

Population study

Tarayre M. (1997): The spatial genetic structure of cytoplasmic (cpDNA) and nuclear (allozyme) markers within and among populations of the gynodioecious *Thymus vulgaris* (Labiatae) in southern France. *American Journal of Botany* 84(12): 1675-1684



Reasons for the study

- comparison of spatial pattern of genetic variability of nuclear and chloroplast markers
 - among populations
 - among patches within one young population
- comparison of pollen and seed flow
- is genetic differentiation related to the proportion of female plants in gynodioecious species ?

Methods

- RFLP
 - 12 populations, 5 plants / population
 - 31 plants from young population (CAB) – 3 patches + continuous surroundings
 - 8 restriction enzymes tested, 3 selected
 - hybridization with cpDNA from *Beta vulgaris*
- allozymes
 - 30 plants from 10 populations
 - CAB – 10 plants per patch + 20 from the surroundings
 - 4 polymorphic loci

Data analyses

- genetic diversity (Nei 1987) – H_S, H_T
 - estimates based on the type of marker inheritance
- F-statistics (FSTAT)
- number of migrants per generation – M
- *pollen/seed flow ratio* (Ennos 1994)

Results – cpDNA variability

- RFLP variability – mainly due to mutations
- 8 chlorotypes – combination of restriction patterns from three enzymes
 - I and II – more widespread than all others (*Tab. 2*)
 - I – in 10 of the 13 studied populations (*Fig. 2, Tab. 3*)
 - VIII – specific for just one population
- identical chlorotypes found in distant populations

Spatial structure of markers

cpDNA

- large part of variability among populations (*Tab. 4*)
- $F_{ST} = 0.238$

allozymes

- variability within populations (*Tab. 5*)
- $F_{ST} = 0.019$

→ gene flow via pollen is greater than that via seeds
(P/S=14)

similar pattern both at population and local scale

Discussion

- even though only low number of plants was analysed
 - extensive cpDNA spatial structure was found
 - up to 4 different chlorotypes within 5 plants from a single population
- CG patch of the new population (CAB) – specific chlorotype
 - not found in surrounding populations
 - may be due to low number of samples
- no relationship between frequency of female plants and cpDNA diversity (*Tab. 6*)

Discussion

- extensive spatial structure
 - colonization over short distances – no seed dispersal adaptations
 - young populations – more female plants, i.e., more seeds
- F_{ST} for cpDNA higher in young population
 - colonization (*founder effect*) increases population differentiation
 - differentiation persists for a long time – limited dispersal

Systematic study

Segraves K.A. et al. (1999): Multiple origins of polyploidy and the geographic structure of *Heuchera grossulariifolia*. *Molecular Ecology* 8:253-262



Reasons for the study

- multiple origins of polyploidy
- identification of original diploids
- spatial pattern of diploid and tetraploid lineages

Methods

- 10 localities – 4 plants / locality and ploidy
- ploidy level – flow cytometry
- RFLP – 12 restriction enzymes
 - hybridization with cpDNA from *Lactuca* and *Petunia*
- PCR-RFLP – *trnK1/2*, *rbcL T1/orf512*
 - restriction with one of 10 endonucleases
- sequencing – *trnL/F*
 - only substitutions considered – indels not included
- *outgroup* – mutations polarisation
- data combination – *strict consensus* – 100 *bootstrap replicates*

Results

- RFLP – 4 mutations of restriction sites, 2 length mutations (*Tab.2*)
- PCR-RFLP – 2 restriction sites in *trnK*
- sequencing – 937 bp
- 13 cpDNA haplotypes – up to 4 in a single population
- 36 *most parsimonious trees* → *strict consensus tree* (*Fig. 2*)
 - at five branches 2n and 4n ploidies together
- almost all 4n have corresponding 2n types
- many incongruencies between phylogeny and geographic distribution of 4n and 2n with the same cpDNA haplotype

Tetraploid origin

- Rapid River population – very different (4-7 mutations) from the rest
 - at least 2 independent polyploid lineages
- up to 7 independent origins
 - because there is 7 different $4n$ haplotypes
- all tetraploid populations – mix of at least two haplotypes
- some of $4n$ populace most closely related to $2n$ populations up to 180 km apart

Discussion

- Rapid River – parental $2n$ haplotype not found – may have been overlooked or has gone extinct
- present day diversity and distribution of $4n$ haplotypes might
 - reflect gene flow in the past
 - be a product of subsequent mutations resulting in divergence between polyploids
- cannot be distinguished between these 2 hypotheses

Haplotype and cytotype distributions

- 4n populations originating from geographically close 2n populations do not exist
- 4 explanations
 - not all haplotypes were sampled (high haplotype diversity)
 - parental 2n haplotypes may be extinct (and thus other 2n haplotypes are considered as most similar)
 - chloroplast introgression through triploid intermediate (...no clear evidence)
 - high level of homoplasmy in spacers (...no – at least in some)
- polyploids did not originate recently – there was a time for migration
 - remnants of past larger (continuous) population
 - gene flow in the past