Biochemical diversity and genetic structure of Dalmatian sage (*Salvia officinalis* L.)

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PANNONIAN PLANT BIOTECHNOLOGY ASSOCIATION

Salvia officinalis L. – Dalmatian sage

- Family: *Lamiaceae*
- Subfamily: *Nepetoideae*
- Tribus: *Mentheae*
- Genus: *Salvia*
- Section: *Salvia*
  (30-40 species – Mediterranean,
  Irano-Turanian region

- natural range of distribution – along the eastern Adriatic coast with inland populations in central parts of Balkan peninsula, central and southern Apennines
- numerous naturalized populations

Importance:

- highly valued essential oil with more than 100 compounds identified
- has the highest essential oil yield among Salvia species
- in the pharmacopoeias of many countries throughout the world
- well known medicinal plant since earliest times (ancinet Egypt, Greece, Roman empire, throughout Middle ages…)

Uses:

- antimicrobal, fungicidal and antiviral activities
- anti-inflamantory
- spasmolytic
- antidiabetic
- skin care
- as insect repellent
- as flavouring and antioxidant (food preservative) agent
- etc.

The toxicity of *S. officinalis* essential oil

- caused by ketone terpenoids – thujone and camphor
- the experimental study in rats: the limit toxic dose of sage essential oil was 300 mg/kg (letal at1.25 mg/kg)
- average man (70 kg) → 21 g of essential oil
- essential oil yield: < 5 g/kg of fresh material

CAUTION: Do not eat more than 4 kg of fresh *S. officinalis* at once and you will be just fine ☺

The composition of the essential oil:

- cis-thujone → 18.0-43.0%
- camphor → 4.5-24.5%
- 1,8 cineole → 5.5-13.0%
- trans-thujone → 3.0-8.5%
- alpha-humulene → >12.0%
- alpha-pinene → 1.0-6.5%
- camphene → 1.5-7.0%
- limonene → 0.5-3.0%
- bornyl acetate → >2.5%
- linalool + linalyl acetate → >1%

(according to ISO 9909)

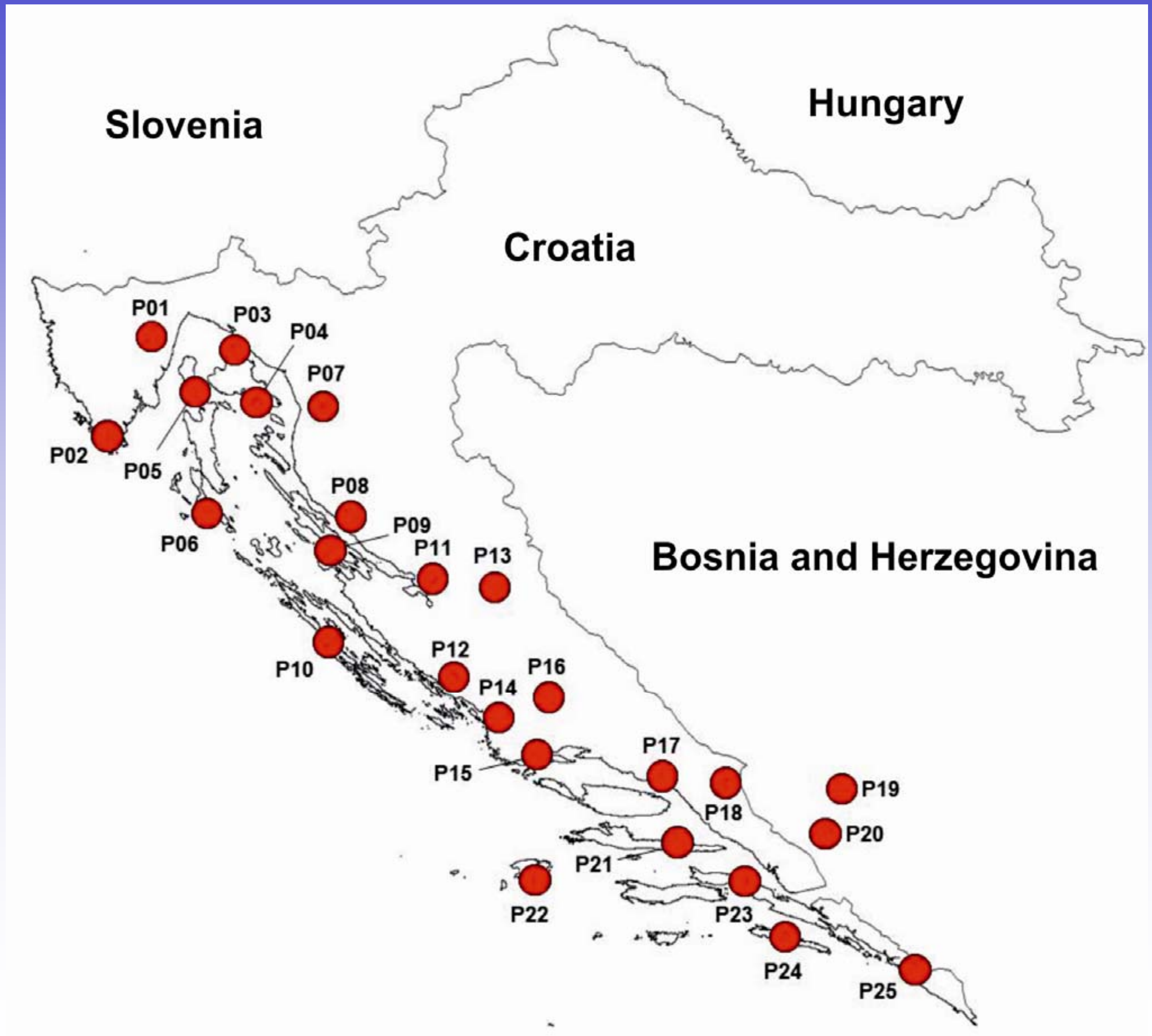
The quantity and composition of *S. officinalis* essential oil is mostly affected by:

- genotype
- environmental factors
- physiological stage
- ratio of leaves/flowers/stems used for distillation
- drying

Aim of research:

- to assess the levels of chemical and genetic diversity of indigenous populations of *S. officinalis* as a background for possible breeding/cultivation program

25 populations, ~ 600 samples
25 samples/ population



Ex situ cultivation! – to discard the possible environmental factors

Essential oil isolation and analysis

Extraction: from dried plant material by hydrodistillation (according to 5th European Pharmacopoeia)

Analysis: Gas Chromatography (GC/FID)

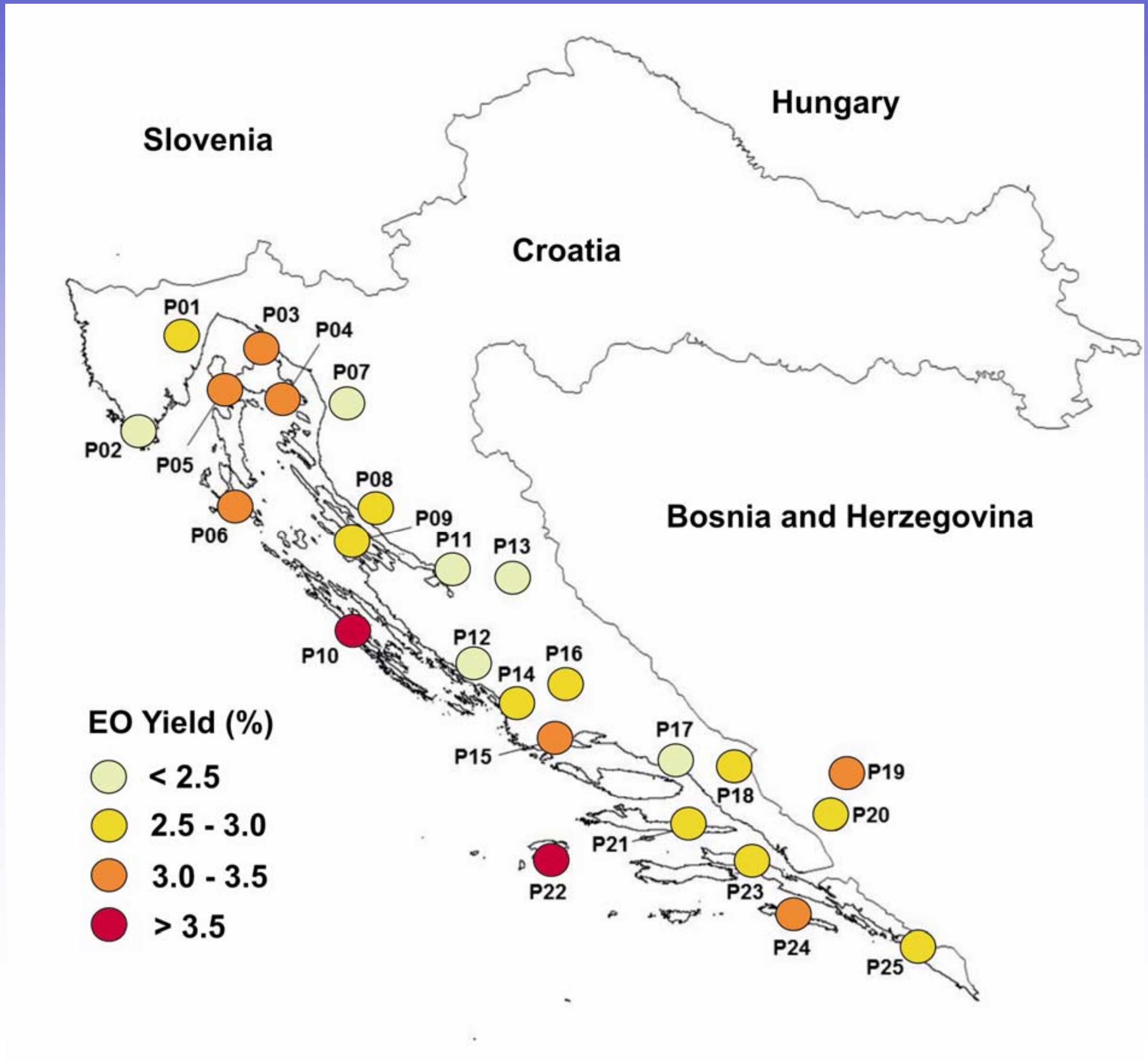
Gas Chromatography-Mass Spectrometry

Microsatellite markers for genetic analisys, eight loci:

SoUZ001	SoUZ011
SoUZ002	SoUZ013
SoUZ003	SoUZ014
SoUZ007	SoUZ019

The essential-oil composition and chemical diversity of indigenous populations of *S. officinalis*

- the essential-oil yield from dried leafs → 1,9% – 3,7% (avarage of 2,8%)



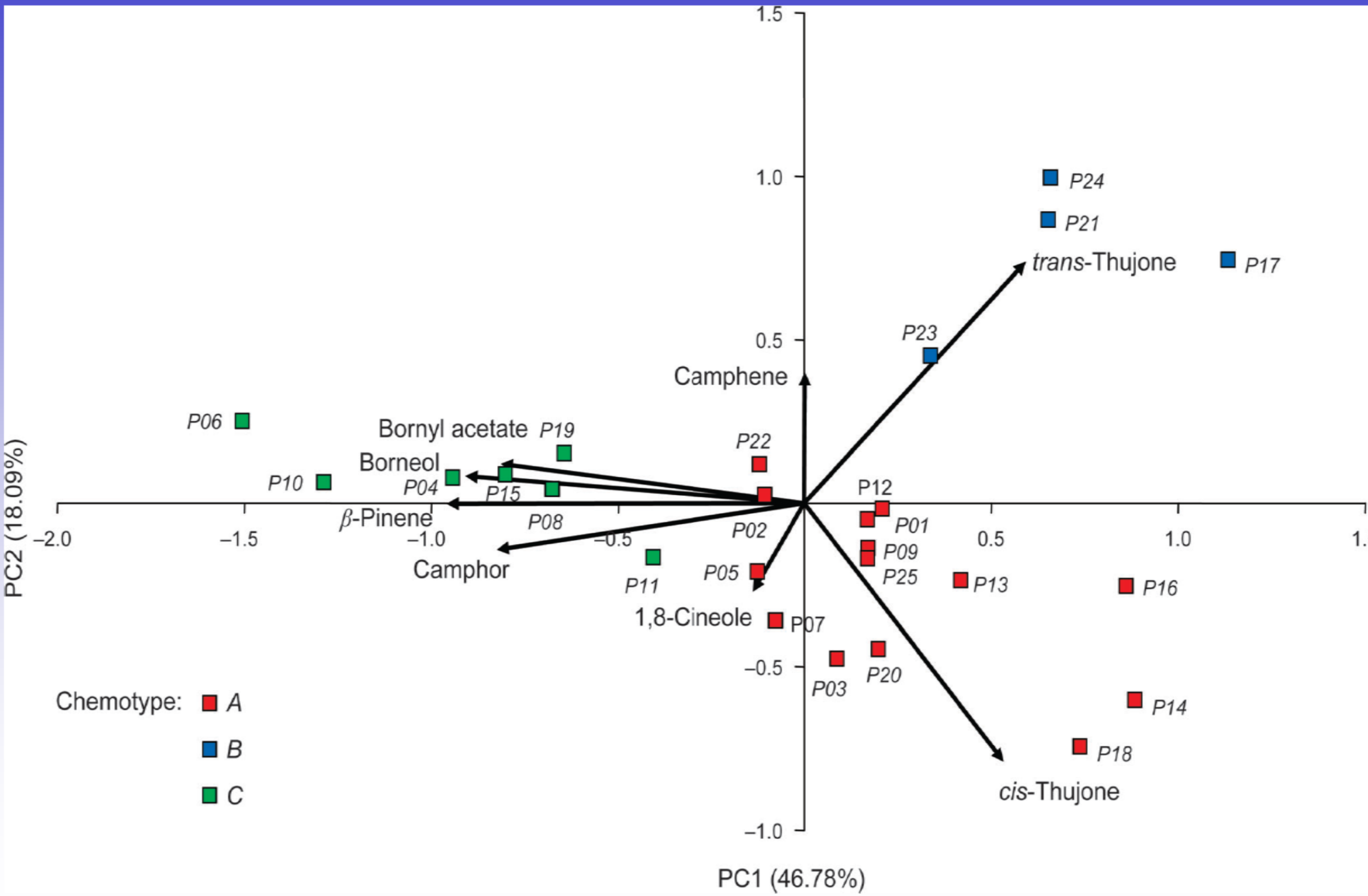
- 62 detected compounds

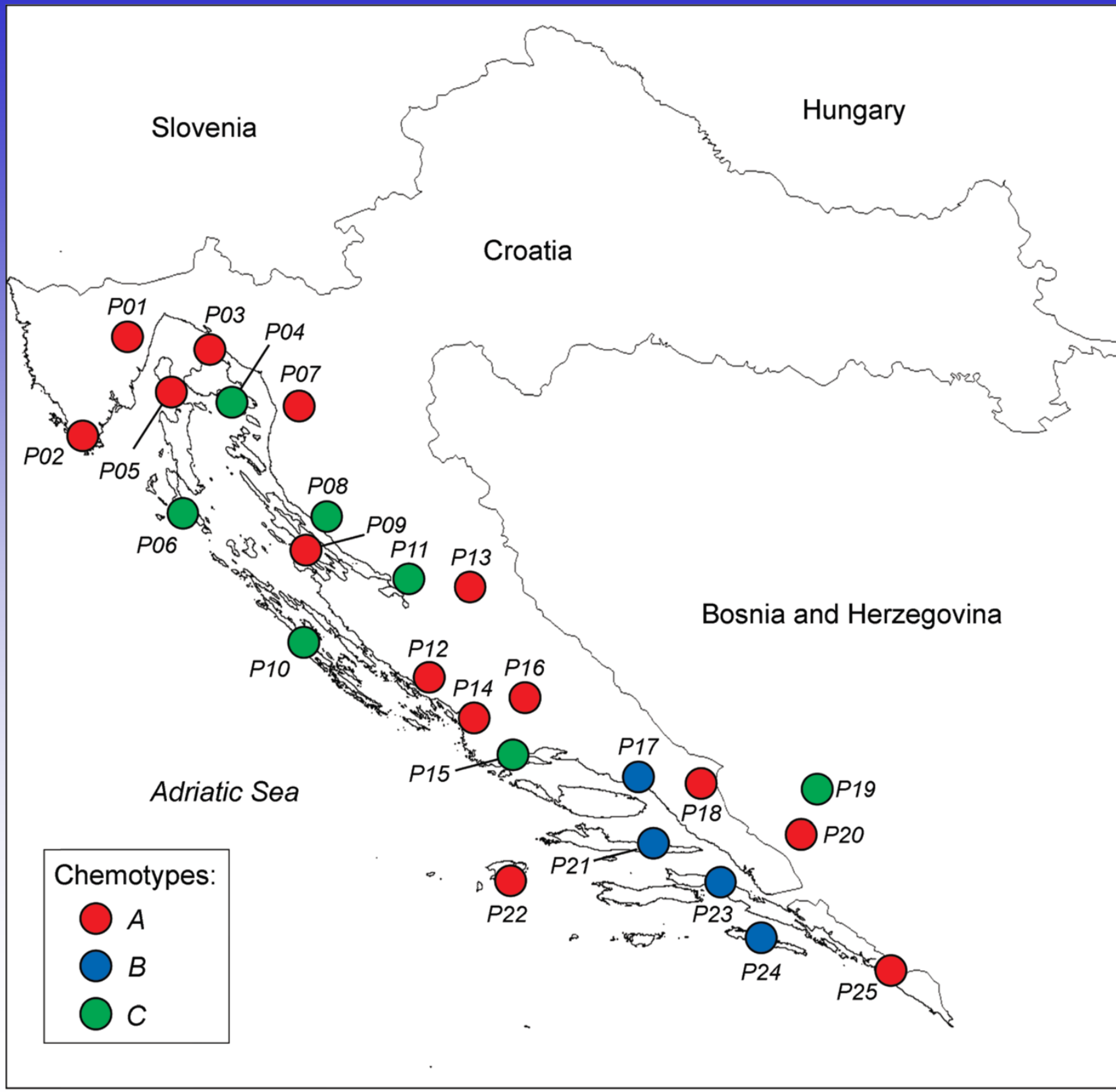
- most abundant compounds: cis-thujone, camphor and trans-thujone

- compounds detected in concentrations higher than 5% in any population were chosen for further analysis (cis-thujone, camphor, trans-thujone, 1,8-cineole, b-pinene, camphene, borneol, and bornyl acetate)

- by using multivariate analyses on the basis of eight major compounds, three chemotypes were distinguished: (A) cis-thujone, (B) trans-thujone and (C) camphor chemotype

Biplot of the PCA Based on the eight main essential-oil compounds

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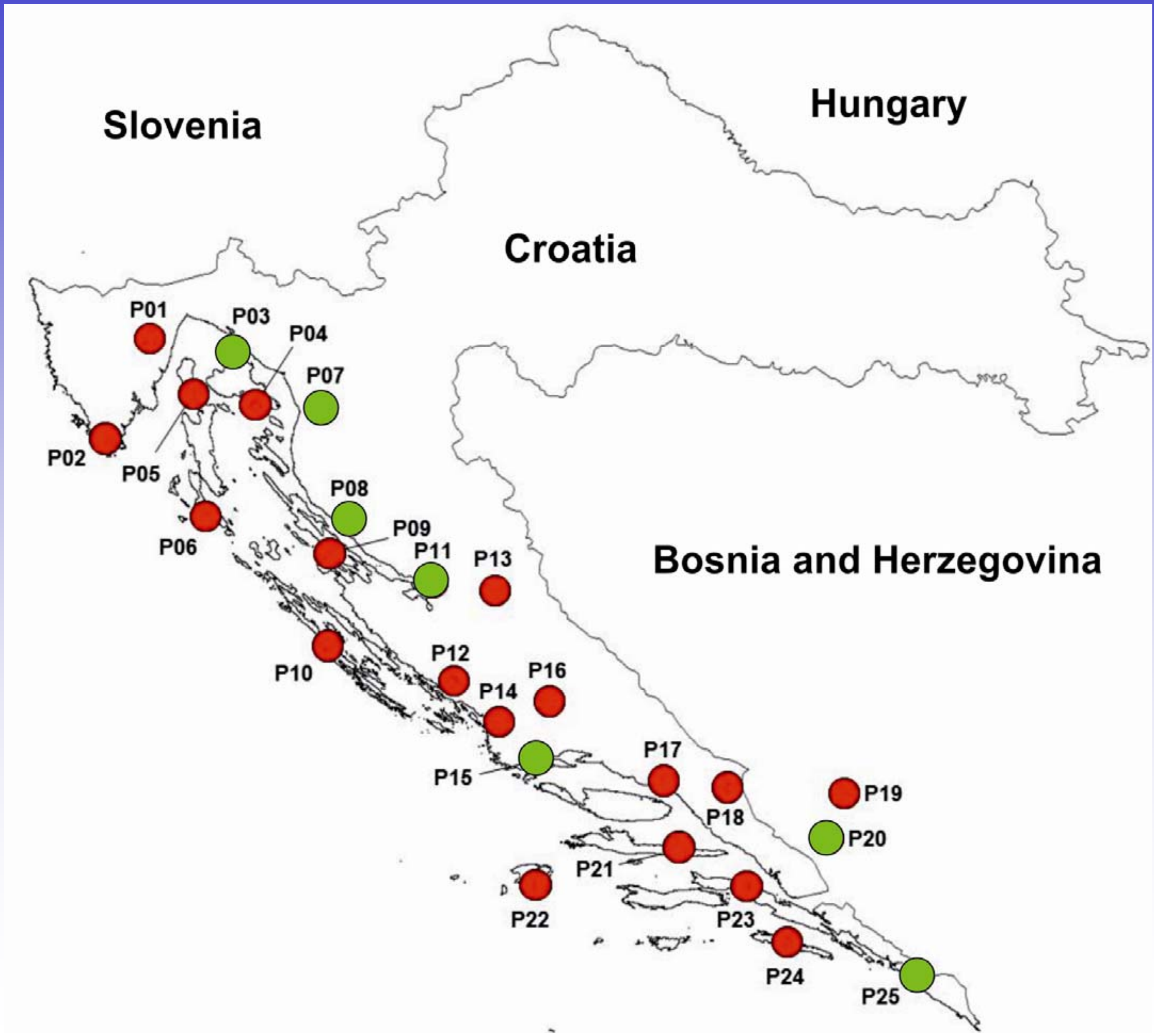
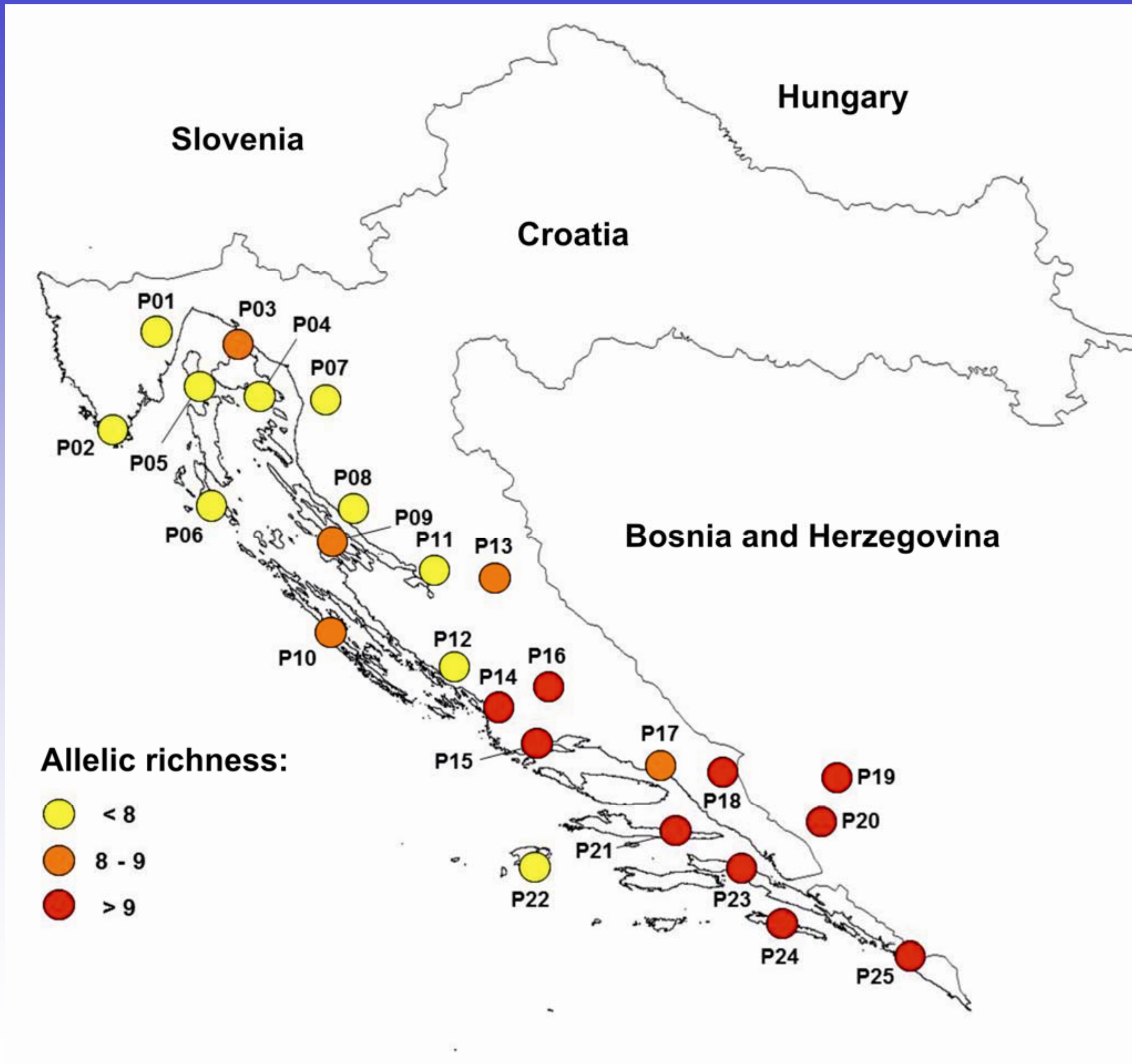
Chemotypes:
A
B
C

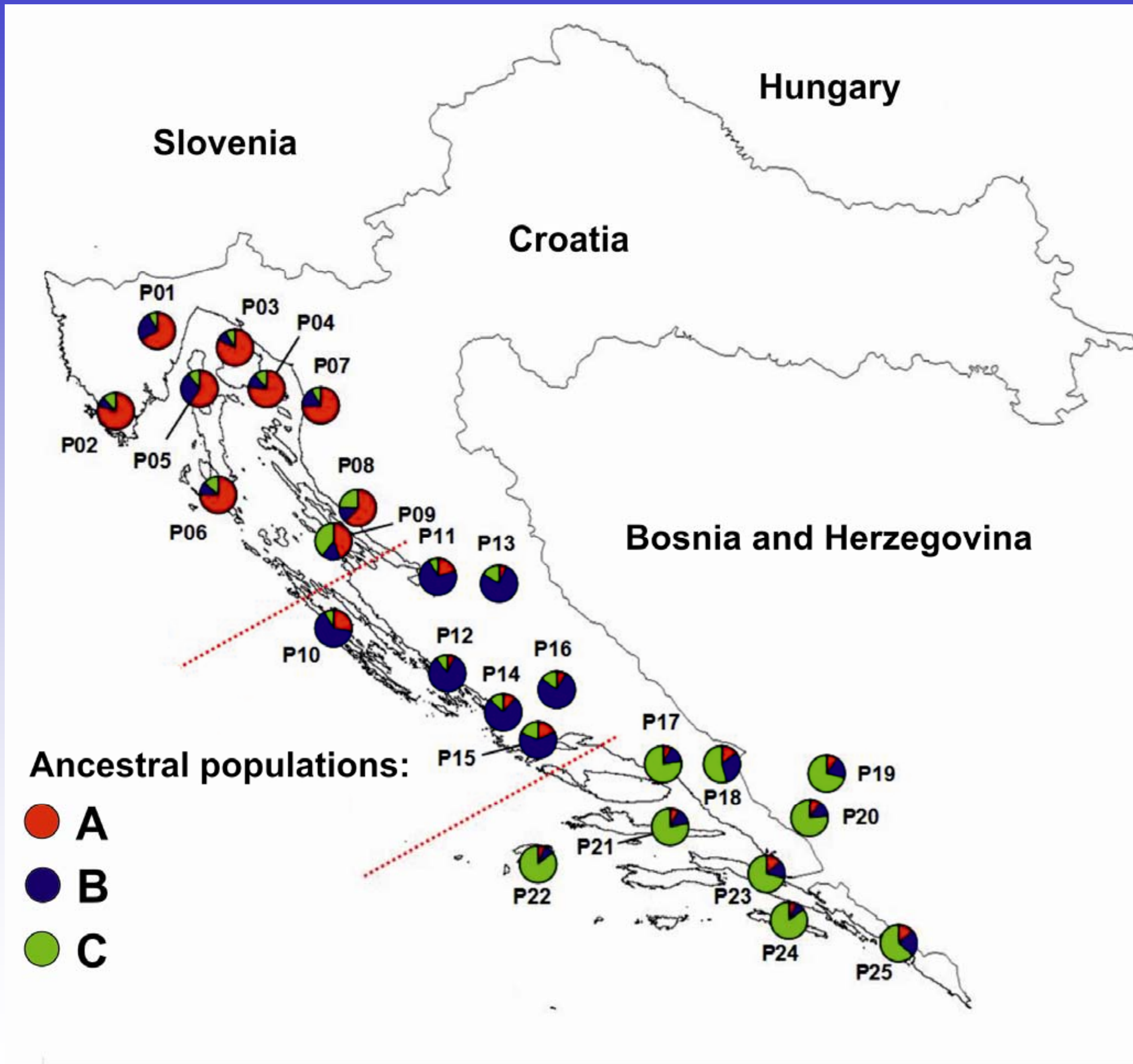
According to ISO 9909

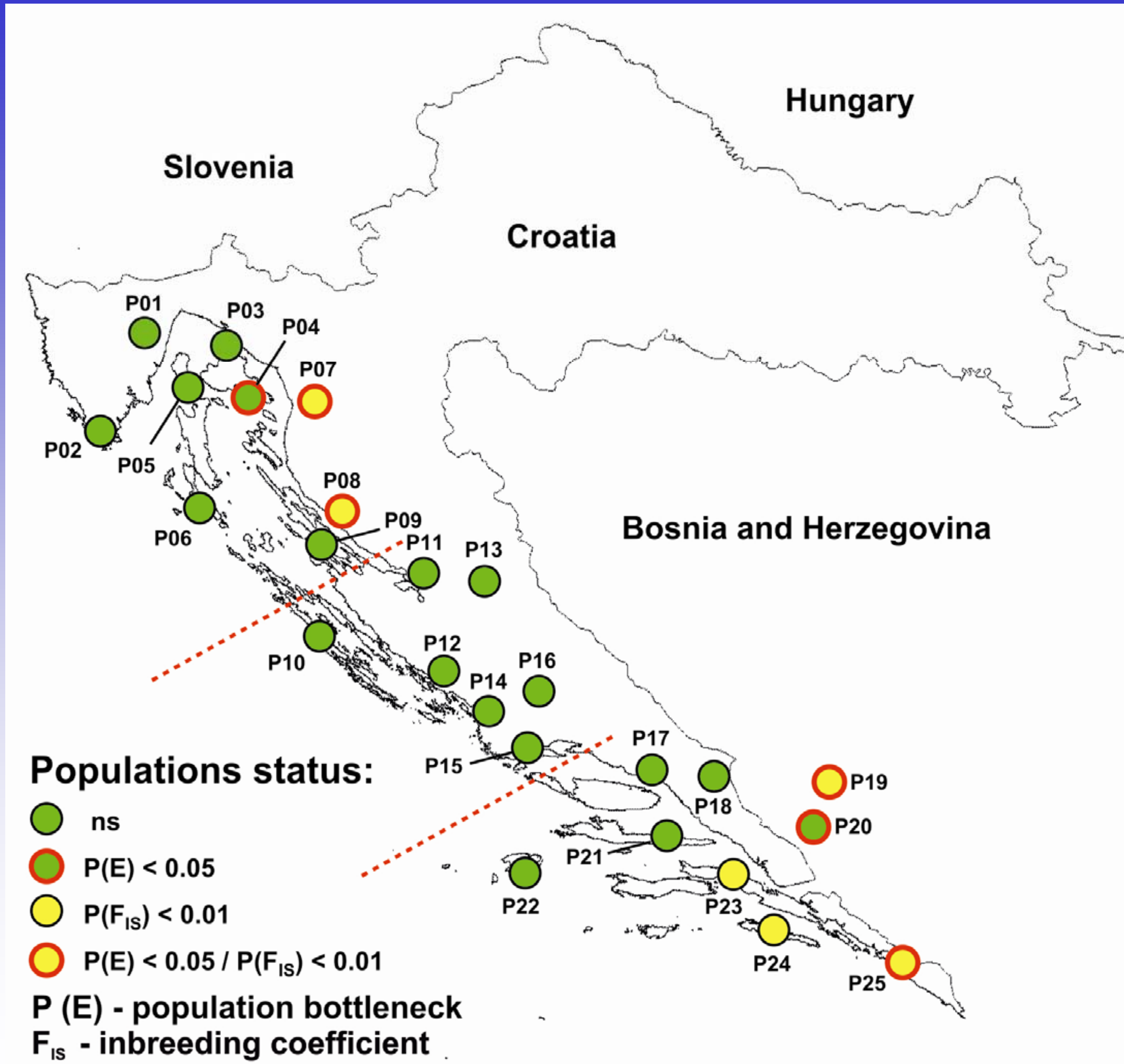


The genetic diversity of indigenous populations of *S. officinalis*



Ancestral populations as revealed by computer program STRUCUTRE

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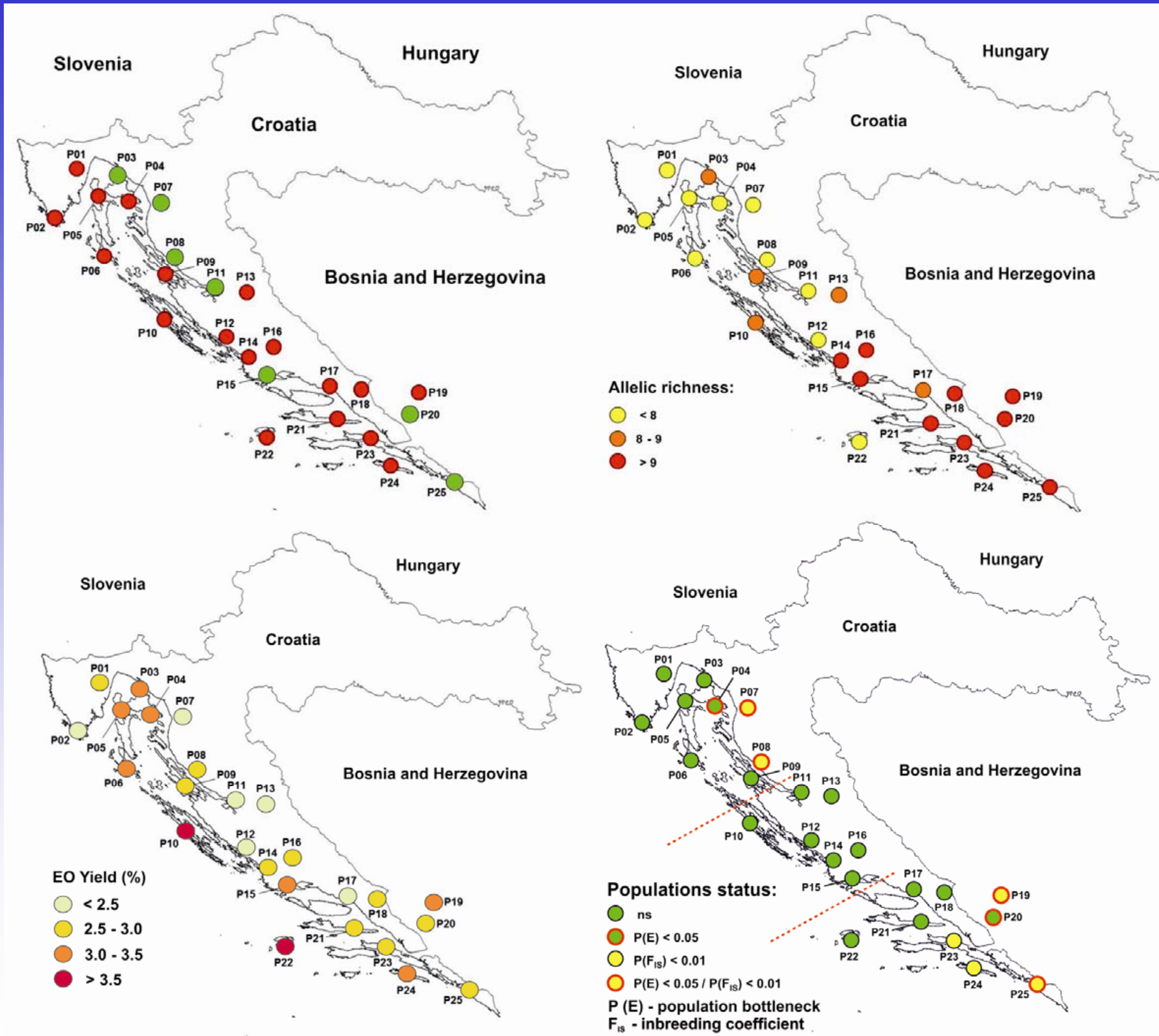
Populations status:
ns
P(E) < 0.05
P(F_{IS}) < 0.01
P(E) < 0.05 / P(F_{IS}) < 0.01
P (E) - population bottleneck
F_{IS} - inbreeding coefficient



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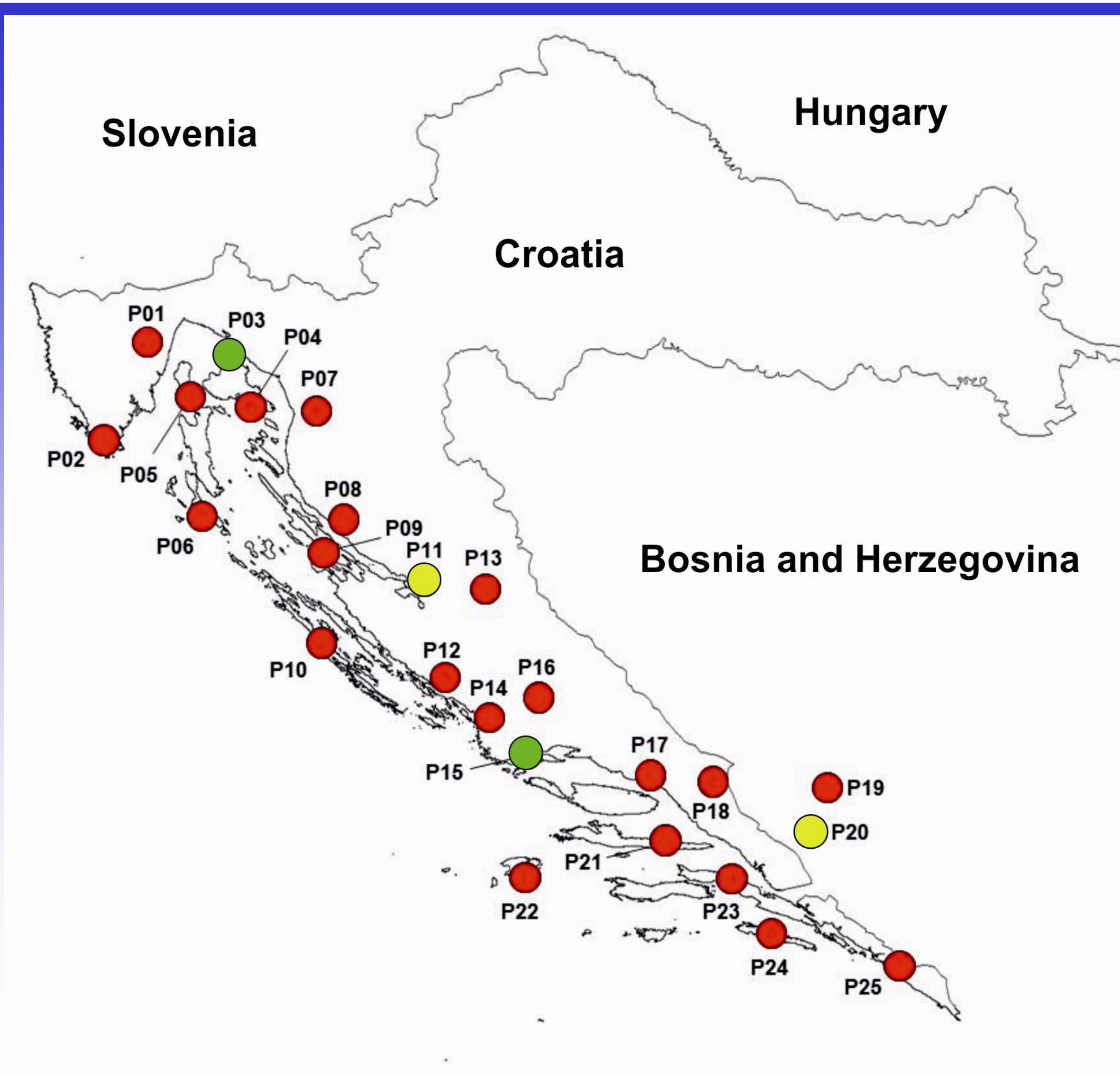
Allelic richness:
< 8
8 - 9
> 9
EO Yield (%)
< 2.5
2.5 - 3.0
3.0 - 3.5
> 3.5
Populations status:
ns
P(E) < 0.05
P(F$_{IS}$) < 0.01
P(E) < 0.05 / P(F$_{IS}$) < 0.01
P (E) - population bottleneck
F$_{IS}$ - inbreeding coefficient



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Thank you for your time ☺