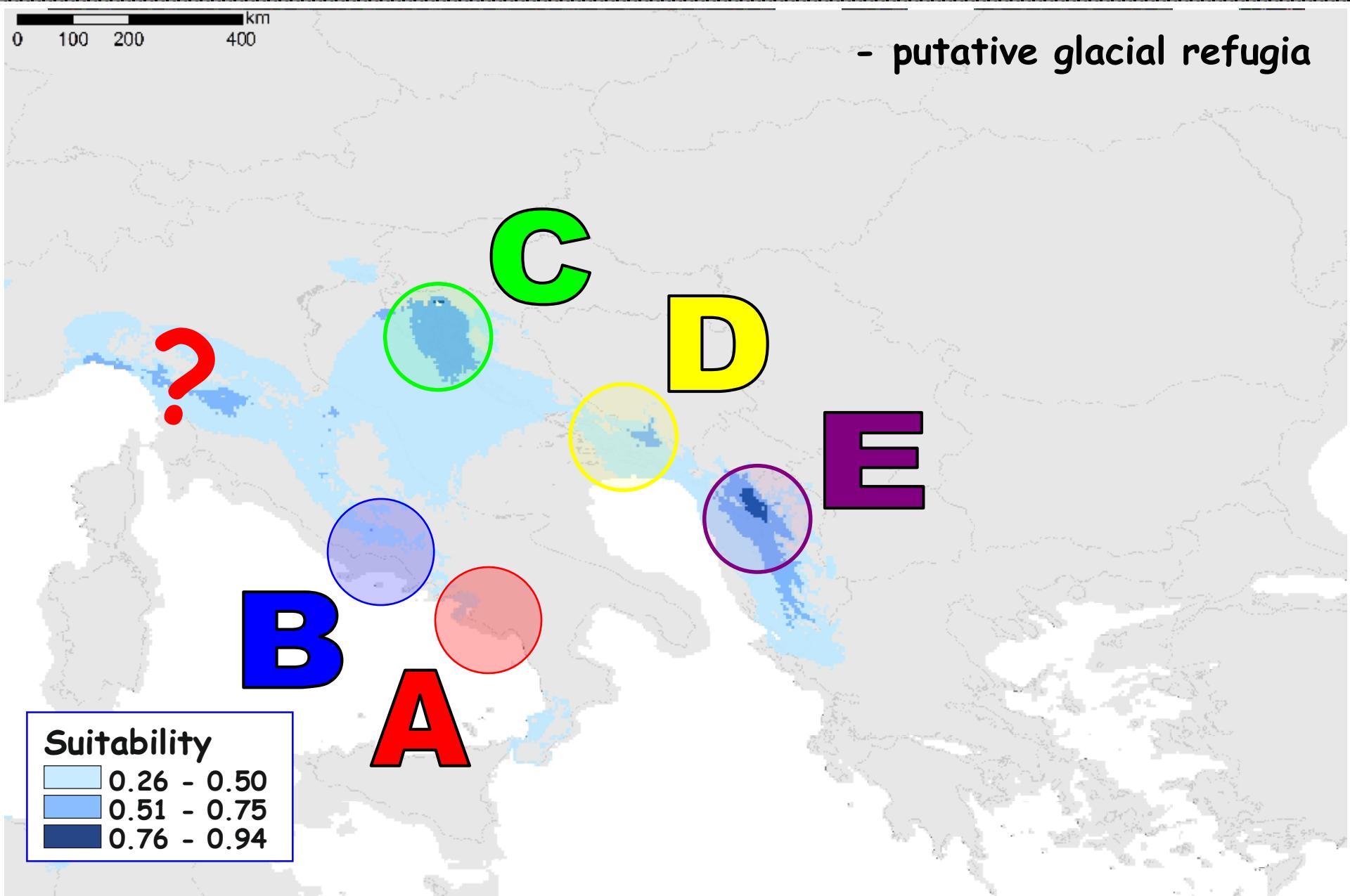
## PAST DISTRIBUTION

- to model potential species distribution during the Last Glacial Maximum (LGM; ~26,500 years BP)
- to identify putative glacial refugia of the species (= region which made possible the survival of the species and allowed a post-glacial re-colonization)
- input:
  - (1) the present model
  - (2) past environmental characteristics of the sites
    - 19 bioclimatic variables
    - bioclimatic data for the LGM developed by  
Paleoclimate Modelling Intercomparison Project Phase II
- two models:
  - CCSM (Community Climate System Model; USA)
  - MIROC (Model for Interdisciplinary Research on Climate; Japan)

# ENM: LAST GLACIAL MAXIMUM MIROC CONDITIONS



# ENM: 26,500 BP



# OUTLINE

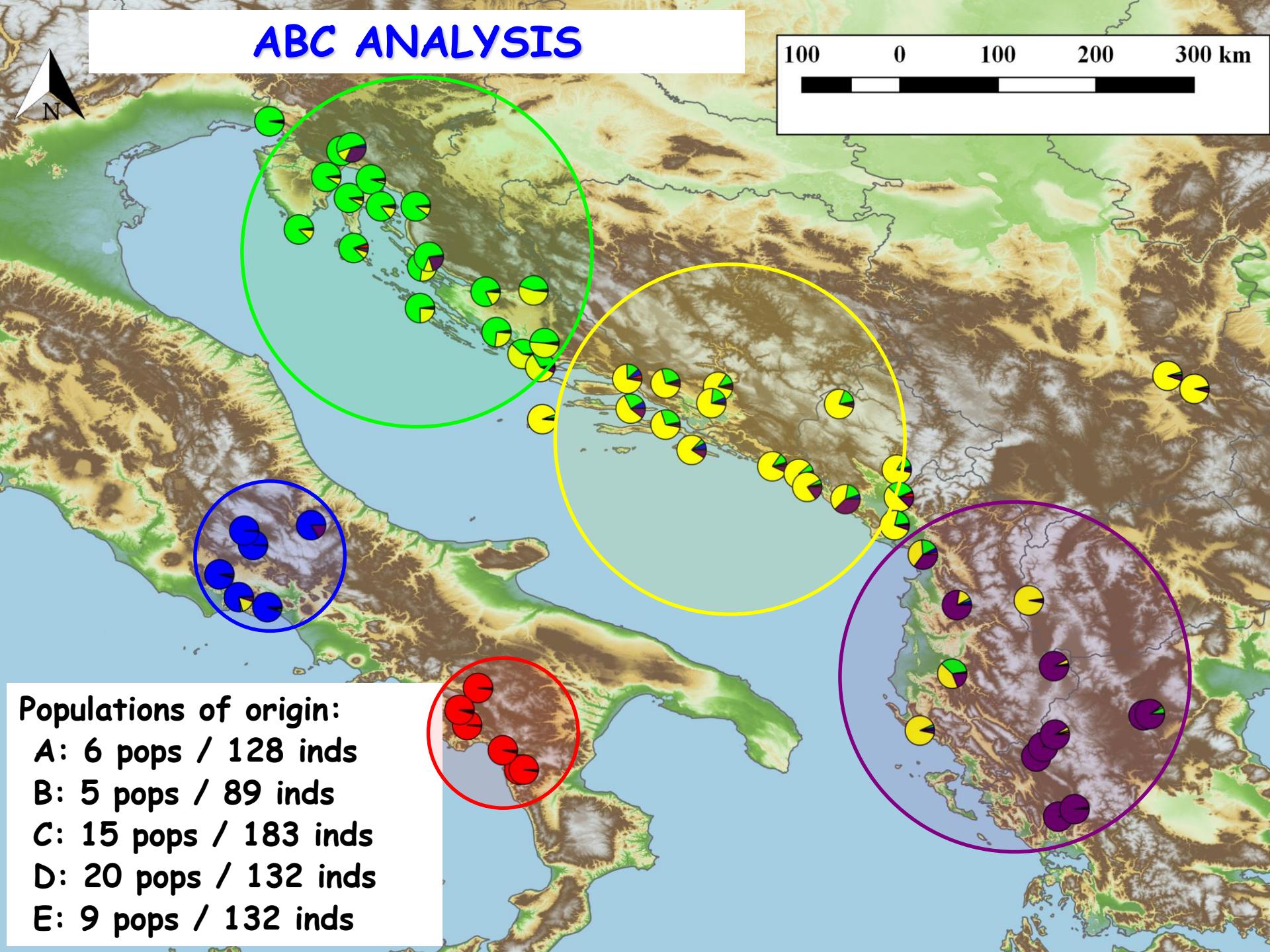
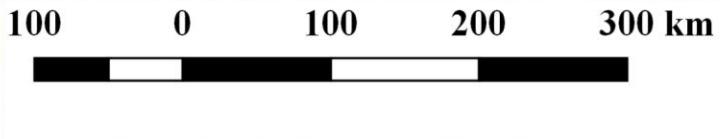
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## (5) DEMOGRAPHIC HISTORY

- inference on demographic history of a species based on approximate Bayesian computation (ABC)
- **input:**
  - molecular data
  - scenarios describing demographic history
- **procedure:**
  - simulate (a large number of) datasets for each scenario
  - compare simulated and observed datasets
  - the most similar simulated dataset is the most likely
- **goal:**
  - compare competing scenarios
    - posterior probability of each scenario
  - estimate parameters for one or more scenarios
    - effective population size; time of divergence

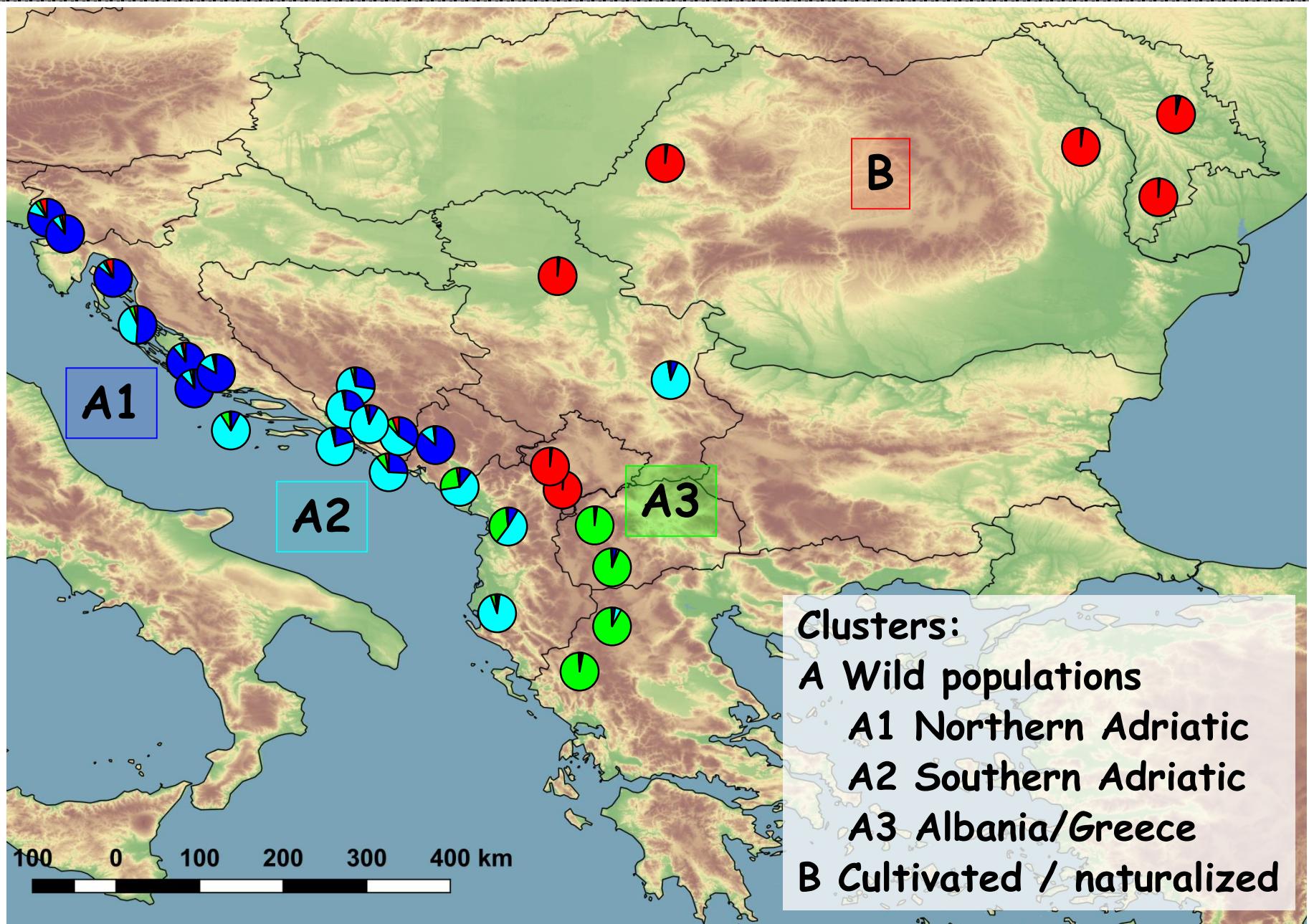
# ABC ANALYSIS



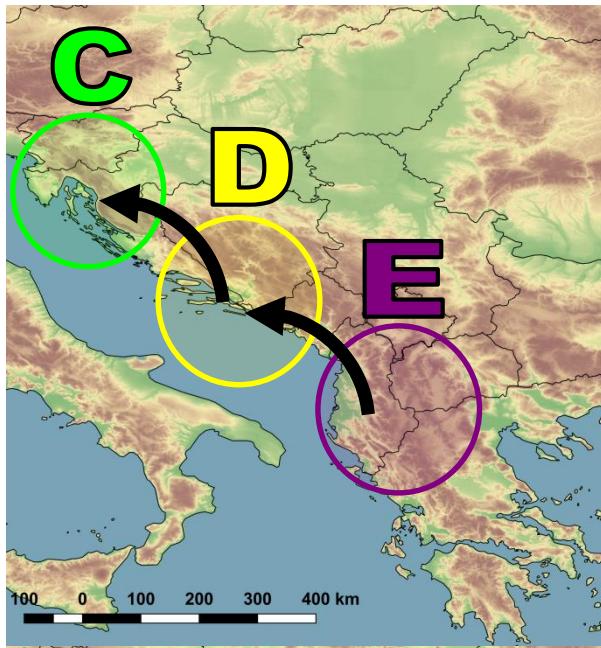
**Populations of origin:**

- A:** 6 pops / 128 inds
- B:** 5 pops / 89 inds
- C:** 15 pops / 183 inds
- D:** 20 pops / 132 inds
- E:** 9 pops / 132 inds

# PREVIOUS RESEARCH: Rešetnik et al. (2016)

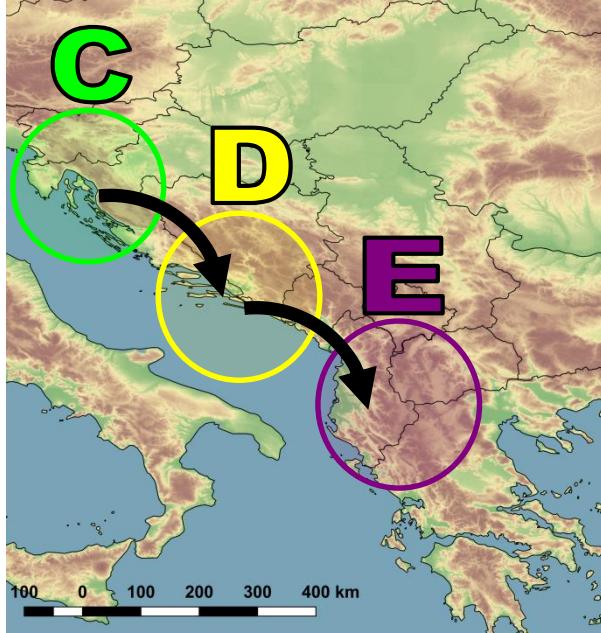
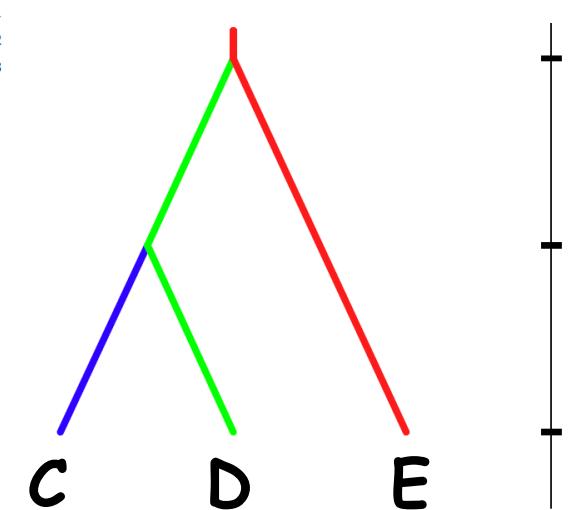


# BALKANS: FIVE SIMPLE HISTORIC SCENARIOS



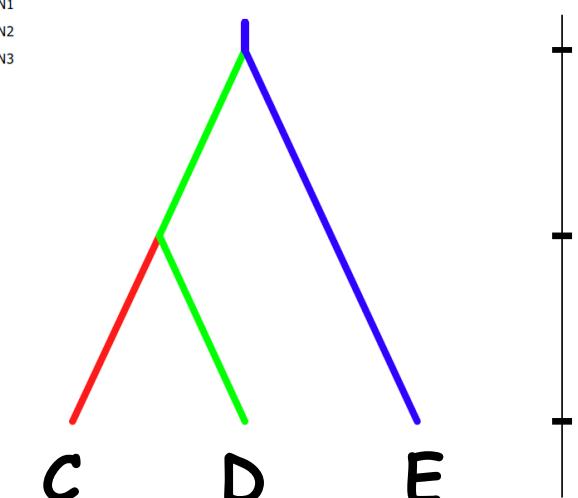
## Scenario 1

Population C is derived from population D, itself derived from population E

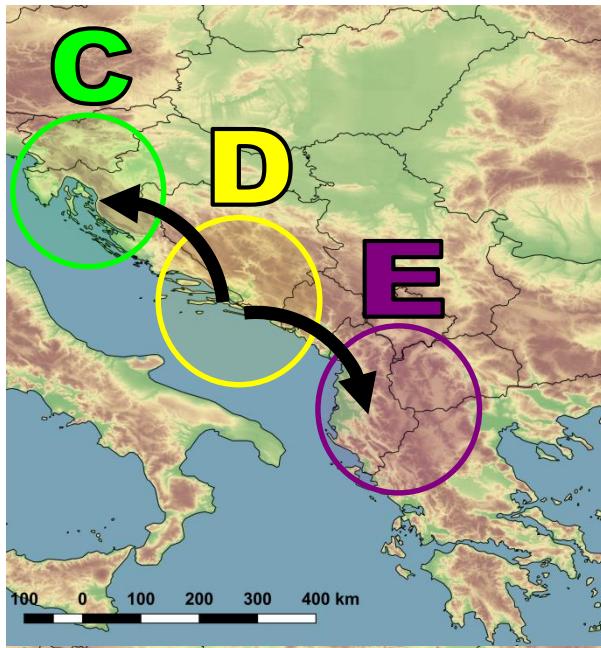


## Scenario 2

Population E is derived from population D, itself derived from population C

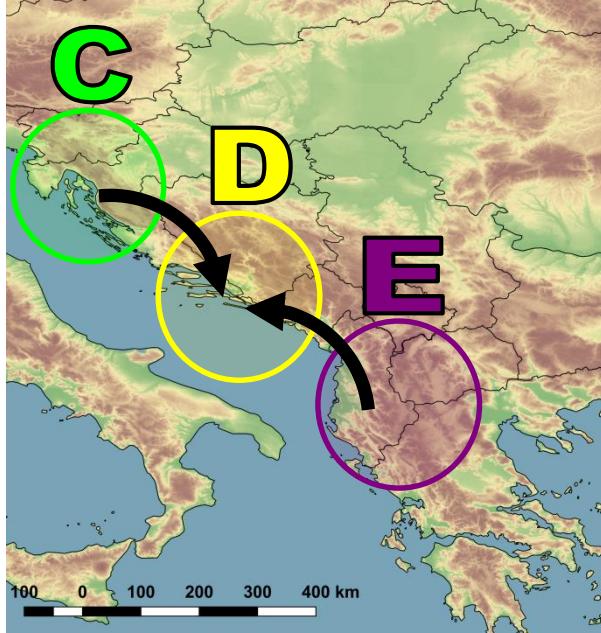
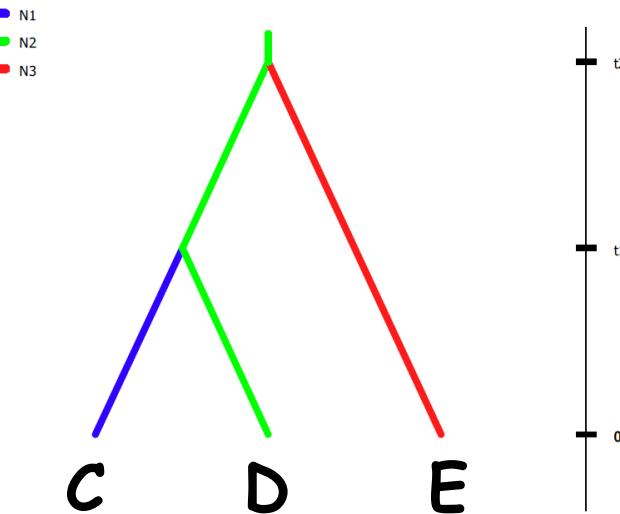


# BALKANS: FIVE SIMPLE HISTORIC SCENARIOS



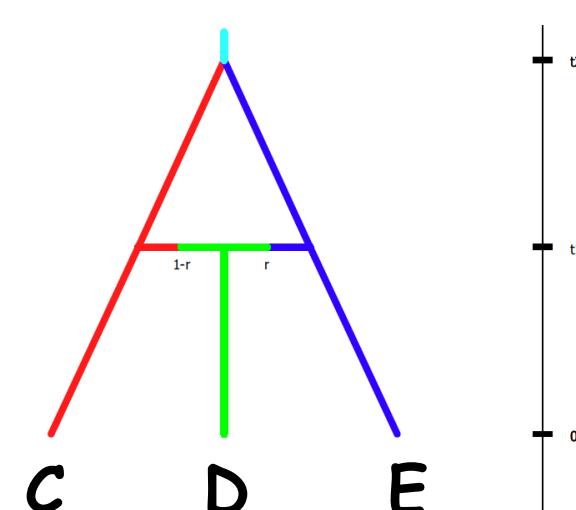
## Scenario 3

Both populations C and E derived independently from population D

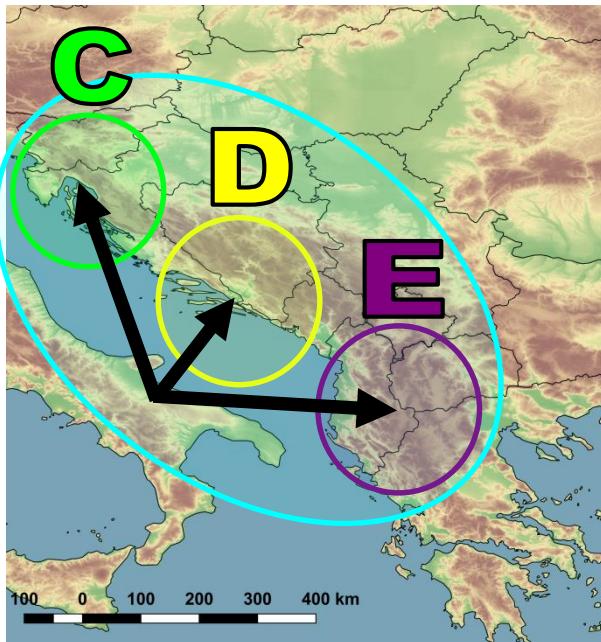


## Scenario 4

Population D was generated by admixture of populations C and E

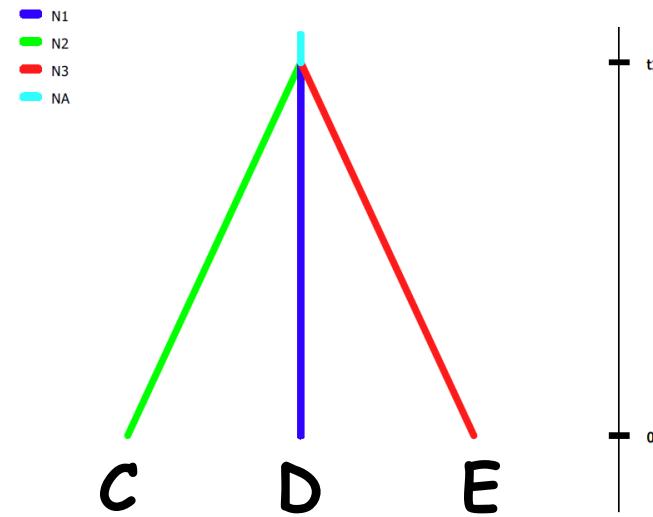


# BALKANS: FIVE SIMPLE HISTORIC SCENARIOS



## Scenario 5

All three populations diverged at the same time



Scenario	Posterior probability (PP)
1	0.114
2	0.009
3	0.107
4	0.007
5	0.764

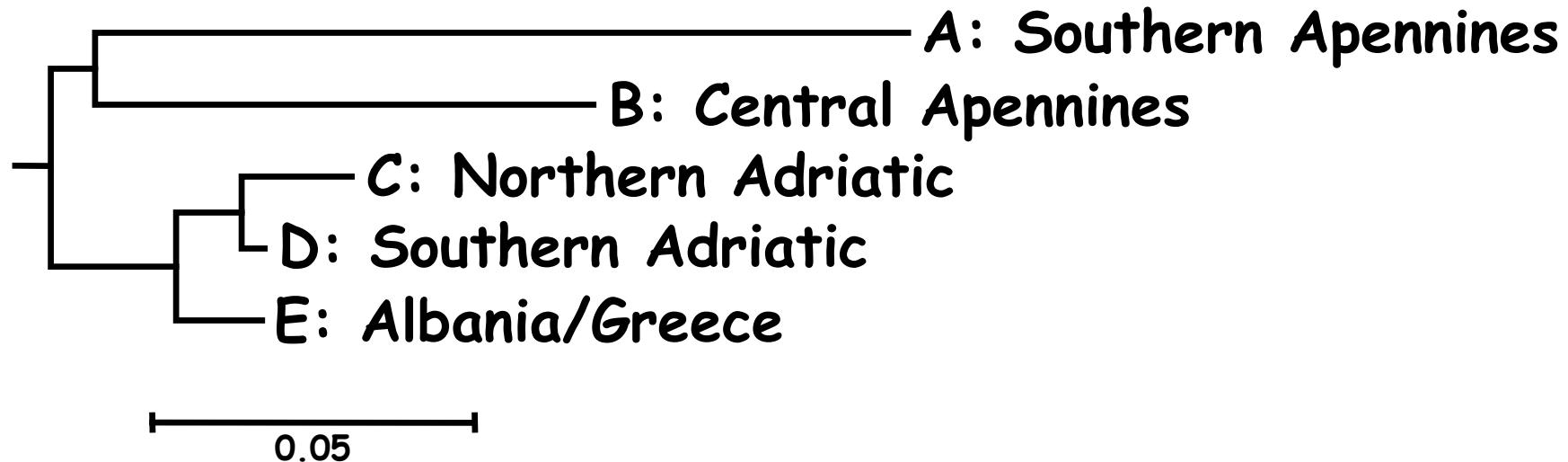
**RESULTS:**

1	0.114
2	0.009
3	0.107
4	0.007
5	0.764

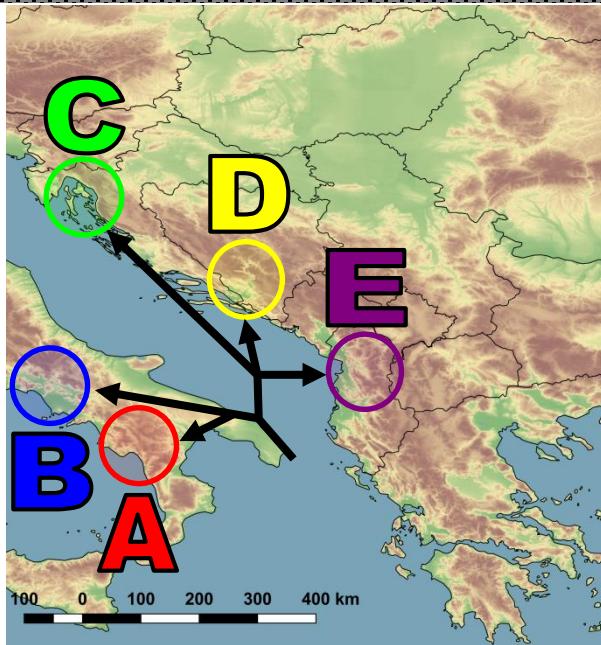
# APENNINES + BALKANS: HISTORIC SCENARIOS ?

Distance measure: net nucleotide distance

Tree algorithm: Neighbor-Joining

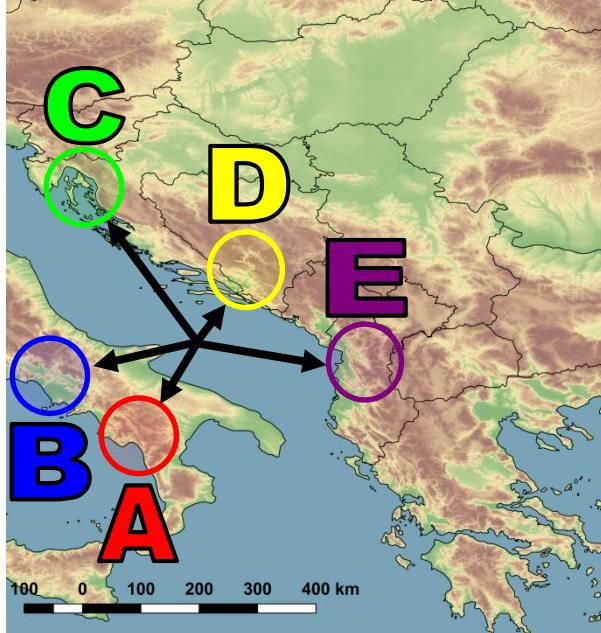
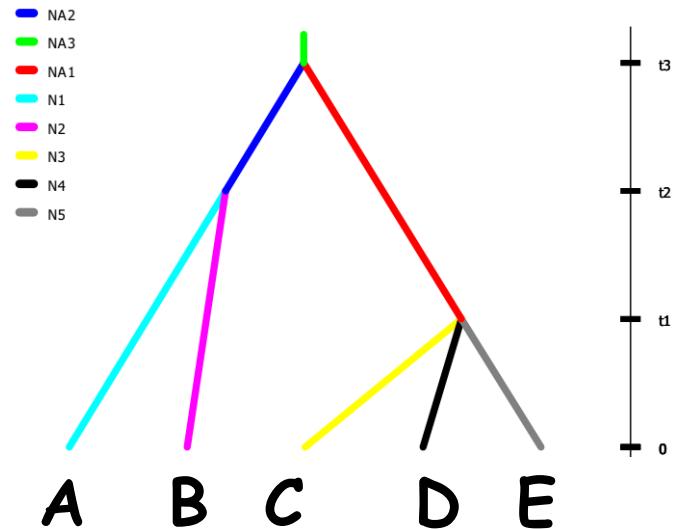


# APENNINES + BALKANS: HISTORIC SCENARIOS



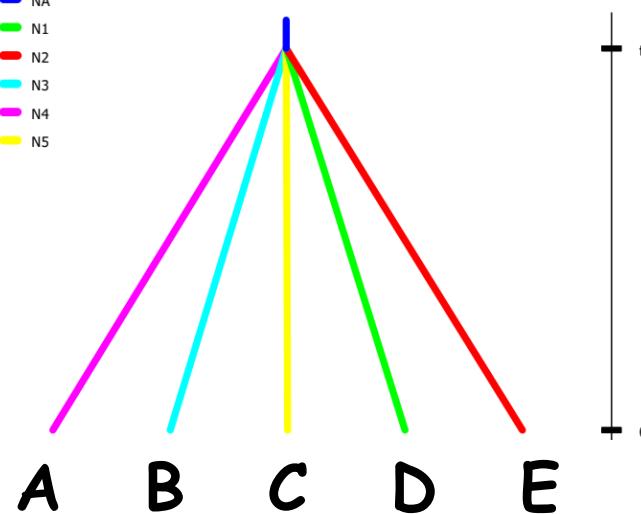
## Scenario 1

Apennine population group diverged from Balkan population group



## Scenario 2

All five populations diverged at the same time



# POSTERIOR PROBABILITIES OF SCENARIOS

Scenario	Posterior probability (Confidence interval)
1	0.384 (0.307-0.462)
2	0.616 (0.538-0.693)

- Scenario 2: All five populations diverged at the same time
- estimation of parameters under Scenario 2
  - effective population size
    - of the ancestral population
    - of the populations of origin (A-E)
  - time of divergence
    - in number of generations

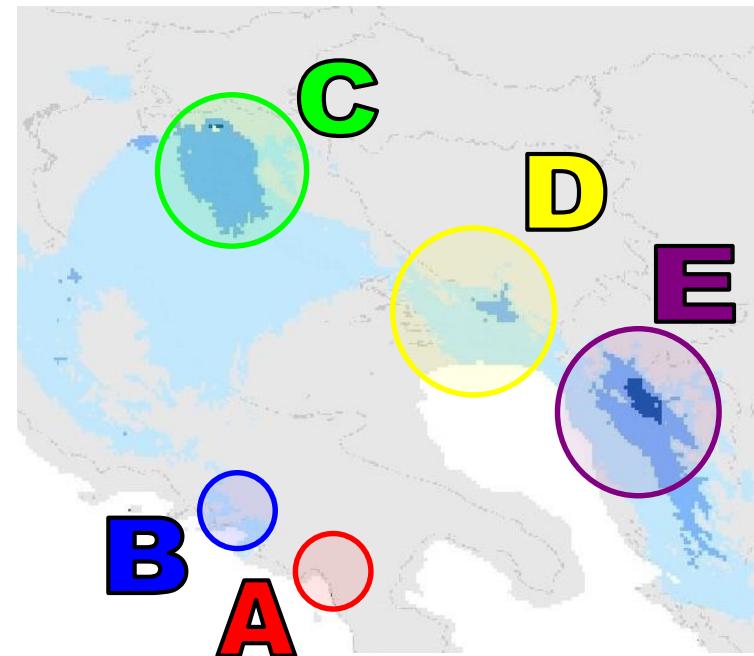
# HISTORICAL RECONSTRUCTION: ANCIENT PAST



Species distribution before  
the last glaciation (?)

Effective populations sizes:

Ancestral	9,280 individuals
A	1,140 individuals
B	940 individuals
C	4,360 individuals
D	8,330 individuals
E	5,220 individuals

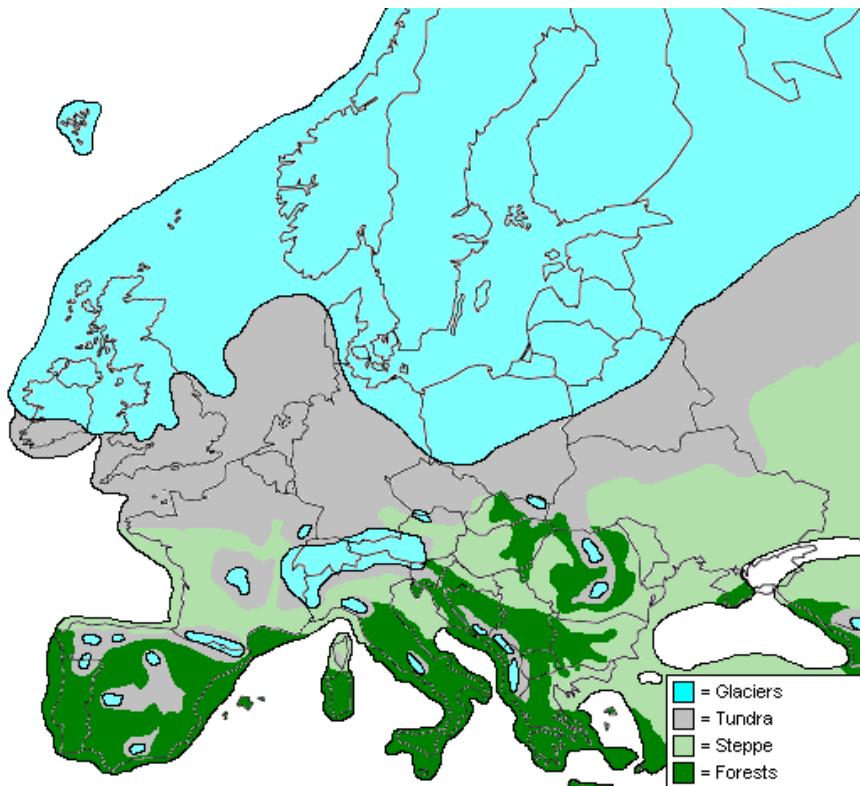


Species distribution during  
the last glaciation

(Last Glacial Maximum; LGM;  
~26,500 years BP)

# HISTORICAL RECONSTRUCTION: GLACIATION

Vegetation map of Europe during the last glaciation



Glacial refugia:

1. Iberian Peninsula
2. Apennine Peninsula
3. Balkan Peninsula

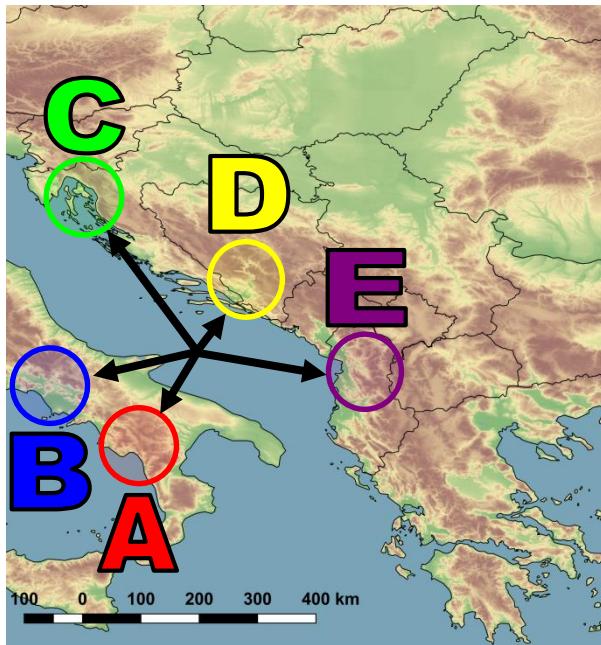
Glacial refugia in the Mediterranean Basin



Glacial (micro)refugia:

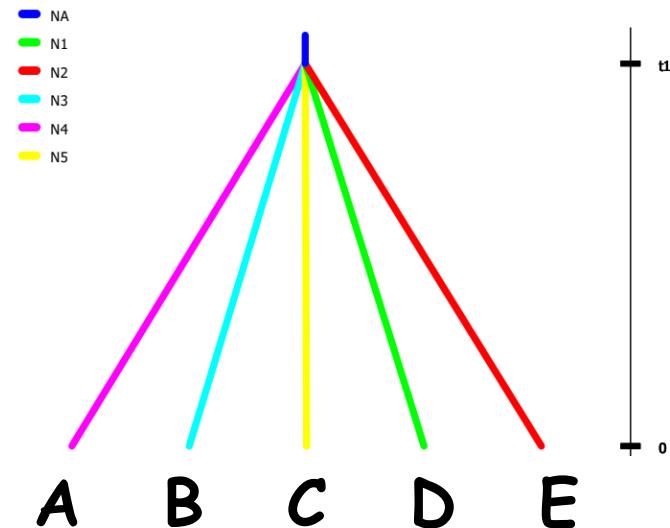
- A** 22. S. Apennines
- B** 21. Campania
- C** 26. N. Istria
- D** 28. S. Bosnia / Biokovo
- E** 29. Montenegro

# HISTORICAL RECONSTRUCTION: DIVERGENCE



## Scenario 2

All five populations diverged at the same time



Time of divergence ( $t_1$ ):

1,220 generations ago

Last glacial maximum (LGM):

26,500 years BP

Generation time of Dalmatian sage:

~20 years (?)



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