



GENETIC STRUCTURE OF THE INSTECTICIDAL PLANT DALMATIAN PYRETHRUM (Tanacetum cinerariifolium Trevir./Sch./Bip.)

Martina Grdiša

University of Zagreb, Faculty of Agriculture, Zagreb, Croatia Centre of Excellence for Biodiversity and Molecular Plant Breeding (CoE CroP-BioDiv), Zagreb, Croatia E-mail: mgrdisa@agr.hr

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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





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



SAMPLING



No.	Population	n
P01	Osor	23
P02	Cres	22
PO3	Mali Lošinj	21
P04	Krk	24
P05	Gornja Klada	22
P06	Senj	19
P07	Pašman	22
P08	Zlarin	25
P09	Primošten	22
P10	Čiovo	19
P11	Šolta	19
P12	Kozjak	20
P13	Brač	22
P14	Hvar	21
P15	Omiš	22
P16	Kotiški Stanovi	13
P17	Lađena	18
P18	Ravna Vlaška	22
P19	Pelješac	14
P20	Mljet	21
	Total	411







(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 *Eco*RI and *Mse*I
- (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



MOLECULAR ANALYSIS



AFLPs (Amplified Fragment Length Polymorphism) 6 primer combinations 593 polymorphic markers 6 of the particular



MOLECULAR DATA



Population	Individual	M001	M002	M003	M004	M005	M006	M593
P01	S001	0	0	1	1	0	0	0
PO1	S002	0	0	1	1	0	0	0
PO1	S003	0	0	1	1	0	0	0
PO1	S004	0	0	0	1	0	0	0
P01	S005	0	0	1	1	0	0	0
PO1	S006	0	0	1	1	0	0	0
PO1	S007	0	0	0	1	0	0	0
PO1	S008	0	0	1	1	0	0	0
PO1	S009	0	0	1	1	0	0	0
PO1	S010	0	0	1	1	0	0	1
•••								
P20	S4 11	1	0	1	0	1	0	0



- 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 $1 - (H_p/H_t)$

Parameter	Value
H _t	0.297
H _p	0.223
(H _p /H _t)	0.750
$1 - (H_p/H_t)$	0.250

- the proportion of diversity within populations (75.00%) was considerably higher than the proportion of diversity among populations



- (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

Parameter	Value
Η _τ	0.116
Hw	0.107
F _{st}	0.078





(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)







- 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 individal, 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

IBD



 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





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

- reduced diversity: small population size; reduced gene flow
- relict populations: isolated from other populations
- altitudinal shifts in species distribution



(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

IBED





- 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

