Epigenetic vs. genetic diversity of stenoendemic short-toothed sage (*Salvia brachyodon* Vandas)

Biruš, I., Liber, Z., Radosavljević, I., Bogdanović, S., Jug-Dujaković, M., Zoldoš, V., Šatović, Z.

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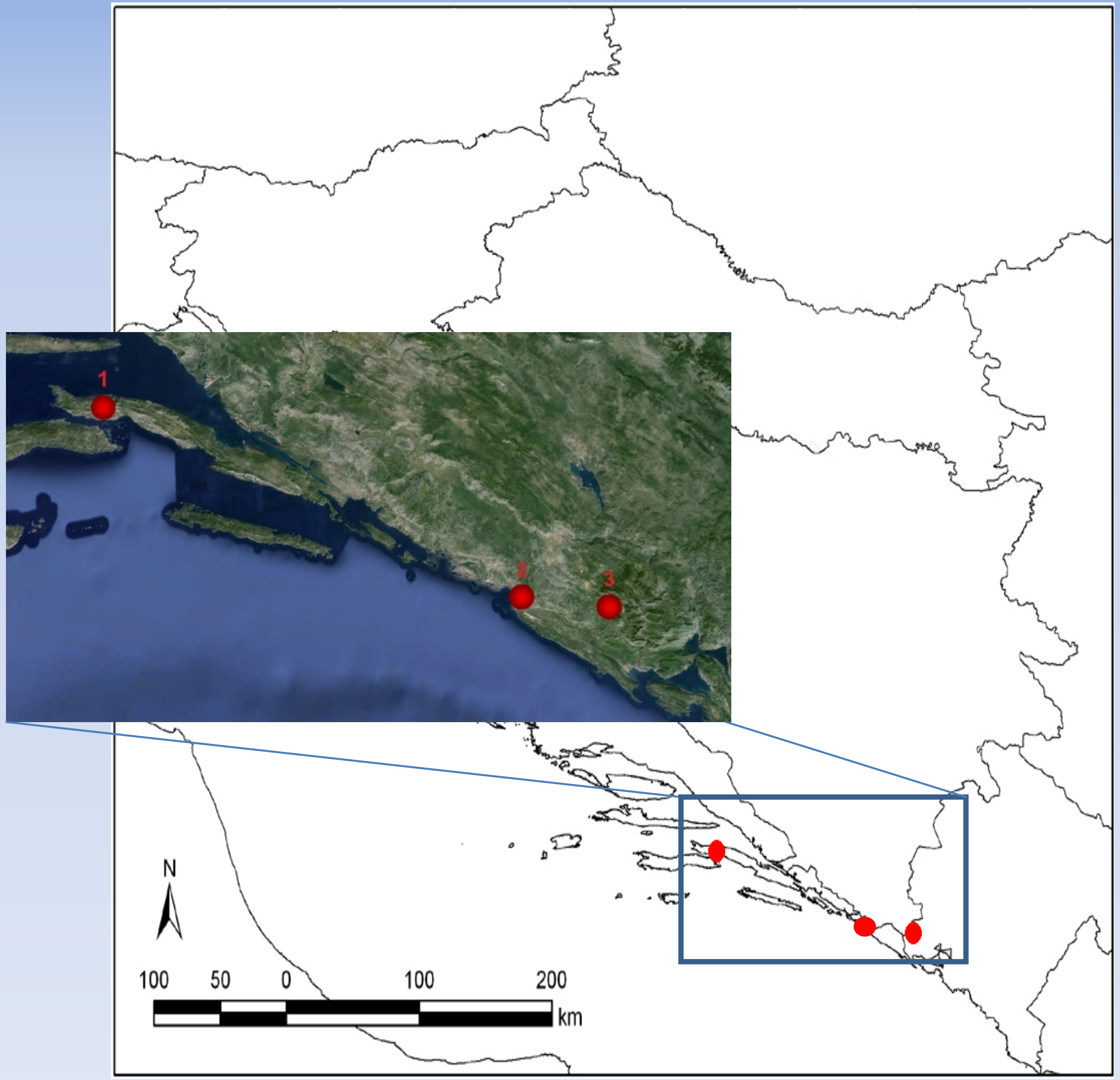
Short-toothed sage (*S. brachyodon* Vandas)

- Illyrian-Adriatic stenoendemic species

- very narrow distribution range

- three known localities:
  1. Sv. Ilija, Pelješac
  2. Velji do, Konavle
  3. Vrbanje, Mt. Orjen, BH/Monte Negro border



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

- propagation germinatively and vegetatively - via stolons (field observation**)**

- in CRO near threatened (NT), in MN endangered species (EN)

narrow endemic species, clonality → limited genetic diversity?

Could epigenetic modifications be the molecular mechanism enabling evolutionary persistence and phenotypic plasticity?

Aims of the Study

- To study the relationship between epigenetic and genetic variation in narrow endemic and clonally propagated species

1. compare the levels of clonal, genetic and epigenetic diversity among populations
2. determine the relationship between epigenetic and genetic distance among individuals
3. determine the amount of epigenetic variation found among genetically identical organisms

Methods

- 3 populations/ 25 individuals per population

(1) Genetic markers

a) Microsatellites

- *Simple Sequence Repeats (SSRs)*

– 8 microsatellites, total of 63 markers

(2) Epigenetic markers

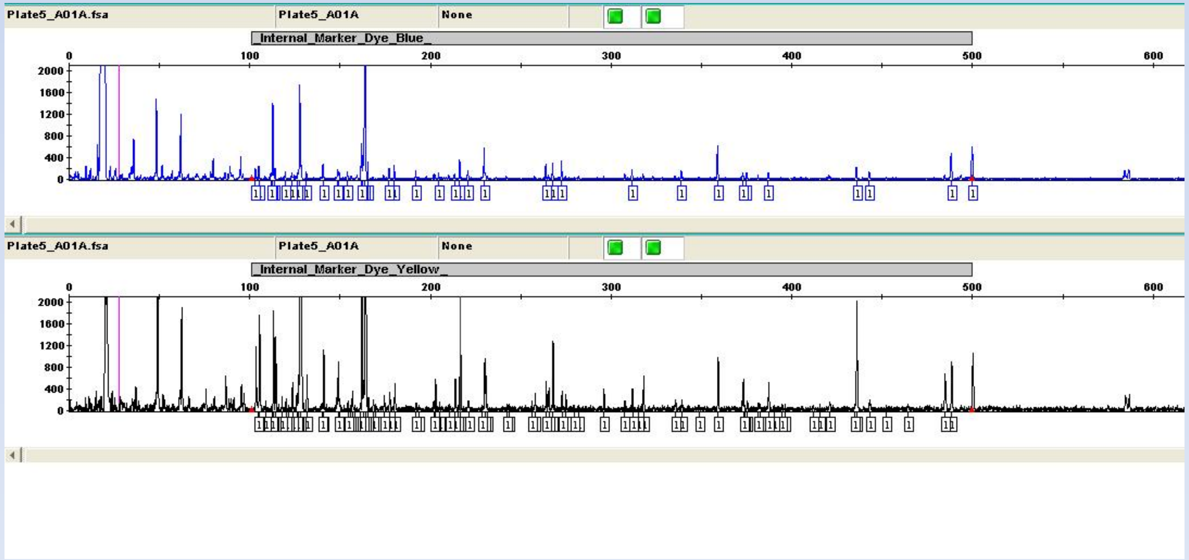
a) MSAP

- *Methylation-Sensitive Amplified Polymorphism (MSAP)*

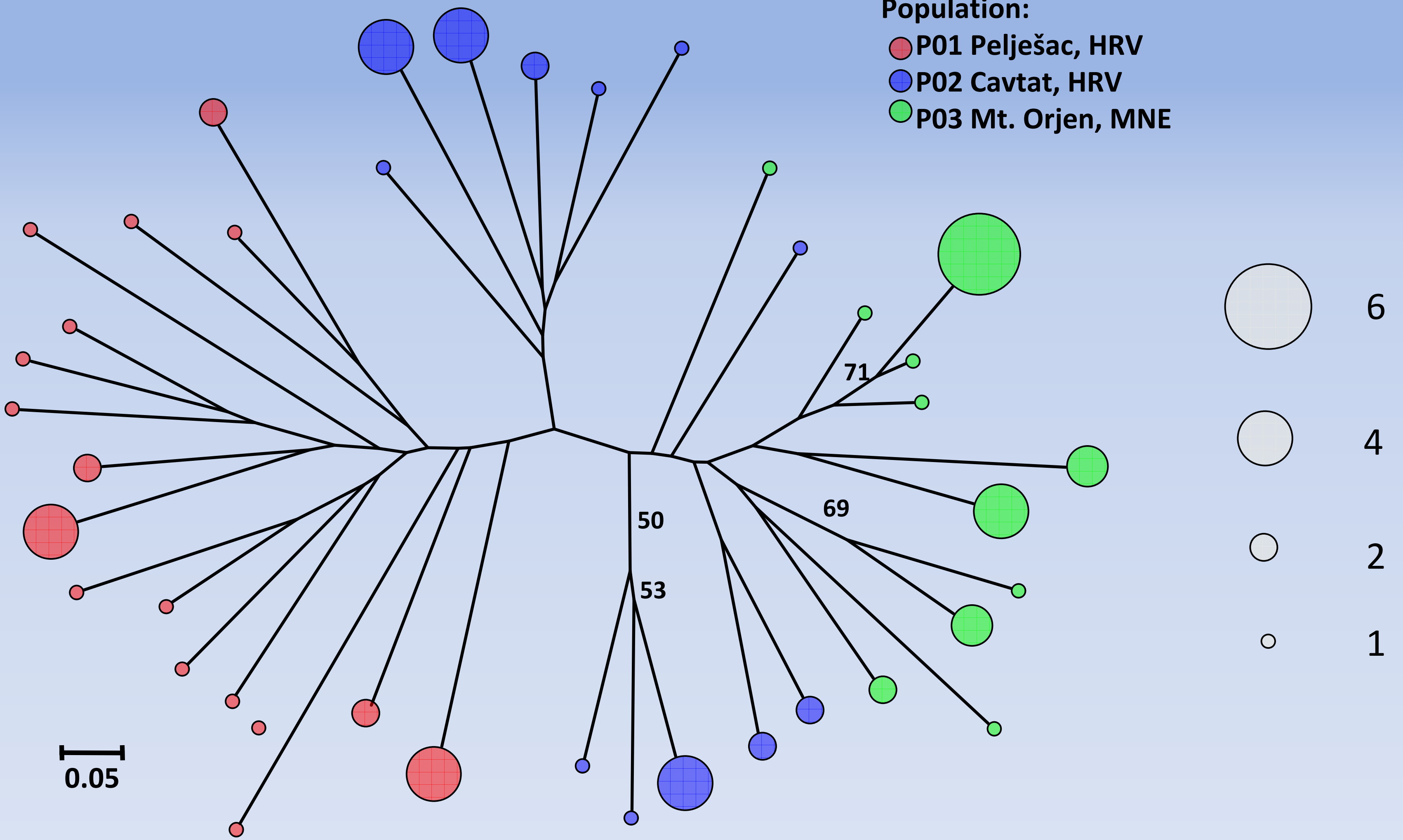
– 4 primer combinations, > 3000 loci

Methods - *Analysis of global genome methylation*

- MSAP (methylation sensitive amplified polymorphism) – modification of AFLP based on differential sensitivity of restriction enzymes (isoshizomeres) such as *Msp*I/*Hpa*II to methylation (5’-CCGG-3’)



Results: TREE BASED ON GENETIC DISTANCES



Unrooted Neighbour-Joining tree of short-toothed sage samples from 3 populations based on D_{PSAM} genetic distance. The size of the circles at the branch tips is proportional to size (no. of individual plant samples) of the clonal lineage.

Results: PROOF OF CLONALITY

Range of *P*-values across different multi-locus genotypes (MLGs)

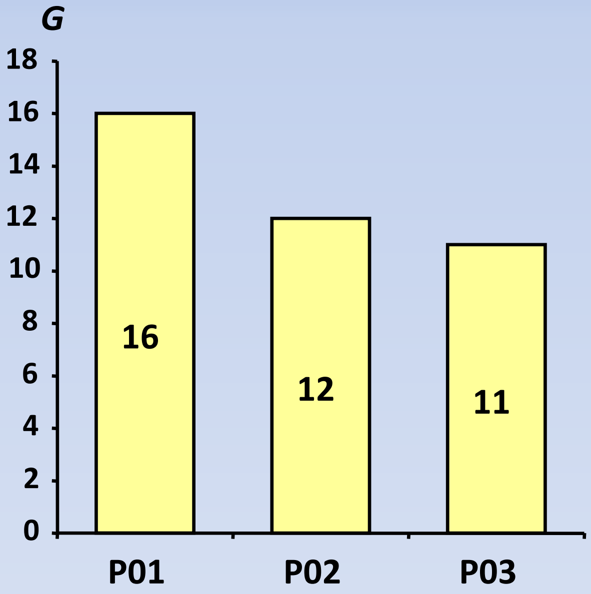
Parameter	P01	P02	P03
P_{gen}	1.24×10^{-11} - 5.55×10^{-7}	9.03×10^{-10} - 3.36×10^{-6}	8.55×10^{-9} - 9.31×10^{-4}
$P_{gen}(F_{is})$	3.80×10^{-11} - 7.99×10^{-7}	1.39×10^{-9} - 3.30×10^{-6}	4.71×10^{-9} - 7.49×10^{-4}
P_{sex} (1 reencounter)	3.66×10^{-8} - 1.39×10^{-5}	2.26×10^{-8} - 8.39×10^{-5}	3.38×10^{-7} - 2.30×10^{-2}
$P_{sex}(F_{is})$ (1 reencounter)	7.41×10^{-8} - 2.00×10^{-5}	3.47×10^{-8} - 8.24×10^{-5}	2.97×10^{-7} - 1.86×10^{-2}

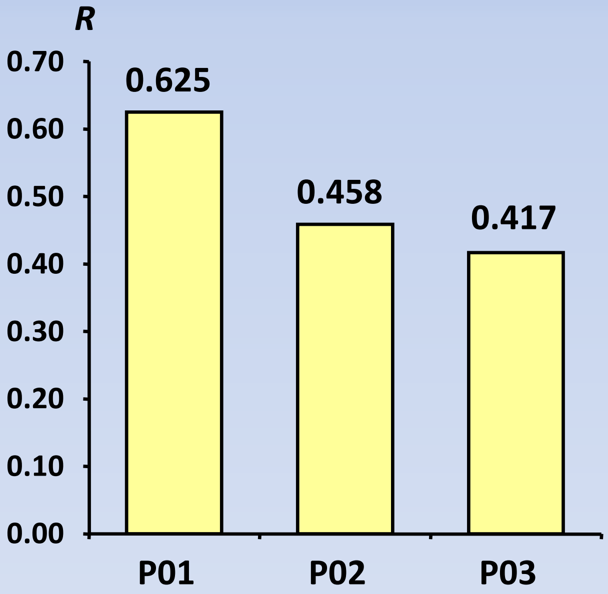
$P < 0.05$ in all cases

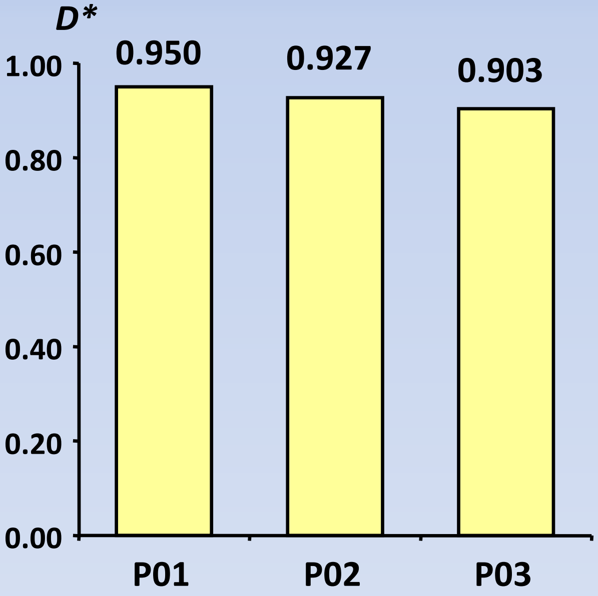
Our marker set has enough power to discriminate among clones: identical MLGs can be considered as pertaining to the same clone!

Results: CLONAL DIVERSITY

No of ramets = 25


Number of genets


Genotypic richness

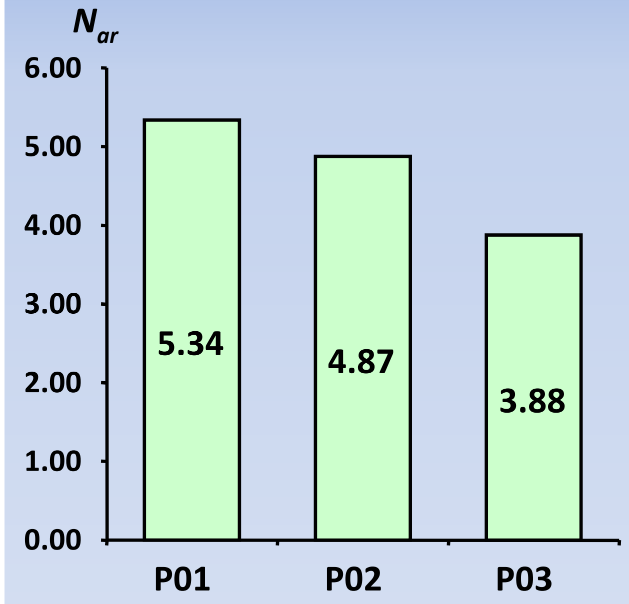

Simpson's complement index

High clonal diversity!

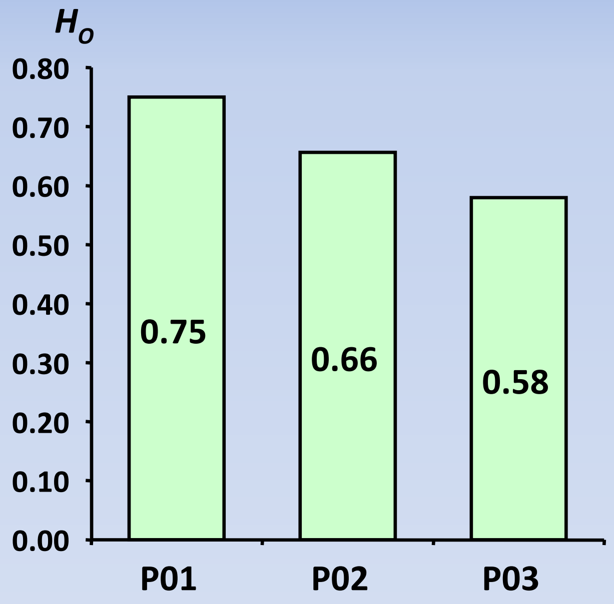
Results: GENETIC DIVERSITY

No. of genets = 16 (P01), 12 (P02), 11 (P03)

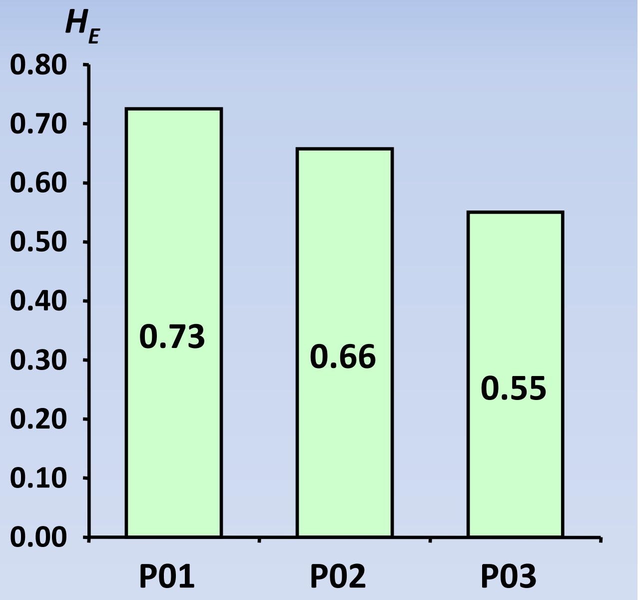
Allelic richness



Observed heterozygosity



Expected heterozygosity



P_{FIS}
P01: 0.32
P02: 0.59
P03: 0.22

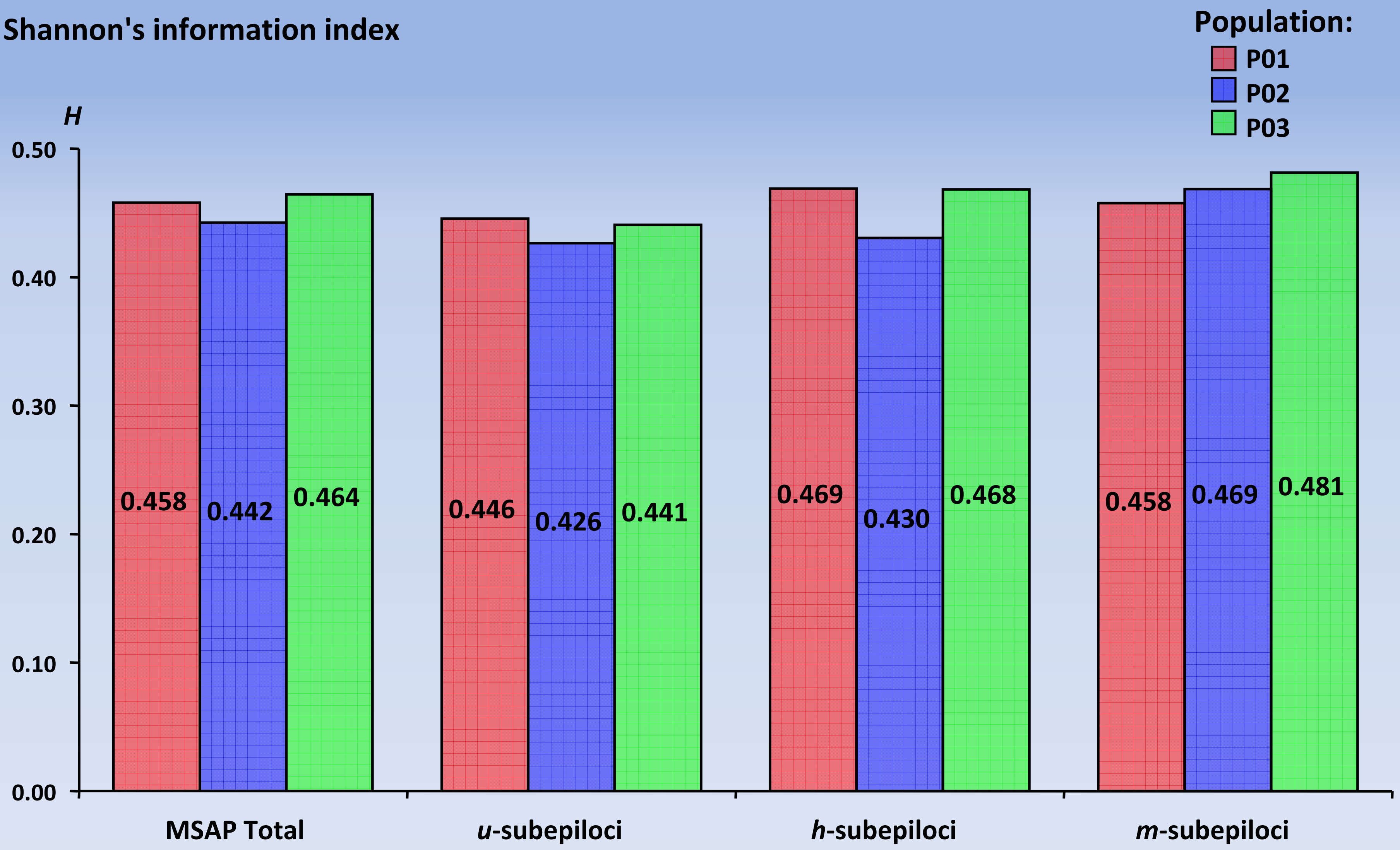
not significant

$P_{Bottleneck}$
P1: 0.19
P2: 0.47
P3: 0.77

not significant

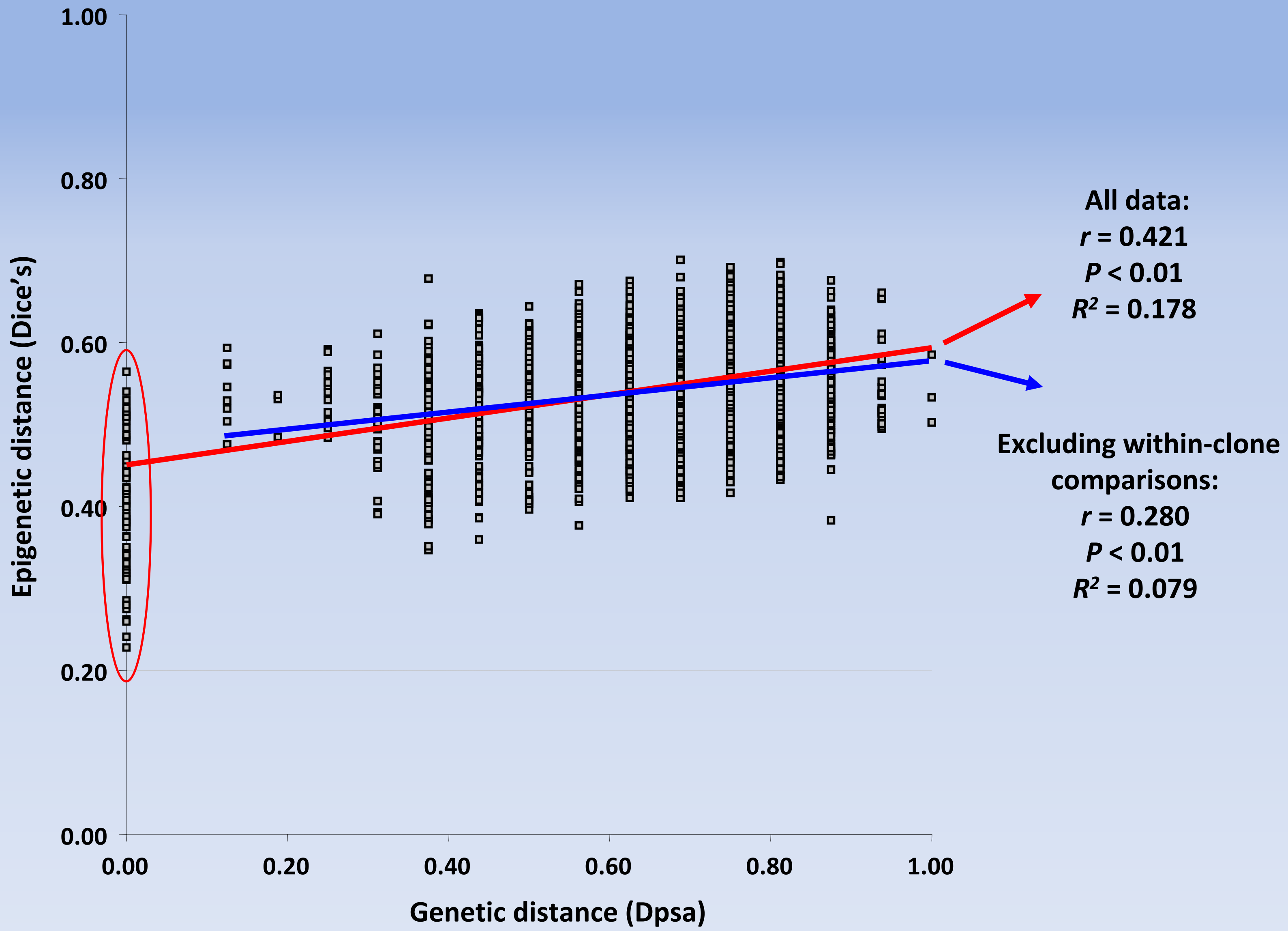
High genetic diversity!
All populations in Hardy-Weinberg Equilibrium!
No signs of recent bottleneck events!

Results: EPIGENETIC DIVERSITY



Similar levels of epigenetic diversity
across populations and across subepiloci!

Results: GENETIC VS. EPIGENETIC DISTANCES



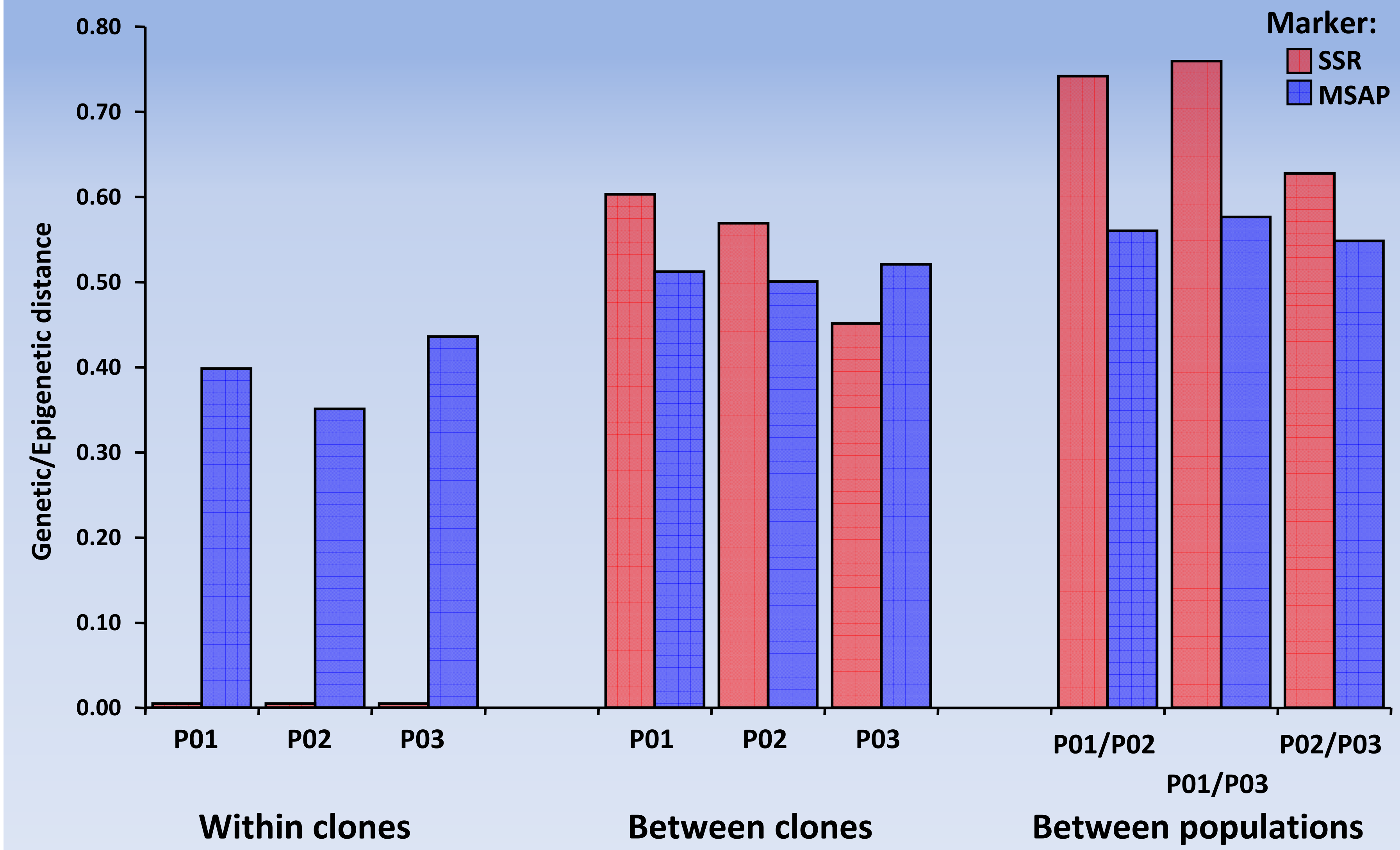
All data:
r **= 0.421**
P **< 0.01**
R^2 **= 0.178**

Excluding within-clone comparisons:
r **= 0.280**
P **< 0.01**
R^2 **= 0.079**

Significant but weak correlation between genetic and epigenetic distances!
Genetic variability explains between 8 and 18% of epigenetic variability!

Results: GENETIC VS. EPIGENETIC DISTANCES

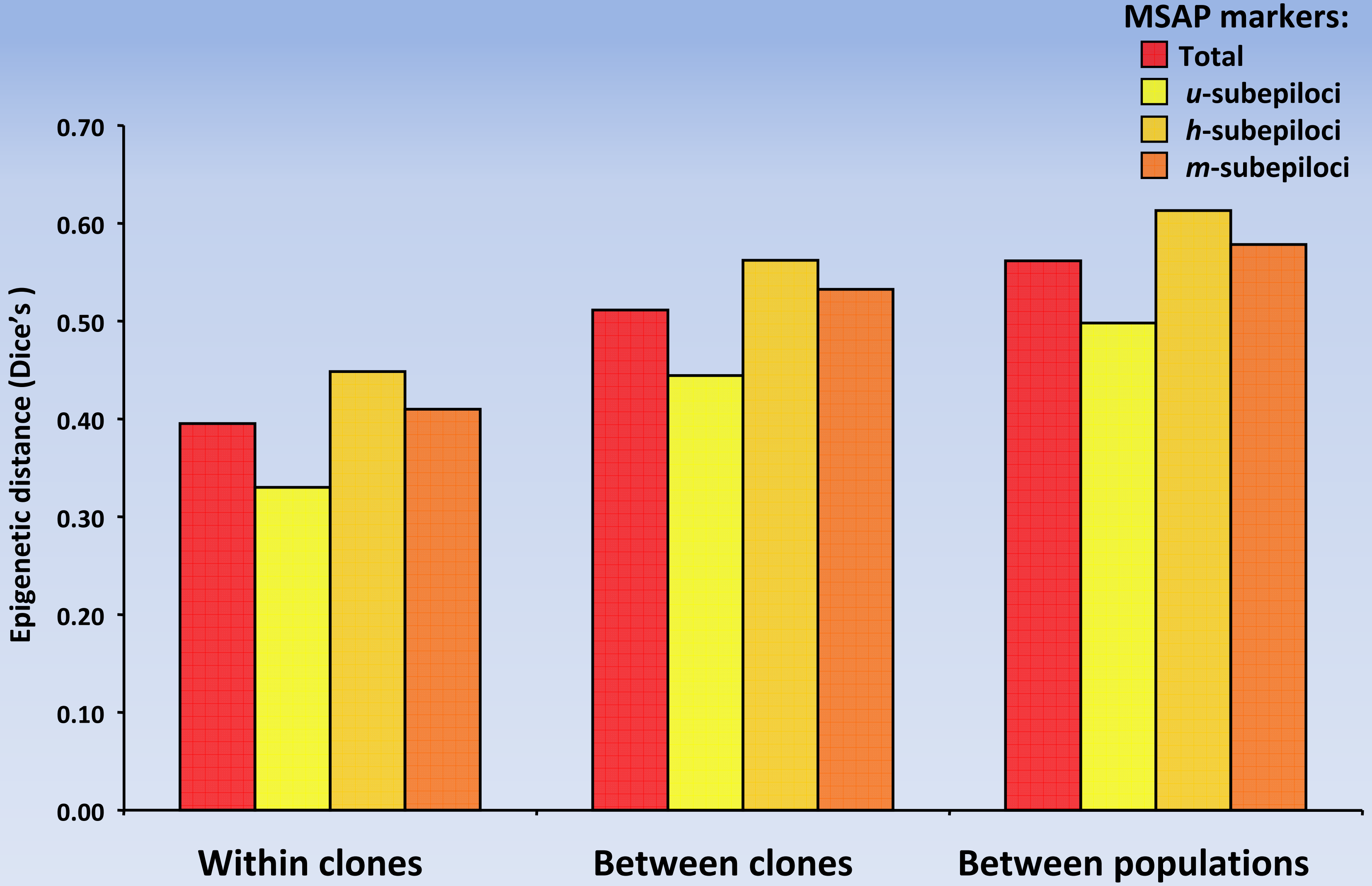
WITHIN CLONES, BETWEEN CLONES AND BETWEEN POPULATIONS



Average epigenetic distance among genetically identical plants only slightly lower than those among genetically different plants!

Results: EPIGENETIC DISTANCES

WITHIN CLONES, BETWEEN CLONES AND BETWEEN POPULATIONS



Similar levels of epigenetic distances
across subepiloci!

Conclusion:

- narrow endemic and clonaly propagated species
  - high both clonal and genetic diversity
  - in HWE; no bottleneck
- epigenetic diversity was more even across populations than clonal and genetic diversity
- significant but weak correlation between epigenetic and genetic distances
- relatively high epigenetic distances even among genetically identical individuals
- epigenetic mechanisms contribute in the evolutionary persistence of these populations

Thank you for your kind attention!!