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

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Dalmatian sage

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

Cultivation:
- throughout 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
(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:
- Bootstrapping

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

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Wild</th>
<th>Cultivated</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Average (Range)</td>
<td>Average (Range)</td>
<td></td>
</tr>
<tr>
<td>Observed heterozygosity ($H_O$)</td>
<td>0.733 (0.54-0.85)</td>
<td>0.590 (0.31-0.79)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Expected heterozygosity ($H_E$)</td>
<td>0.751 (0.61-0.85)</td>
<td>0.545 (0.38-0.61)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Inbreeding coefficient ($F_{IS}$)</td>
<td>0.025 (-0.08-0.12)</td>
<td>-0.067 (-0.30-0.17)</td>
<td>&lt; 0.01</td>
</tr>
</tbody>
</table>

- 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

<table>
<thead>
<tr>
<th>Source of variation</th>
<th>% of total variation</th>
<th>(\phi)-statistics</th>
</tr>
</thead>
<tbody>
<tr>
<td>Among groups: wild vs. cultivated</td>
<td>11.94</td>
<td>0.119***</td>
</tr>
<tr>
<td>Among populations within groups</td>
<td>9.78</td>
<td>0.111***</td>
</tr>
<tr>
<td>Within populations</td>
<td>78.28</td>
<td>0.217***</td>
</tr>
<tr>
<td>Including only wild populations:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Among populations</td>
<td>10.82</td>
<td>0.108***</td>
</tr>
<tr>
<td>Within populations</td>
<td>89.18</td>
<td></td>
</tr>
</tbody>
</table>
(E) **Bottleneck**

- reduced number of alleles
- rare alleles are preferentially lost, but these do not affect expected heterozygosity / 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:

<table>
<thead>
<tr>
<th>If</th>
<th>Gene diversity</th>
<th>Sign of</th>
</tr>
</thead>
<tbody>
<tr>
<td>$H_E &gt; H_{EQ}$</td>
<td>excess</td>
<td>population bottleneck</td>
</tr>
<tr>
<td>$H_E &lt; H_{EQ}$</td>
<td>deficiency</td>
<td>population expansion</td>
</tr>
</tbody>
</table>
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

Allelic richness:
- < 5
- 5 - 7
- 7 - 9
- > 9

Bottleneck
\[ P < 0.01 \]
(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)

K = 2

K = 3

Wild

Cultivated (naturalized)

Adriatic

MKD/GRC
Proportion of Membership (Q)

K = 2

K = 3

K = 4

Cultivated

Northern 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

Baričević Dea, Ljubljana, Slovenia
Batîr Rusu Diana, Suceava, Romania
Carović-Stanko Klaudija, Zagreb, Croatia
Chatzopoulou Paschalina, Thessaloniki, Greece
Dajić-Stevanović Zora, Belgrade, Serbia
Gonceariuc Maria, Chisinau, Moldova
Grdiša Martina, Zagreb, Croatia
Greguraš Danijela, Zagreb, Croatia
Ibraliu Alban, Tirana, Albania
Jug-Dujaković Marija, Split, Croatia
Krasniqi Elez, Prishtinë, Kosovo
Murtić Senad, Sarajevo, Bosnia and Herzegovina
Pećanac Dragana, Banja Luka, Bosnia and Herzegovina
Radosavljević Ivan, Zagreb, Croatia
Rešetnik Ivana, Zagreb, Croatia
Stefkov Gjoshe, Skopje, Macedonia
Stešević Danijela, Podgorica, Montenegro
Šoštarić Ivan, Belgrade, Serbia
Varbanova Kana, Sadovo, Bulgaria
Liber Zlatko, Zagreb, Croatia
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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