Patterns, sources and ecological implications of clonal diversity in apomictic *Ranunculus carpaticola* (*Ranunculus auricomus* complex, Ranunculaceae)

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Overview of Language

- apomicts: asexuals that reproduce via seeds
- vegetatively reproducing: asexuals that reproduce without seeds (i.e. budding)
- apospory- embryo comes from somatic cells
- AFLP: Amplified Fragment Length Polymorphism
- SSR: Simple Sequence Repeat

The Organism

- *Ranunculus carpaticola* Soó is part of the *R. auricomus* complex
- Grows in Eurasia
- Has sexual diploids/polyploids and apomictic polyploids
- Studies have shown aposporous reproduction
- Hexaploid apomictic populations could be hybrids of diploid *R. carpaticola* and *R. cassubicifolius*

Why central Slovakia?????!!!!

- It may provide a model system to study possible backcrossings to sexuals as a source of variation in apomicts
- The putative ancestors growing nearby might simplify the origin of the apomicts in the area
- Frequent sampling in a biogeographically homogenous region allows the study of spatial and ecological differentiation in clones in various types of surrounding vegetation

Goals of this Study

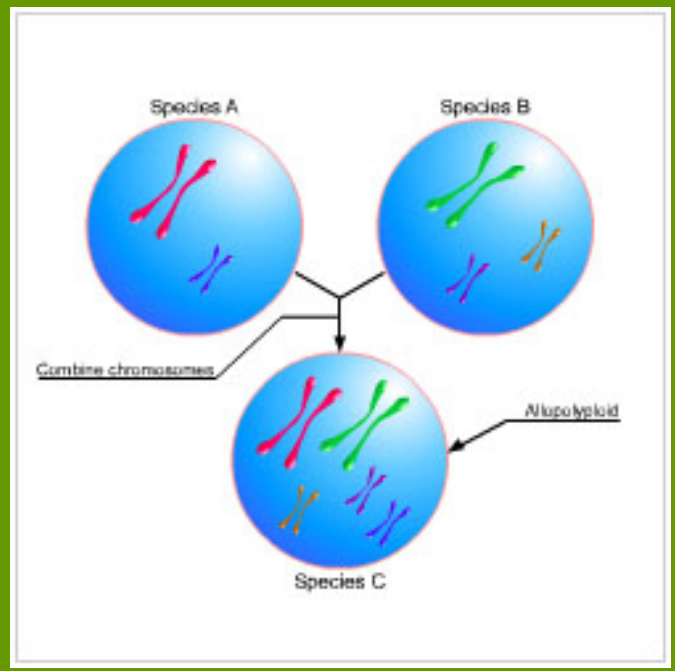
- Is genotypic variation within populations indicative of the mode of reproduction?
- How is sexual vs. apomictic mode of reproduction distributed in central Slovakia?
- To what extent is genetic variation within populations due to facultative sexuality and to mutations?
- How is clonal diversity partitioned within and among populations?
- Do apomictic lineages evolve *in situ*, or have they spread between sites instead?
- How is genetic diversity of apomicts correlated with habitat differentiation and geographical patterns?

Sampling

- Collected plants from 8 sites (10 sites total)
- Normally 20-40 individuals
- Some plants came from experimental garden (previously collected from these sites)

Determination of Ploidy Level

- Feulgen DNA image densitometry
- DNA flow cytometry
- Chromosome counts
- Basically they counted chromosomes



Results for Ploidy Level

- Feulgen densitometry did not work due to a staining inhibitor
- Could see they were polyploid, but how many?
- Must be greater than tetraploid and pentaploid, so hexaploid
  - Values were above tetraploid and around pentaploid, but due to staining inhibitor, hexaploid was assumed
- Flow cytometry gave hexaploid results as well

DNA Genotyping

- AFLP was done with 3 primer combinations
  - *Eco*RI ACC (NED)-*Mse*I CATA
  - *Eco*RI ACA (6-FAM)-*Mse*I CTGA
  - *Eco*RI ACG (HEX)-*Mse*I CTCG
- 2 Microsatellites were used
  - 1407 (TC/GA)
  - 3313 compound dinucleotide (TC/GA and TG/CA)

Locus no.	GenBank Accession no.	Primer sequence (5′–3′)	T_a (°C)	Repeat motif	SR
1407	DQ118795	F: ATGGAGATTGGTGTTCAGTG R: CAGCAACCACCTTCTTCAAC	54	$(GA)_{30}$	11–67
3313	DQ118820	F: GTTCTTGCTTGCGGAGATT R: AGCTGGTAAAGACACACACA	52	$(TC)_{28}(TG)_{40}$	35–99

- PCR and polyacrylamide gel
  - Used presence/absence for AFLP
  - Used allele size and repeat number for SSR

AFLP Analysis

- Total number of fragments
- Number of unique fragments
  - only occurring in one population
- Number of fixed unique fragments
  - occurring in all individuals in one population
- Percentage of polymorphic fragments from the total ($\%_{POLY}$)
- Neighbour-joining dendrogram
- Number of genotypes per population (G)
- Mean number of pairwise differences within each population (π)
- Diversity: probability that two randomly chosen individuals will be genetically different (D)
  - 0=uniform and 1=diverse
- Proportion of distinguishable genotypes (PD)
  - PD=G/N (number of individuals)
- Genotypic evenness (E)
  - 0=all individuals have different genotype or one dominant genotype and others are represented by a single individual
  - 1=all clones are represented by the same number of individuals
- Matrix Incompatibility (MI)
  - Incompatibilites with clonal evolution

AFLP Data

- 249 clear bands (54-480 bp)
  - 214 bands (85.94%) polymorphic
- 35 multilocus genotypes
  - Clones
  - Restricted to within populations
- 4 of 8 populations had one genotype, the rest had dominant genotype
  - In populations with rare genotypes, they differ from dominant by one mutation
  - IVAC stands out
- All diploid individuals had different genotypes

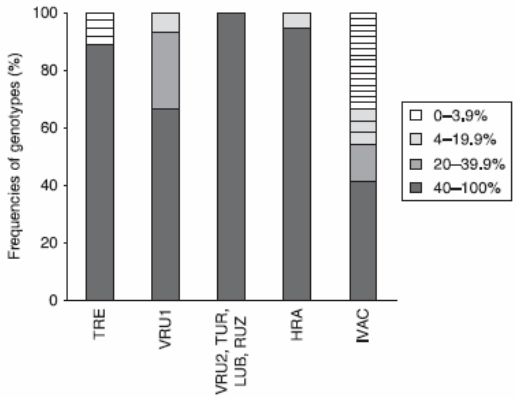
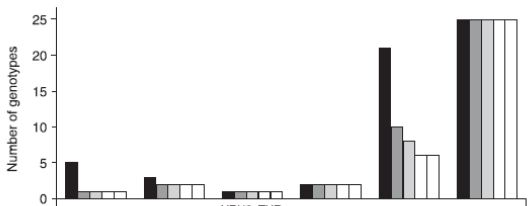


Fig. 2 Frequency distribution of AFLP genotypes within eight facultative apomictic populations in central Slovakia. Shading indicates different frequencies of genotypes found in each population. No genotype was found in more than one population.



	6x					2x
M	D					
0	0.21	0.5	0	0.11	0.82	1
1	0	0.4	0	0.11	0.66	1
2	0	0.4	0	0.11	0.63	1
3,4	0	0.4	0	0.11	0.62	1

Fig. 3 Bar diagram showing the number of AFLP genotypes found within populations for different number of allowed mutations (M), inferred with GENOTYPE software. Genotypic diversity (*D*) values calculated with GENODIVE are given for different M. Diploid sexual populations REV1 and REV2 show similar patterns, with the only difference that for the first, 27 individuals were analysed, and for the second, 25; on this graph only REV2 is shown.

AFLP Data

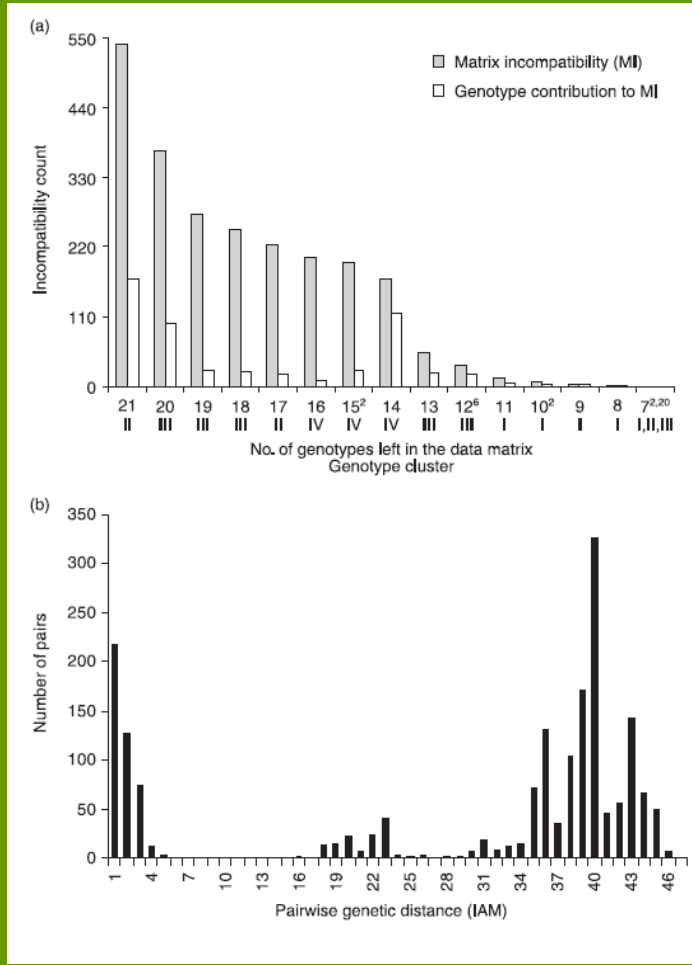
- MI for IVAC and TRE because at least 4 genotypes were present
- IVAC shows a sexual recombination event
- TRE shows no recombination event (MI=0)

Table 3 Descriptive statistics of 10 *Ranunculus carpaticola* populations in central Slovakia based on AFLPs. *N*, population sample size; $\%_{POLY}$, percentage of polymorphic fragments; *G*, no. of genotypes; *D*, genotype diversity; π, mean number of pairwise differences; *PD*, proportion of distinguishable genotypes; *E*, genotypic evenness; $MI_{(i)}$ (initial) matrix incompatibility

Populations	6x								2x	
	TRE	VRU1	VRU2	TUR	LUB	RUZ	HRA	IVAC	REV1	REV2
N	37	30	30	9	11	17	19	48	27	25
No. of fragments	99	109	87	88	87	89	102	131	149	150
$\%_{POLY}$	1.61	8.03	0	0	0	0	10.04	29.32	44.58	44.98
Unique fragments (fixed)	8 (8)	10 (6)	0 (0)	4 (4)	1 (1)	0 (0)	4 (0)	7 (0)	18 (0)	17 (0)
G	5	3	1	1	1	1	2	21	27	25
D	0.21	0.5	0	0	0	0	0.11	0.82	1	1
π	0.22	7.82	0	0	0	0	2.63	23.79	26.04	26.14
PD	0.14	0.1	0.03	0.11	0.09	0.06	0.11	0.44	1	1
E	0	0.65	0	0	0	0	0	0.49	0	0
MI_i	0	—	—	—	—	—	—	541	1631	1552
No. of genotypes left at $MI = 0$	5	—	—	—	—	—	—	7	0	0

IVAC

- Slope is discontinuous in first graph but data is easily seen in the second graph
- First peak is due to distance between similar genotypes, probably different due to mutation
- Second and third peaks are due to recombination events



Dendrogram

- Clear differentiation between diploids and polyploids
- IVAC is in 4 subclades
- No clear difference between two diploid populations

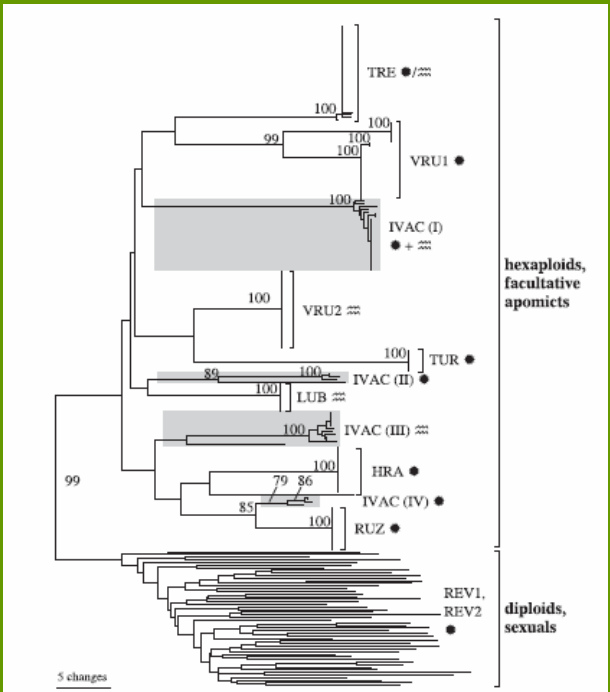


Fig. 5 AFLP neighbour-joining dendrogram of 249 individuals of *Ranunculus carpaticola* from central Slovakia. The numbers are bootstrap values higher than 85% (10 000 replicates). Clades of IVAC are marked in grey and numbered with roman numerals. The habitat of populations is indicated: ● — forest; ≈ — meadow; ●/≈ — margin of forest; ● + ≈ — mixed.

SSR Data Analysis

- Number of alleles
- Mean allele size
- F- and R-statistics
- Rousset's distance between individuals

SSR Data

- Both loci tested were highly polymorphic
- Showed as many genotypes as individuals
- Markers were consistent with assumed ploidy levels
- Clone mates shared number of bands
  - TRE and IVAC, but only a single band at a single locus
- Results were consistent with AFLP

Table 5 Descriptive statistics of 10 *Ranunculus carpaticola* populations in central Slovakia based on two SSRs. *N*, population sample size; H_O, observed heterozygosity; *G*, no. of genotypes; *D*, genotype diversity; $\hat{a}$, mean within-populations pairwise Rousset's distance between individuals

Populations	6x								2x	
Locus 3314/1407	TRE	VRU1	VRU2	TUR	LUB	RUZ	HRA	IVAC	REV1	REV2
N	37	30	30	9	11	17	19	48	23	24
Mean allele size	57.4/44.6	63.9/37.8	63.5/37.8	60/44.2	57.9/36.6	73.5/49.3	70.5/33.7	58.9/38.7	67.8/39.2	66.6/42.8
No. of alleles	22/21	24/28	18/12	15/11	12/4	17/13	21/12	45/41	13/17	18/26
H_O	1	1	1	1	1	1	1	1	0.61	0.52
G	37	30	27	8	10	17	18	48	23	24
D	1	1	0.99	0.97	0.98	1	0.99	1	1	1
$\hat{a}$	0.361	0.408	0.128	0.328	0.169	0.335	0.282	0.483	0.834	1.014
No. of mutations/total no. of alleles scored*	0.5	0.32–0.47	0.32	0.4	0.38	0.51	0.4	0.36–0.42	—	—
No. of TPM mutations for *G* = 1 within AFLP clones*	9	5–11	8	6	4	9	4	13–12	—	—

*determined only for clone mates, and separately for different AFLP clones within population.

Findings

- Apomixis is the rule and outcrossing the exception
- Populations do not have diversity within, but between populations diversity is high
  - Based heavily on AFLP rather than microsatellites
  - Genetic variation detected by microsatellites is neutral
  - High mutation rates in microsatellite evolution
  - Apomictic diversity was lower than other apomicic species
    - Recent establishment and descent from a single individual
  - Limited gene flow between populations
- Polyploids have high heterozygosity when compared to diploids
  - Accumulation of mutations
    - Enhanced due to polyploidy
  - Backcrossing is unlikely due to geographic limitations
  - Hybrid origins
  - IVAC had comparable diversity
    - Rare events of sexual recombination in older populations (facultative sexuality)
    - Two founding clones
- Clones show stronger habitat differentiation than sexual diploids
  - Frozen niche variation model vs. General-purpose genotypes model
  - Clones have undergone adaptive change in order to reduce competition
  - Polyploids have higher ecological vigour
    - Maybe ploidy rather than sexuality
- IVAC maybe a center of origin for other apomictic populations
  - Scattered in several clusters

My Questions

- Should this be a species?
- Seems like the polyploids occupy more niches than the diploids- supports general-purpose hypothesis rather than frozen niche hypothesis, contradictory?