Epigenetic vs. Genetic Diversity in Natural Plant Populations: A Case Study of Croatian Endemic *Salvia* Species

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April 18-20, 2016 Belgrade, Serbia

Epigenetics « » Ecology « » Evolution

(1) In plants, epigenetic variations based on DNA methylation are often transmitted across generations

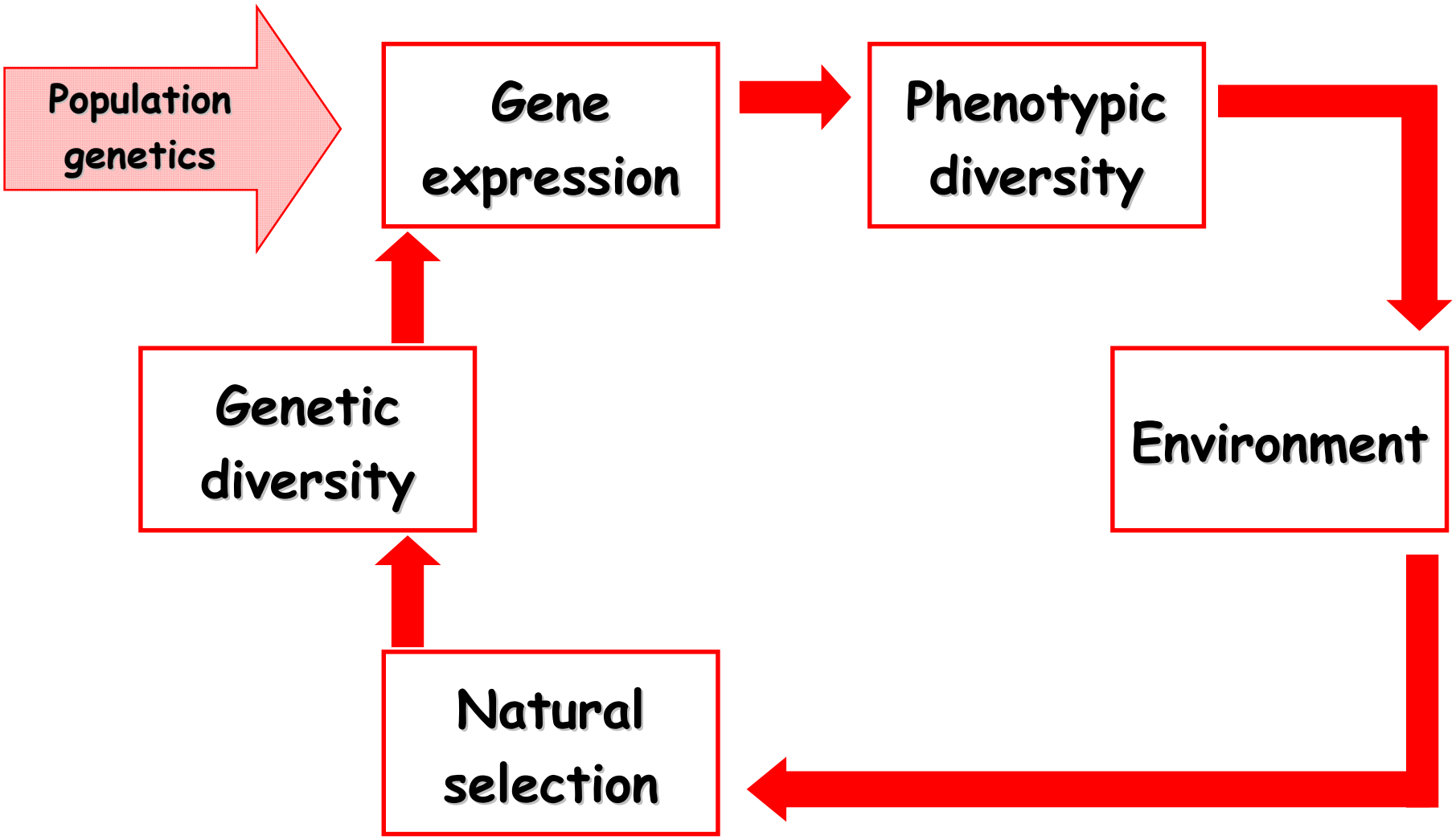
(2) Epigenetic changes are mostly independent from variation in the DNA sequence

(3) Epigenetic changes are environmentally induced

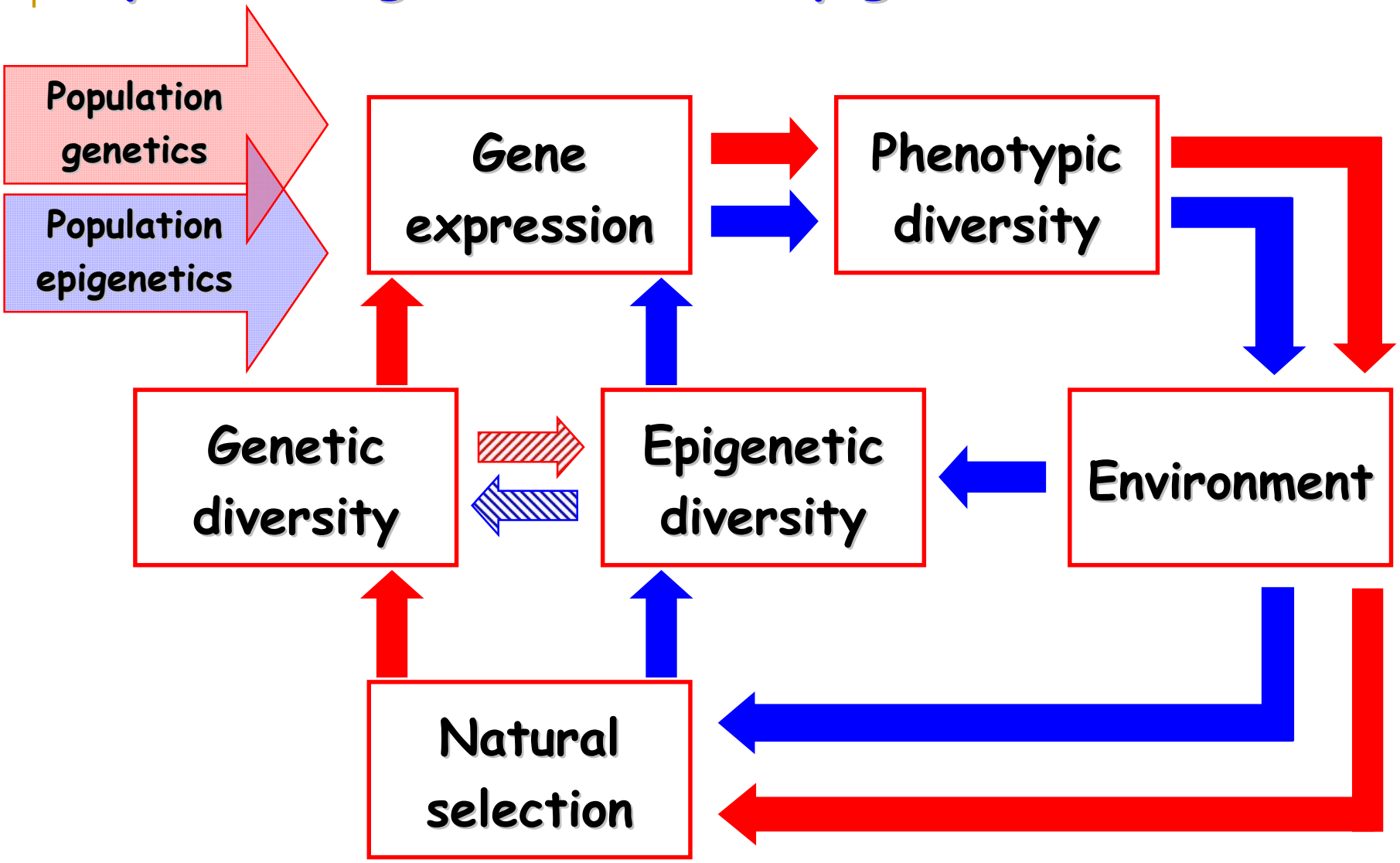
(4) Epigenetic changes can be influenced by genomic perturbations (e.g. inter-specific hybridisation)

Epigenetic change could play an important role in natural selection, adaptation and plant evolution

Population genetics



Population genetics and epigenetics



Questions

(1) How is epigenetic variation distributed within and among populations in comparison to genetic variation?

(2) Are there ascertainable patterns of epigenetic variation related to particular environmental factors?

(3) What is the level of epigenetic variation among genetically identical organisms of a clonal species?

(4) Do natural hybrids show higher epigenetic variability than their parental species?

Plant material

- wild populations:

(1) Dalmatian sage (*Salvia officinalis* L.)

(2) Short-toothed sage (*S. brachyodon* Vandas.)

(3) Auriculate sage (*Salvia* x *auriculata* Mill.)
= Dalmatian sage (*S. officinalis* L.)
x Greek sage (*S. fruticosa* L.)

Molecular markers

(1) Genetic markers

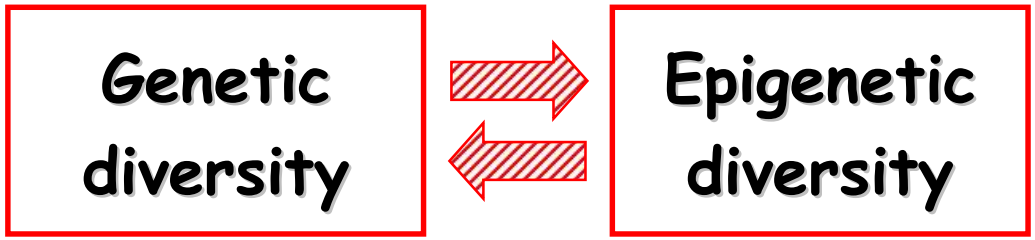
- Simple Sequence Repeats (SSR; microsatellites)

(2) Epigenetic markers

- Methylation-Sensitive Amplified Polymorphism (MSAP)
- a modification of the Amplified fragment length polymorphism method (AFLP) based on the differential sensitivity of isoschizomeric restriction enzymes to site-specific cytosine methylation

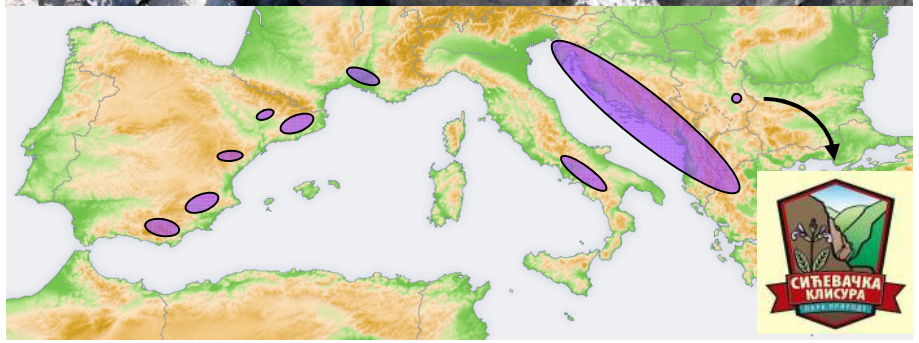
Question 1

(1) How is epigenetic variation distributed within and among populations in comparison to genetic variation?



(1) Dalmatian sage

- *Salvia officinalis*
- perennial allogamous subshrab
- distibution: northern coasts of the Mediterranean
- samples:
  - 25 populations
    - 23 Croatia
    - 2 BiH
  - 25 plants/population

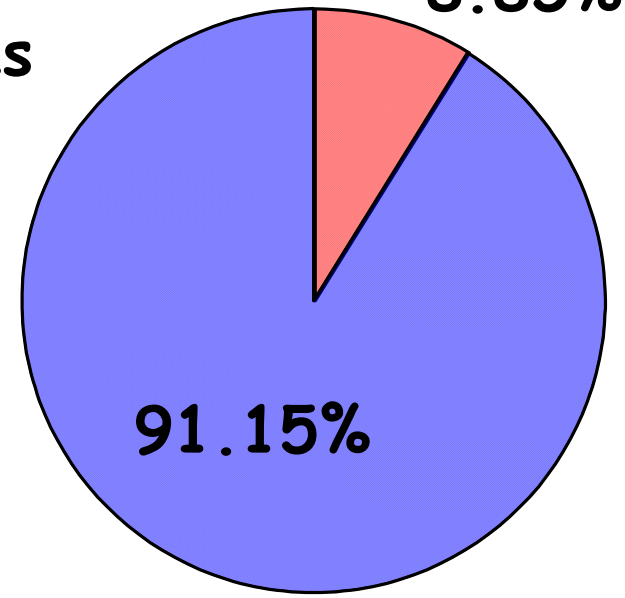


Partition of genetic/epigenetic variability

- Analysis of molecular variance (AMOVA):
  - % variance among populations
  - % variance within populations

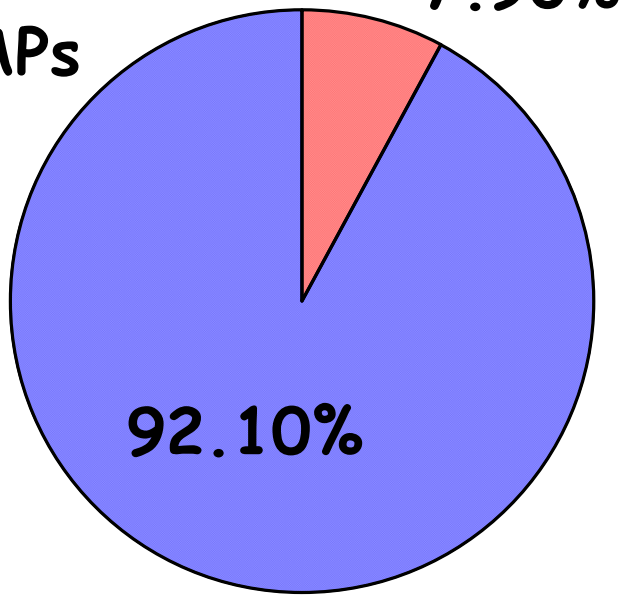
Genetic

SSRs



Epigenetic

MSAPs

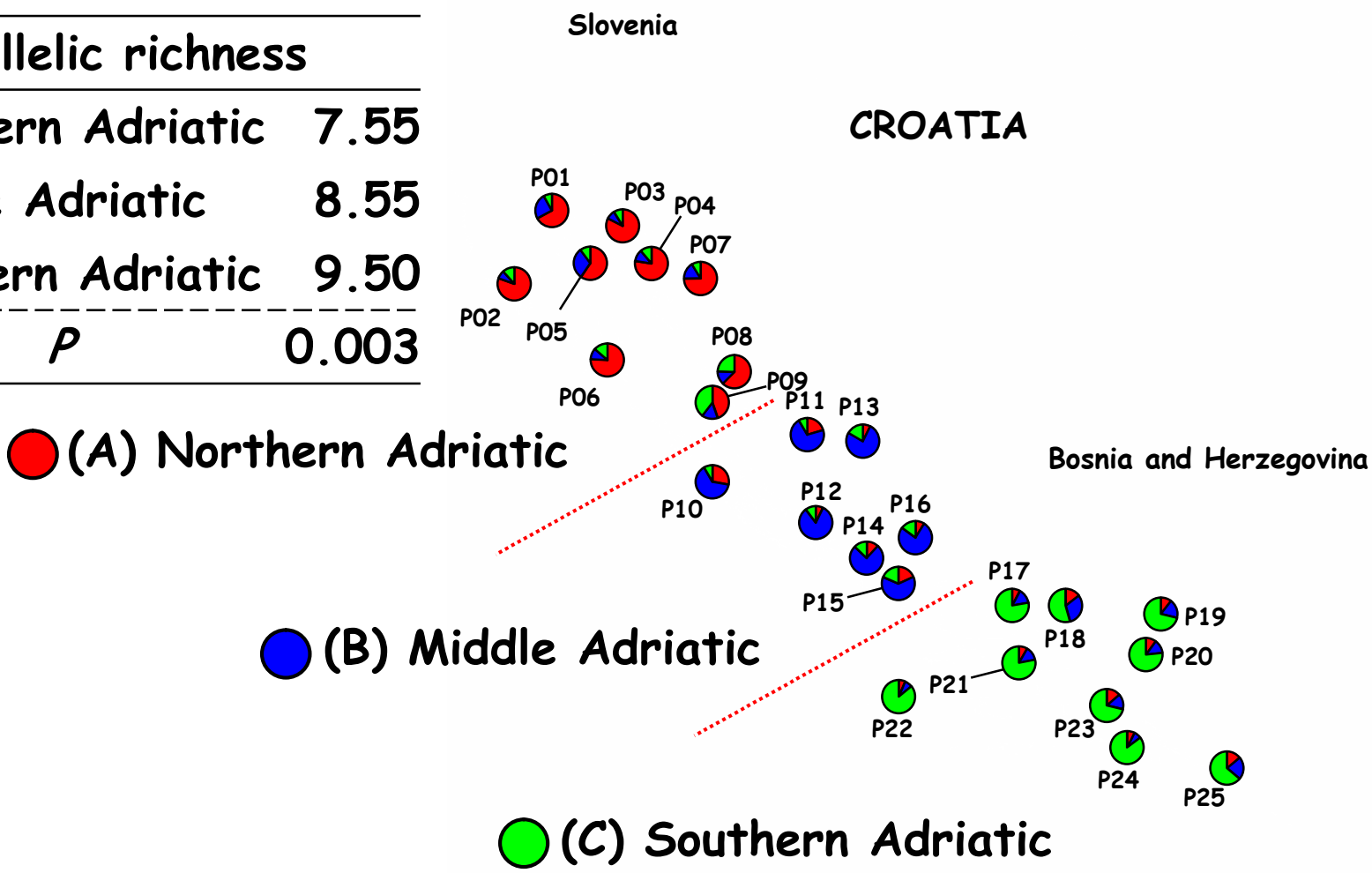


- similar partition of genetic and epigenetic diversity

Genetic structure

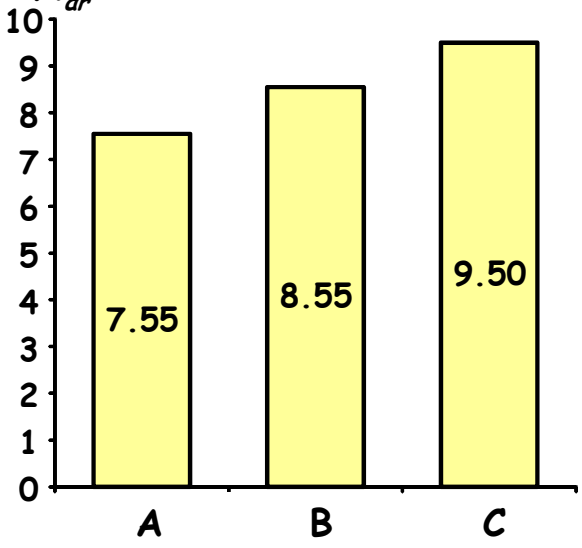
- three clusters:

Allelic richness	
Northern Adriatic	7.55
Middle Adriatic	8.55
Southern Adriatic	9.50
P	0.003

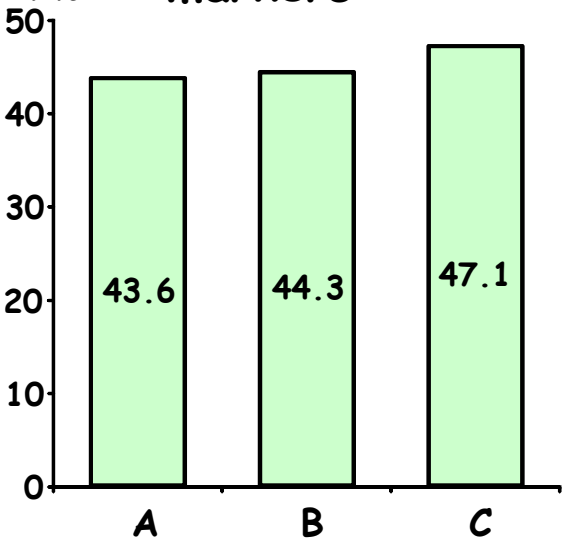


Genetic vs. epigenetic diversity

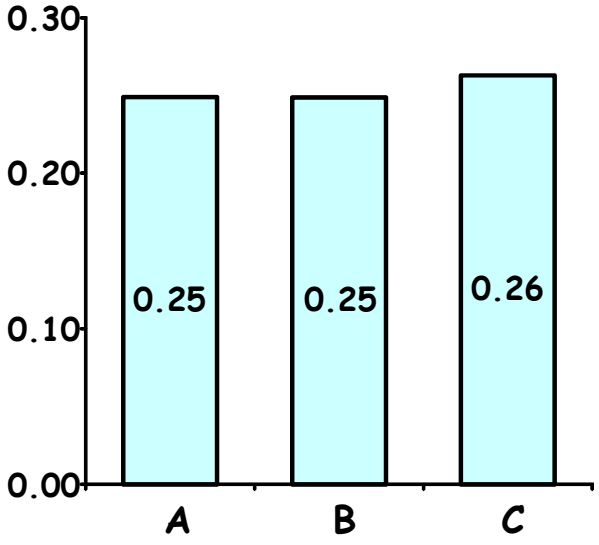
SSR
Allelic richness



MSAP
% polymorphic markers



MSAP
Shannon's information index



Correlation based on 25 populations:

SSR N_{ar} / MSAP $P\%$

$r = 0.24$

$R^2 = 0.05$

SSR N_{ar} / MSAP H

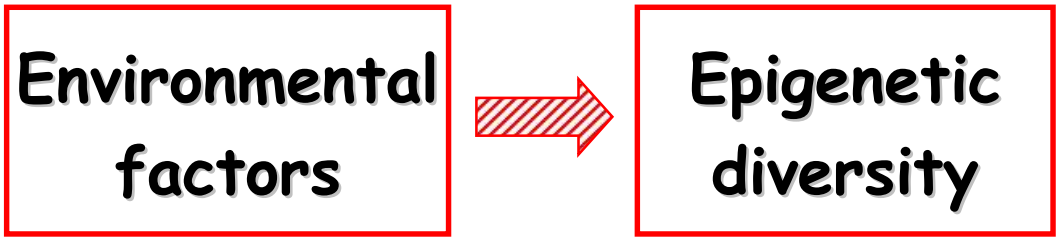
$r = 0.31$

$R^2 = 0.09$

- weak correlation between genetic and epigenetic diversity

Question 2

(2) Are there ascertainable patterns of epigenetic variation related to particular environmental factors?



Environmental factors and epigenetic diversity

(1) Environmental data

- 19 bioclimatic variables
- WordClim database (www. worldclim.org)
- 25 sampling sites

(2) Logistic regression

- multiple univariate logistic regressions
- the probability of presence of an allelic variant of a polymorphic marker given the environmental conditions of sampling locations
- software Samβada

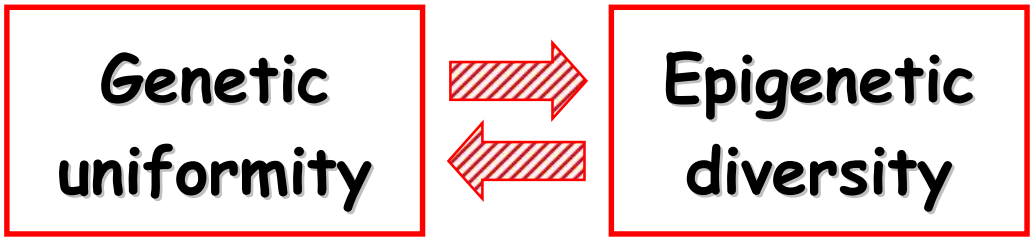
Epigenetic markers vs. bioclimatic variables

No.	Bioclimatic variable	No. MSAPs
BIO1	Annual Mean Temperature	0
BIO2	**Mean Diurnal Range**	**8**
BIO3	**Isothermality**	**8**
BIO4	Temperature Seasonality	4
BIO5	Max Temperature of Warmest Month	0
BIO6	Min Temperature of Coldest Month	2
BIO7	Temperature Annual Range (P5-P6)	4
BIO8	Mean Temperature of Wettest Quarter	5
BIO9	Mean Temperature of Driest Quarter	0
BIO10	Mean Temperature of Warmest Quarter	1
BIO11	Mean Temperature of Coldest Quarter	0
BIO12	Annual Precipitation	6
BIO13	Precipitation of Wettest Month	3
BIO14	**Precipitation of Driest Month**	**13**
BIO15	**Precipitation Seasonality**	**8**
BIO16	Precipitation of Wettest Quarter	3
BIO17	**Precipitation of Driest Quarter**	**13**
BIO18	**Precipitation of Warmest Quarter**	**12**
BIO19	Precipitation of Coldest Quarter	5

- 36 significant MSAP markers (related to multiple traits):
  - (1) Precipitation-related traits
  - (2) Traits describing temperature/precipitation range

Question 3

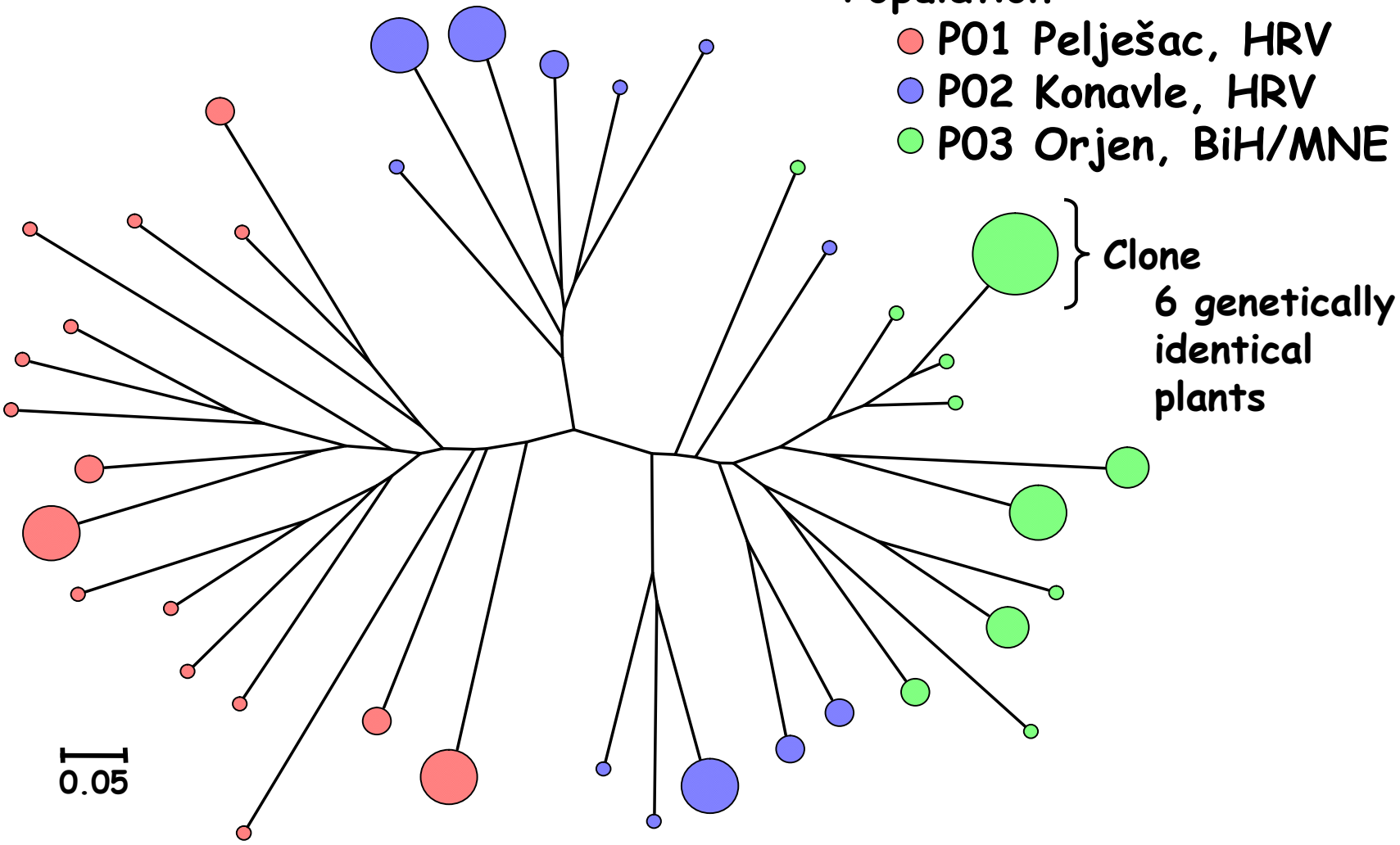
(3) What is the level of epigenetic variation among genetically identical organisms of a clonal species?



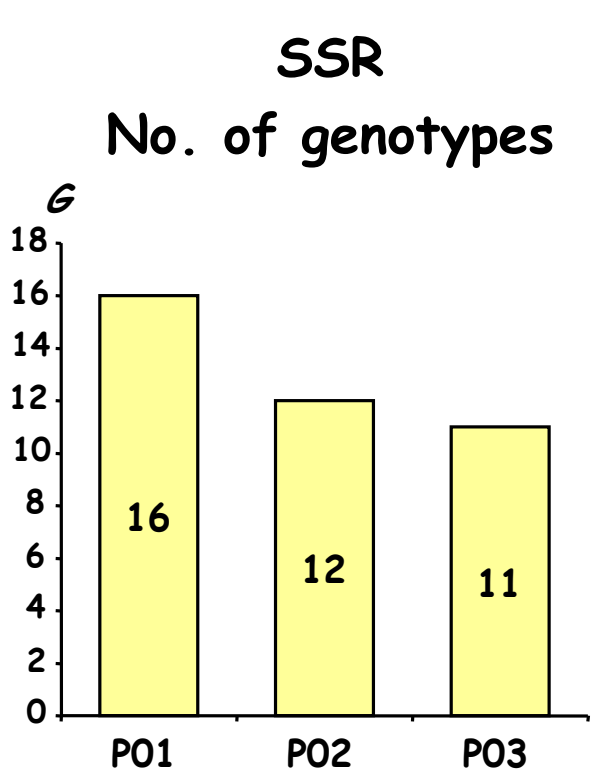
(2) Short-toothed sage

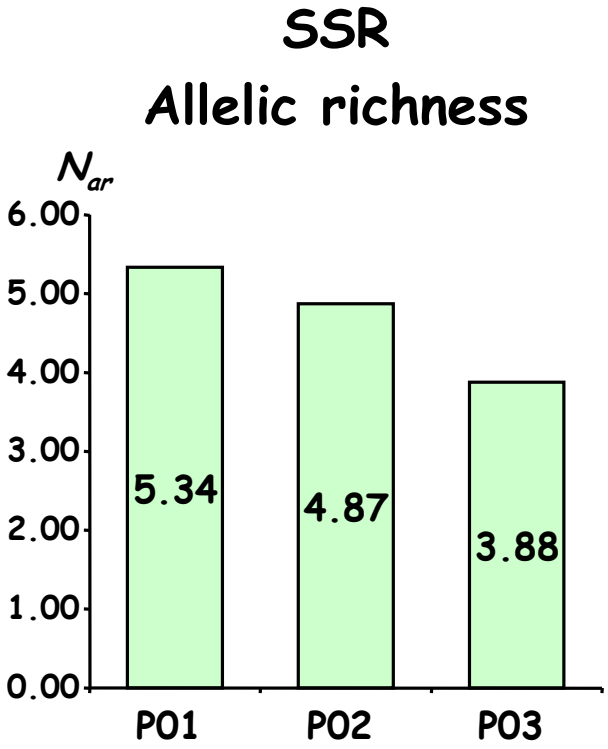
- *Salvia brachyodon*
- three known localities:
  1. Sv. Ilija, Pelješac Croatia
  2. Velji Do, Konavle, Croatia (2013)
  2. Mt. Orjen, BiH/Montenegro
- propagation:
  generative
  vegetative
- samples: 25 per pop.

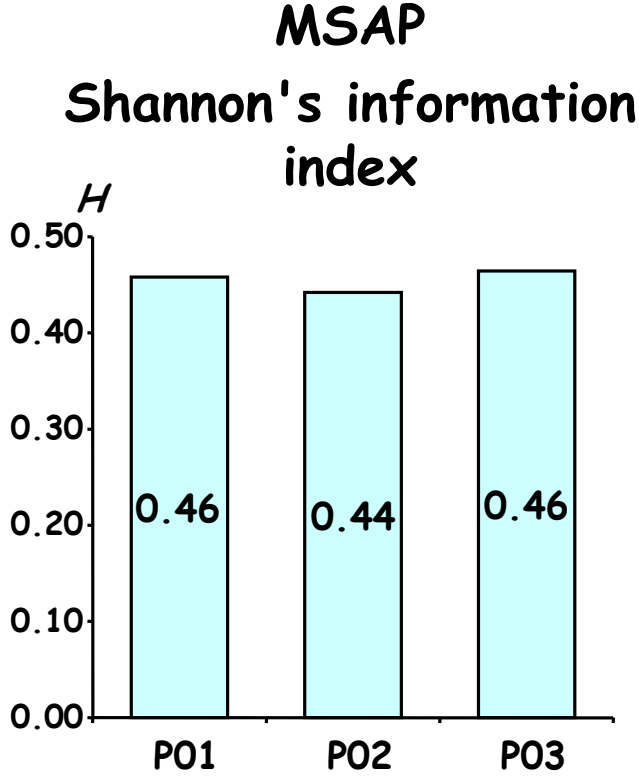
Genetic distance



Genetic vs. epigenetic diversity

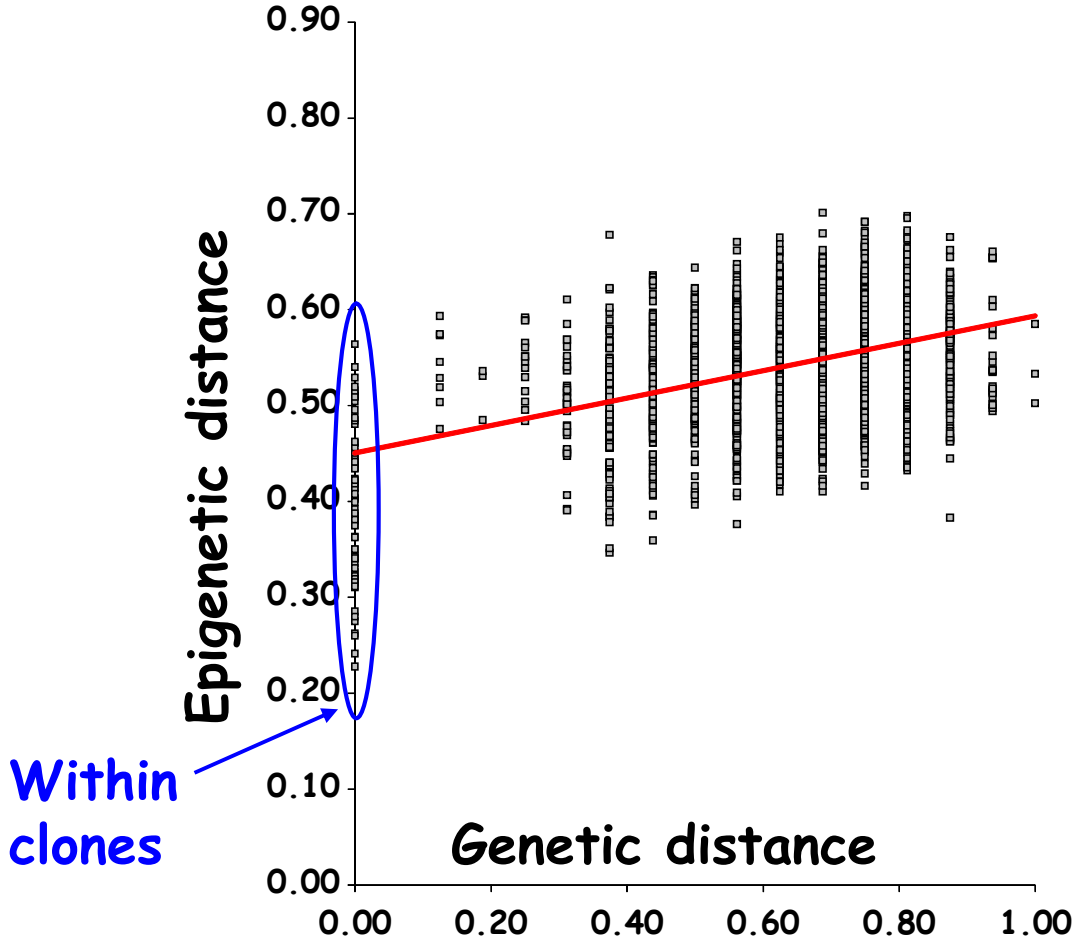






- considerable differences in clonal and genetic diversity
- small differences in epigenetic diversity

Genetic vs. epigenetic distance



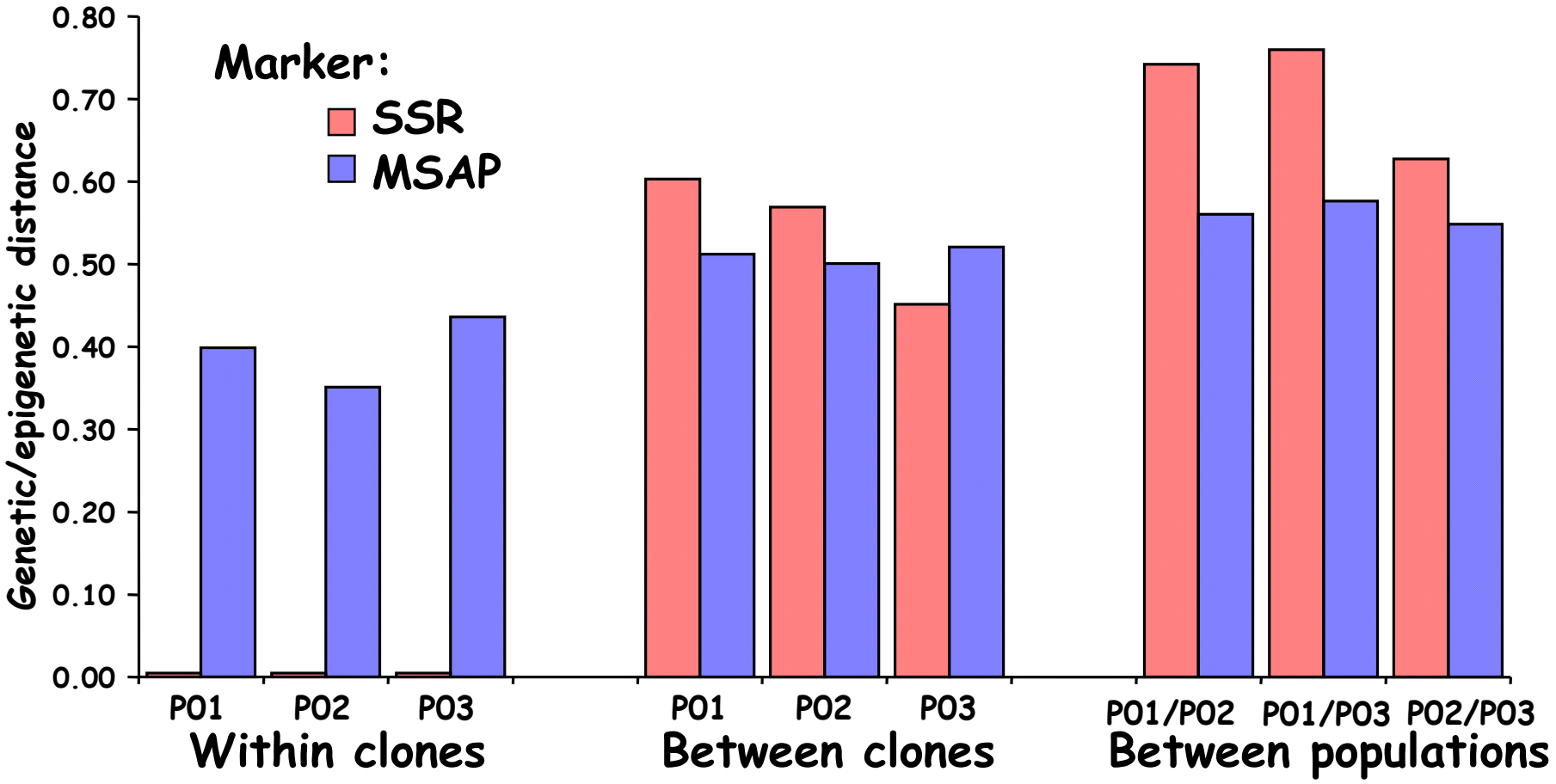
SSR D_{psa} / MSAP D_{Dice}

r = 0.42

R^2 = 0.18

- moderate correlation between genetic and epigenetic distances

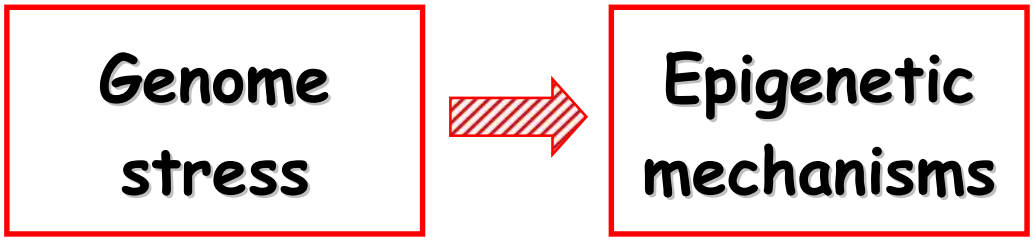
Genetic vs. epigenetic distance



- considerable epigenetic distances between genetically identical plants

Question 4

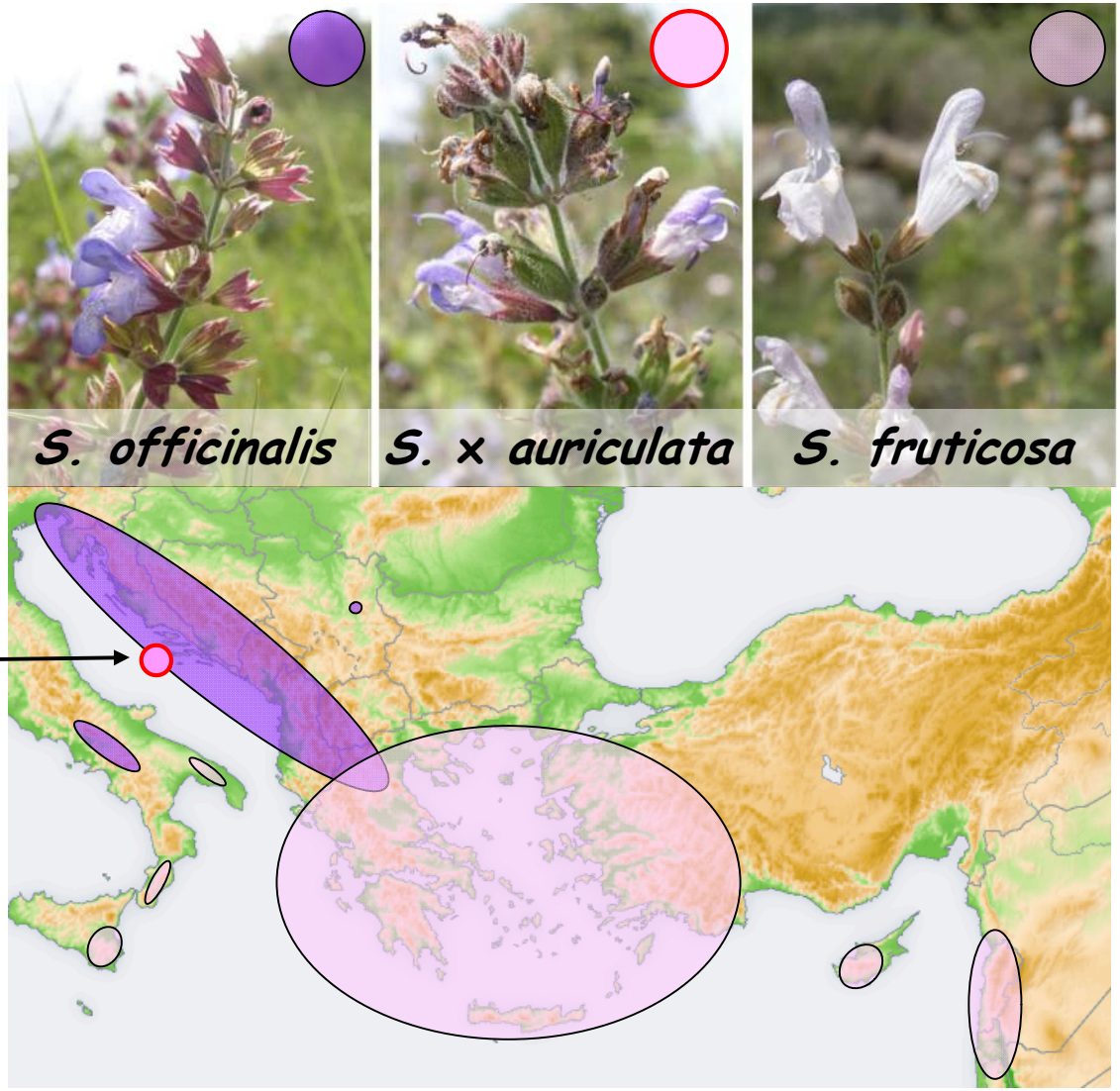
(4) Do natural hybrids show higher epigenetic variability than their parental species?



(3) Auriculate sage

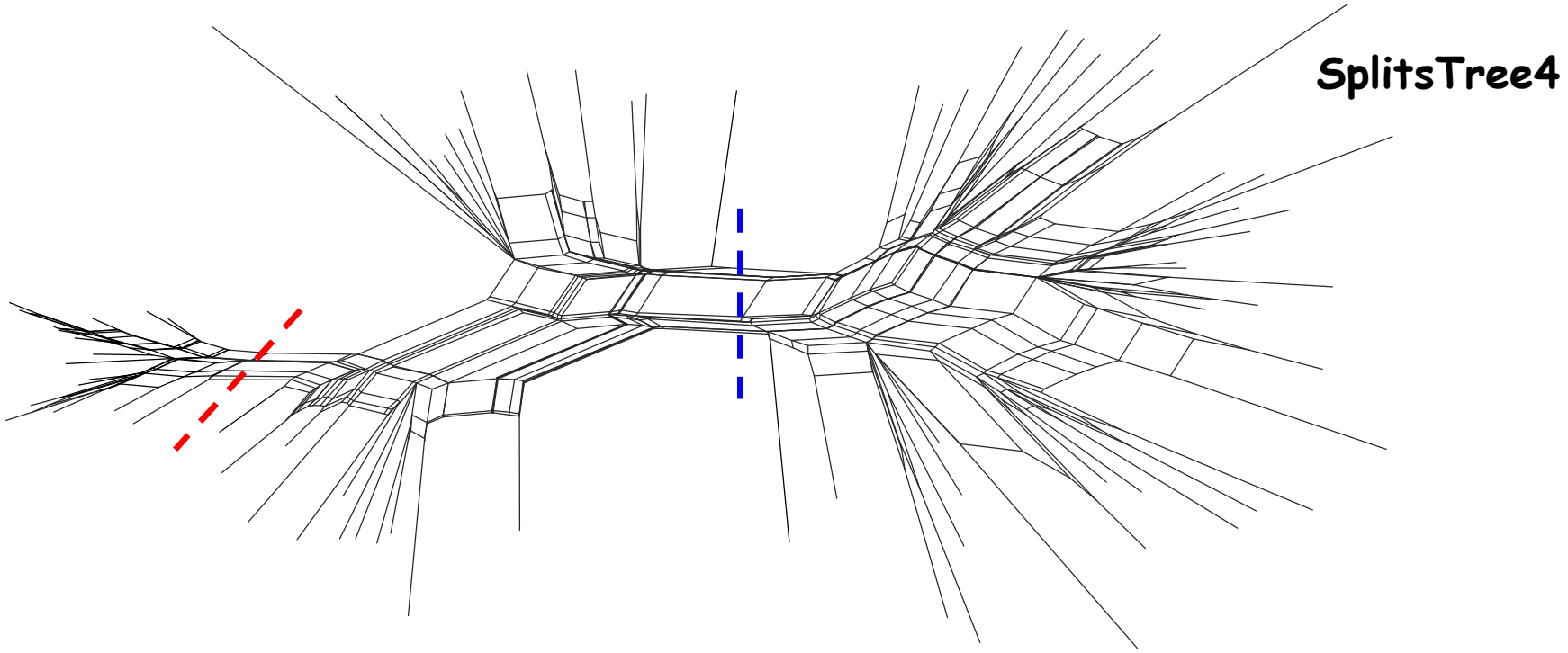
- *S. x auriculata*
- hybrid species:
  Dalmatian (So)
  x Greek sage (Sf)
- known from
  artificial
  crossings
- natural hybrids:
  island of Vis

- samples:
  16 So
  25 Sxa
  38 Sf



Morphological diversity

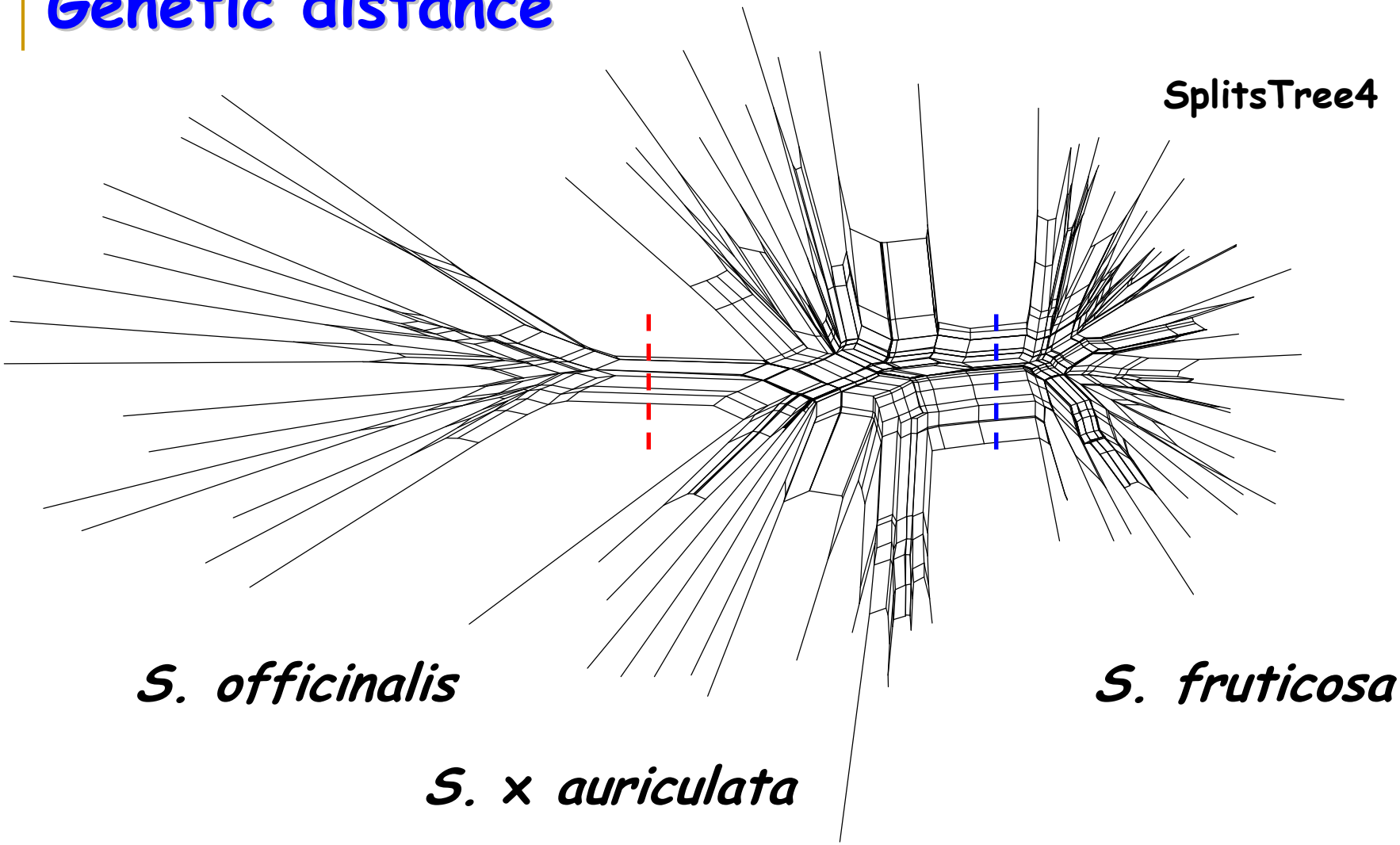
- based on 22 qualitative and 19 quantitative traits



S. officinalis *S. x auriculata* *S. fruticosa*

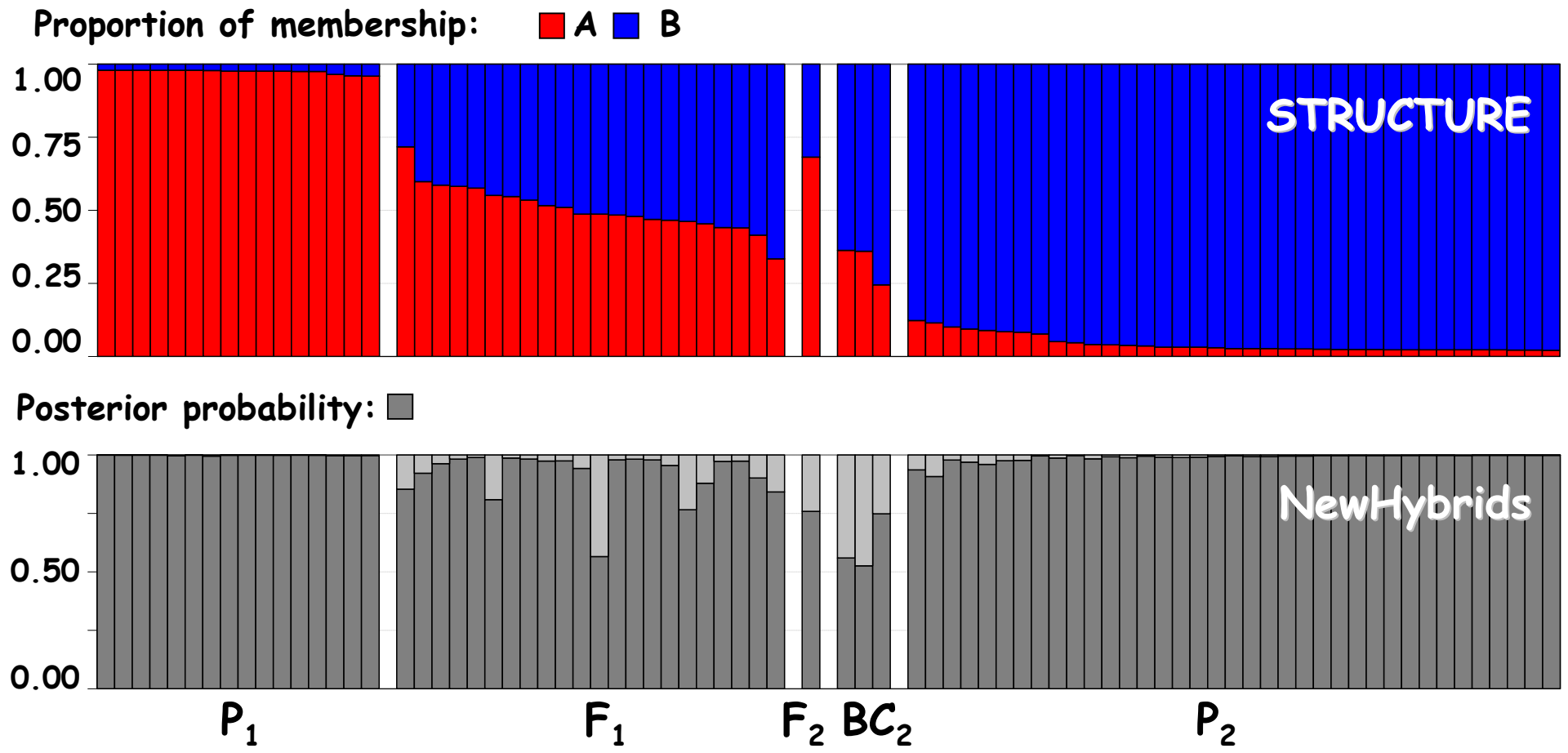
- high morphological diversity of Sf

Genetic distance



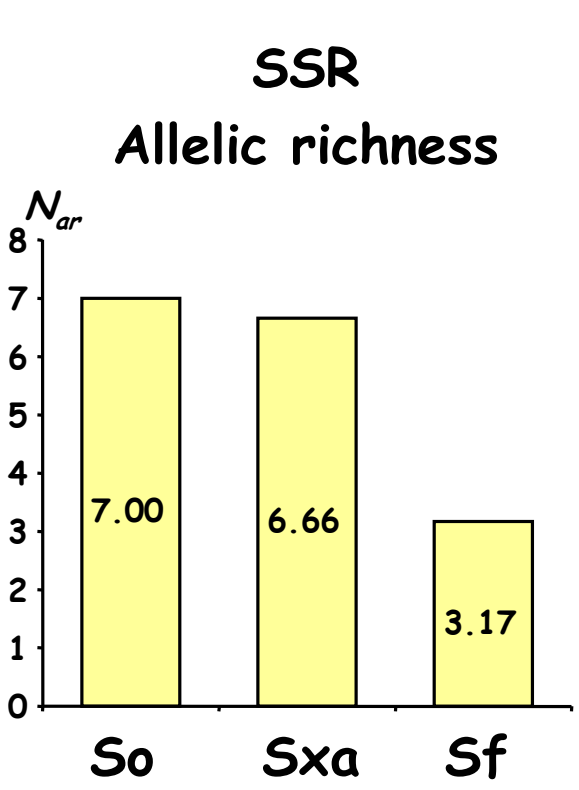
- low genetic diversity of Sf

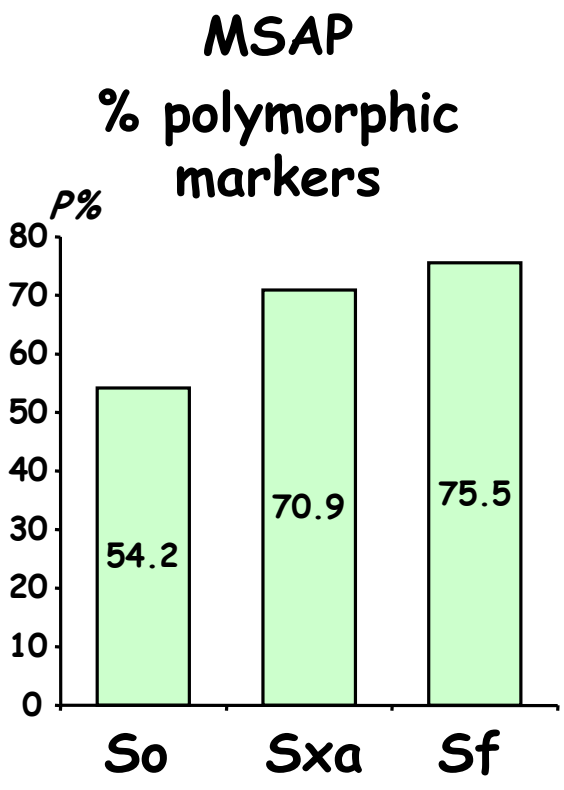
Genetic structure

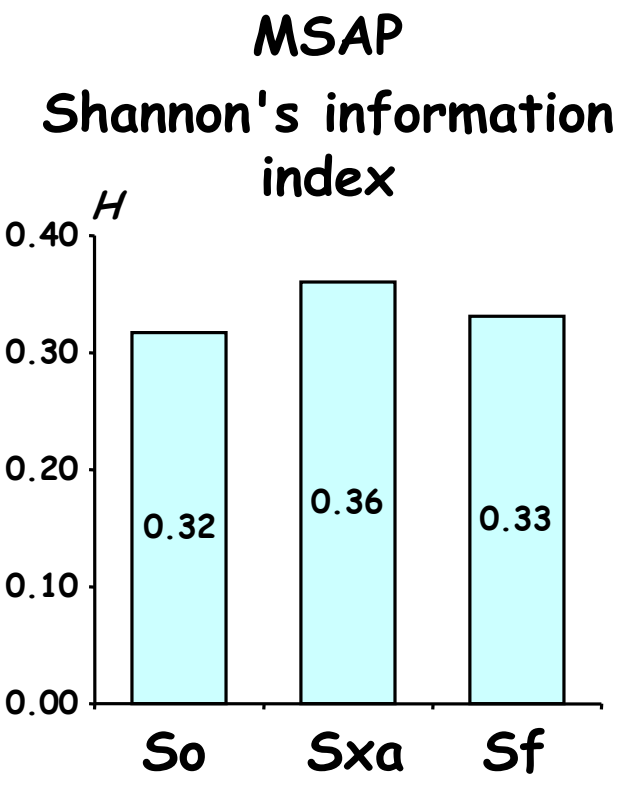


- most of the hybrids belong to F_1 generation

Genetic vs. epigenetic diversity







- the highest % polymorphic markers in Sf
- higher level of epigenetic diversity of hybrids in comparison to parental species

It seems that...

(1) So: Partitions of genetic and epigenetic diversity within and among populations follow the same pattern, but there the correlation between genetic and epigenetic diversity is weak

(2) So: Epigenetic variation is related to environmental factors that are crucial for survival of populations

(3) Sb: Relatively high epigenetic distances among genetically identical individuals may contribute to evolutionary persistence of populations in clonal species

(4) Sxa: Low genetic diversity but pronounced phenotypic plasticity of greek sage could be explained by epigenetic mechanisms

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The project is financed by Croatian Science Foundation

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Please, visit our project's web-site at: hirc.botanic.hr/EpiSalvia/en/

Komiža, island of Vis