

Population study

Tribsch A., Schönswetter P. & Stuessy T. (2002):
Saponaria pumila (Caryophyllaceae) and the Ice
Age in the European Alps. *American Journal of
Botany* 89(12): 2024-2033



Reasons for the study

- distribution changes caused by glaciation
- where are glacial refugia located ?
- can the plants survive on nunataks inside the continuous ice shield ?
- are isolated populations product of long-term isolation or present-day long distance dispersal ?

Methods and data analyses

- 33 populations, 5 individuals / population
- AFLP
 - 9 combinations of selective primers tested
 - 3 combinations selected
- Shannon diversity index – H_{Sh}
- rare fragments – f_r (occurred in less than 20 individuals)
- unique fragments – f_u (in 1 population only)
- UPGMA (Nei) – Popgene
- NJ (Nei & Li) – TreeCon
- PCoA (Jaccard) – SPSS
- Mantel test – R-Package
- AMOVA

Results

- 3 primer combinations – 223 fragments
- H_{Sh} – increase from W to E (*Fig. 2*)
- rare and unique fragments – mainly in the E (*Fig. 3*)
- UPGMA, PCoA – 4 groups consistent with geographical distribution (E, CE, CW, D) (*Fig. 4+5*)
- AMOVA (*Table 4*)
 - about 50% of diversity within populations
 - 22% among population within a region
 - 27% among regions
- Mantel test (*Fig. 7*)
 - $R_M = 0.44$ (*isolation by distance*)
 - significant differences between E and other groups

Discussion

- 4 groups (PCoA) – 4 isolated refugia ? → NO
 - region E – partially covered with ice → refugium
 - CW – completely covered with ice → no refugium
- greatest boundary – E × CE
 - limestone massive – barrier
 - deep valley
 - forested parts
- drastic successive depauperation from the E to W
 - *bottleneck* (Pleistocene isolation) or *founder effect* (recent long-dispersal) ?
 - to be distinguished with the presence of unique markers

Geographical pattern of genetic diversity

East (E)

- high within-population diversity, many unique markers
→ i.e., refugium
- population 33 – high f_r , low H_{Sh} , i.e., typical „old“ depauperate population, isolated at present

Center-East (CE)

- not clear pattern
- significantly differentiated from E
- moderate to high level of genetic diversity
- 9 unique markers
- suggested refugium (peripheral nunataks) rather than postglacial recolonization

Center-West (CW)

- genetically depauperated
- without unique fragments
- recent recolonization
(positive correlation with CE and D)

Dolomites (D)

- moderate H_{Sh} , rare fragments
- probable refugium
- population 1 – extremely depauperated – recently

Reconstruction of distribution changes

- long time center in the Eastern Alps
 - only local glaciation
 - recurrent glacial survival *in situ*
- CE colonized before the last glaciation
- distribution during last ice age
 - eastern peripheral refugium (E)
 - peripheral nunataks (CE)
 - southern Dolomites (D)
- during postglacial
 - short-distance dispersal – without loss of variability
 - stopped by the limestone massif (within E)
 - in the easternmost part – fragmentation
 - westernmost part – recent *long-distance dispersal* – genetic depauperation

Systematic study

Schenk M.F. et al. (2008): Phylogenetic relationships in *Betula* (Betulaceae) based on AFLP markers.
Tree Genetics & Genomes 4: 911–924



Reasons for the study

- taxonomically controversial group
- reconstruct phylogeny – relationships among subgenera and species
- hybridization
- previous studies (matK, ITS and ADH sequences) – limited variation, incongruencies
- use of AFLP for systematic studies

Methods

- 99 samples – 23 species, 5 hybrids + 4 outgroup
- flow cytometry – ploidy level determination
- AFLP – 3 primer combinations
- absence/presence of fragments
 - only intense and well-separated bands scored
 - 8 duplicates as a control

Data analyses

- phenetic analyses (NTSYSpc)
 - Dice & Jaccard similarity coefficients + NJ
 - cophenetic coefficients – correlations between matrix and tree – *goodness of fit*
 - bootstrap – 1,000 replicates (PAUP)
- subgen. *Betula*
 - PCoA (Dice)
 - STRUCTURE 2.2 – clusters of species, hybrids
 - admixture analysis, correlated allele frequencies
 - K=1-17, 3 replicates, burn-in 50,000 and 100,000 data collection
- phylogenetic (cladistic) analysis (PAUP)
 - limited dataset (without hybrids) + diploids only
 - heuristic search for MP (*most parsimonious*) tree – 10,000 bootstrap replicates
 - consensus tree

Results

- 3 primer combinations – 321 variable bands
- Dice + NJ – best combination (highest cophenetic coefficient)
 - four major clades
- PCoA
 - 3 axes 29.6% of the variation – five groups
 - cultivars and hybrids – between groups
- STRUCTURE – $K=3$ – largely consistent with PCoA
- phylogenetic analysis
 - 12 MPTs of 721 steps – strict consensus computed
 - similar topology with NJ

Discussion – AFLP for phylogeny

- sequence markers – limited variation
- AFLP
 - polymorphic markers
 - high reproducibility
 - genome wide sampling
- more than 200 parsimony informative markers
- four subgenera distinguished – higher support than ITS
- failed to resolved relationships among them
- high congruence between ITS and AFLP
- AFLP – complementary information on hybridization

Discussion

four subgenera

- *Betulenta* – *B. lenta*, *B. alleghaniensis*
- *Betulaster* – *B. maximowicziana*, *B. nigra*?
- *Neurobetula*
 - Group I – *B. chichibuensis*, *B. schmidtii*
 - Group II – *B. costata*, *B. ermanii*, *B. davurica*, *B. utilis*... - should be merged with subgen. *Betula*
- *Betula* – four/three species groups within subgenus
 - *B. pendula*, *B. platyphylla*, *B. populifolia* – conspecific?
 - group E – intermediate position – hybrids/polyploids

Discussion – Evolution

unclear relationships among groups (lack of support)

- occurrence of hybridization and introgression
 - homogenizing effect
 - band sharing – shared evolution or sharing parental genomes in natural hybrids – hard to differentiate
- major speciation events – within very short time frame
 - homoplasy limits phylogenetic resolution
 - relationships may remain unresolved (forever...)