GENETIC STRUCTURE OF THE INSECTICIDAL PLANT
Dalmatian Pyrethrum
(Tanacetum cinerariifolium Trevir./Sch./Bip.)

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DALMATIAN PYRETHRUM
(Tanacetum cinerariifolium /Trevir./Sch. Bip.)

- family Asteraceae
- perennial, outcrossing plant species
- thermophytic
- indigenous to the Eastern coast of the Adriatic Sea
- distribution range expands from the coastal region to 200 m, but in some cases, it is also present in higher mountainous Mediterranean zones at elevation above 500 m
- **natural habitat:** extremely degraded habitats with shallow rocky soils
DALMATIAN PYRETHRUM  
(Tanacetum cinerariifolium /Trevir./Sch. Bip.)

**PYRETHRIN** - natural insecticide
- 6 active ingredients: pyrethrin I i II, cinerin I i II, jasmolin I i II
- pyrethrin I i II - the most active
- contact insecticide
- acting on the nervous system of insects
- causing *knock-down* effect and death
- highly biodegradable when exposed to light, water and air, and does not accumulate in food chains and ground water
- an efficient and environmentally safe method of insect control
DALMATIAN PYRETHRUM
(Tanacetum cinerariifolium /Trevir./Sch. Bip.)

- documented history in Croatia
- cultivation and use of Dalmatian pyrethrum and its products in households and agricultural systems
- ca. 1850: cultivation began near Dubrovnik and rapidly expanded along the Dalmatian coastal region and the islands
- 'Flores Chrysanthemi' - dried flowers powder
- 1915-30: golden era of 'Dalmatian Insect Powder'
- from 1930: rapid decrease in production; synthetic pesticide DDT
DALMATIAN PYRETHRUM
(Tanacetum cinerariifolium /Trevir./Sch. Bip.)

- population decline
- threatened by anthropogenic habitat loss and degradation related to factors such as urbanization and habitat conversion
- strictly protected plant species
- gathering from the wild strictly forbidden
- exception scientific investigations
<table>
<thead>
<tr>
<th>No.</th>
<th>Population</th>
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<tbody>
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<tr>
<td>P02</td>
<td>Cres</td>
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<tr>
<td>P03</td>
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<td>21</td>
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<tr>
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<td><strong>Total</strong></td>
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MOLECULAR ANALYSIS

(1) Seed collecting and field trial
- leaf tissue for DNA extraction
- 20 populations / 411 samples

(2) DNA extraction

(3) Molecular marker analysis
- AFLPs (Amplified Fragment Length Polymorphism)
- 6 primer combinations
  
  VIC-EcoRI-ACGC+MseI-AGA, NED-EcoRI-AGA+MseI-CAG,
  VIC-EcoRI-ACG+MseI-CGA, FAM-EcoRI-ACC+MseI-CGA,
  NED-EcoRI-AGA+MseI-CGA, PET-EcoRI-ATG+MseI-CGA
method for detecting polymorphisms in the DNA

Steps:

(1) **Restriction**
   - restriction enzymes *EcoRI* and *MseI*

(2) **Ligation** of specific adapters to restriction sites

(3) **Preamplification**

(4) **Selective amplification** (6 primer combinations)

(5) **Separation** of the amplified fragments - Capillary electrophoresis

(6) **Scoring** of amplified fragments; present (1); absent (0) - binary matrix
AFLPs (Amplified Fragment Length Polymorphism)
6 primer combinations
593 polymorphic markers

Amplified fragment of the particular size is:

Ind1
Present = 1

Ind2
Present = 1

Ind3
Present = 1

Ind4
Absent = 0

Ind5
Absent = 0
## MOLECULAR DATA

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<th>M002</th>
<th>M003</th>
<th>M004</th>
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</table>
- the genetic diversity within populations was estimated by determining:

(1) Proportion of polymorphic loci (%P)
(2) Shannon's information index (I)
(3) Expected heterozygosity ($H_E$)
(4) Number of private bands ($N_{pr}$)
(5) Frequency down weighted marker value (DW)
GENETIC DIVERSITY

(1) Proportion of polymorphic loci (%P)
   average: 40.57%
   min: 26.14% (P16-Kotiški stanovi)
   max: 52.11% (P4-Krk)

(2) Shannon's information index (I)
   total diversity ($H_t$)
   average intra-population diversity ($H_p$)
   diversity within populations ($H_p/H_t$)
   diversity among populations 1-($H_p/H_t$)

<table>
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<tr>
<th>Parameter</th>
<th>Value</th>
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<td>$H_t$</td>
<td>0.297</td>
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<td>$H_p$</td>
<td>0.223</td>
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<td>($H_p/H_t$)</td>
<td>0.750</td>
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<td>1-($H_p/H_t$)</td>
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- the proportion of diversity within populations (75.00%) was considerably higher than the proportion of diversity among populations
### GENETIC DIVERSITY

(3) Expected heterozygosity ($H_E$), gene diversity

- range $0.092$ (P16-Kotiški stanovi) - $0.131$ (P3-Mali Lošinj)

- $H_T$ total gene diversity
- $H_W$ mean gene diversity within populations
- $F_{ST} = (H_T - H_W) / H_T$ Wright’s index of genetic differentiation

<table>
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<td>$H_W$</td>
<td>0.107</td>
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<tr>
<td>$F_{ST}$</td>
<td>0.078</td>
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</table>
(4) Number of private alleles ($N_{pr}$): alleles detected in a single population
- out of 593 alleles scored 36 were private
- from 0-4; max. 4 - Mali Lošinj (Northern Adriatic)

(5) Frequency down weighted marker values (DW)
- average: 45.64
- min. 18.92 (P16-K. stanovi)
- max. 95.20 (P3-M. Lošinj)

- high DW: high amount of rare markers; long-term isolated populations
- low DW: recently diverged populations
GENETIC RELATIONSHIPS

- Nei's standard genetic distance
- Fitch-Margoliash (FM)

- direct relationship between the populations and their geographical origins

- 2 clades: Northern-Southern (bootstrap 88%)
- clear separation of Mt. Biokovo populations (bootstrap 99%)
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

PROCEDURE:

(1) Estimation of the number of Ks populations of origin
   (1.1) Choose different values of K
   (1.2) Compute posterior probabilities for each K
   (1.3) Choose the most likely number of populations (K)

(2) Assignment
   (2.1) Assign individuals to populations (K)
   (2.2) For each individual, estimate the proportion of genome derived from the different population of origin
STRUCTURE AT $K = 2$ and $K = 3$

- proportions of membership ($Q$) of each individual plant in each cluster (population of origin):
- each individual plant is represented by a single vertical line divided into colors representing different clusters
- the length of the colored segment shows the individual’s estimated proportion of membership in that cluster
STRUCTURE AT $K = 2$ AND $3$

$K = 2$:
- A Northern Adriatic
- B Middle/Southern Adriatic

$K = 3$:
- A Northern Adriatic
- B Middle/Southern Adriatic
- C Mt. Biokovo
the geographical structuring of populations is also supported by the IBD analysis

(1) Isolation by distance (IBD)

- Mantel’s test
- to compute and test the linear correlation between the matrix of the natural logarithm of geographic distances (in km) between pairs of populations and the matrix of pairwise \( F_{ST}/(1-F_{ST}) \) ratios

- a significant correlation between geographic distance and genetic variation
- 5.6% of the genetic differentiation resulted from isolation by distance (IBD)
- three genetically distinct groups indicate that the species survived in multiple refugial areas followed by a period of isolation(s) - restricted gene flow among refugial populations

- Northern Adriatic - higher $H_E$, $DW$, number of private alleles - ancient origin and significant isolation through a recent period of evolution

- migrations from the southern parts to the northwest during the last interglacial period!? 

- suitable microhabitats - strong differentiation from the gene pool of origin

**Southern Adriatic** - generally lower $H_E$, $DW$
- evidence of ancient populations in the southern parts of the Adriatic basin?
- human activity and the excessive gathering is responsible for such low gene diversity and $DW$
- reduced diversity: small population size; reduced gene flow
- relict populations: isolated from other populations
- altitudinal shifts in species distribution

**Mt Biokovo populations**
- separated, well-defined gene pool
- above 1200 m.a.s.l.
- atypical climatic conditions for a thermophytic species
- geographically and ecologically marginal areas of the species distribution
- lowest $H_E$, high DW
(1) Isolation by environmental distance (IBED)
- to explain the genetic distance patterns through the differences in the bioclimatic variables among the sampling sites
- WorldClim database
- 19 bioclimatic variables (11 temperature- and 8 precipitation-related) representing the annual trends, seasonal variations and extremes in temperature and precipitation
- bioclimatic variables were used for the Principal Component Analysis (PCA) and subsequently to calculate the environmental distances among populations based on principal component scores rather than original values to avoid intercorrelation among variables
- **12.3%** of the genetic differentiation among populations followed the pattern of isolation by environmental distance (IBED)

- Mt. Biokovo populations - typical case of environmental isolation
THANK YOU FOR YOUR ATTENTION!