Hybridization in *Hieracium* s.l. (Lactuceae, Asteraceae)

Judith Fehrer, František Krahulec, Anna Krahulcová, Jindřich Chrtek Jr.

Institute of Botany, Academy of Sciences of the Czech Republic, 25243 Průhonice near Prague, Czech Republic

Hieracium in general

- Two kinds of apomixis (seed production without fertilization) in different subgenera
- Sexual as well as apomictic species
- Several ploidy levels in most species
- Base chromosome number x=9
- Basic species: unique morphological characters, some species comprising diploids
- Intermediate species: combination of morphological characters of two or more basic species, most species not comprising diploids, supposed hybridogenous origin

Hieracium subgen. *Pilosella*

- Facultatively apomictic or sexual species (apomixis of aposporous type)
- Hybridizations frequent (recent and past)
- Most, possibly all apomicts produce viable pollen (frequent ♂ gene flow)
- Residual sexuality in facultative apomicts (additional possibility of ♀ gene flow)
- Reduced and/or unreduced gametes (♀ and ♂) involved in rise of hybrid progeny
- Species form a large hybridogenous complex irrespective of relationships

Hieracium subgen. *Hieracium*

- Near-obligate apomictic or sexual species (apomixis of diplosporous type)
- Hybridizations frequent in the past, rare recently
- Only some apomicts produce viable pollen (possibility of ♂ gene flow)
- Sexuality restricted to recently rare, often allopatric diploids
- Reduced ♀ and reduced or unreduced (?) ♂ gametes involved in rise of hybrid progeny
- Hybridizations very rare recently (only between diploids or diploid ♀ and polyploid ♂)

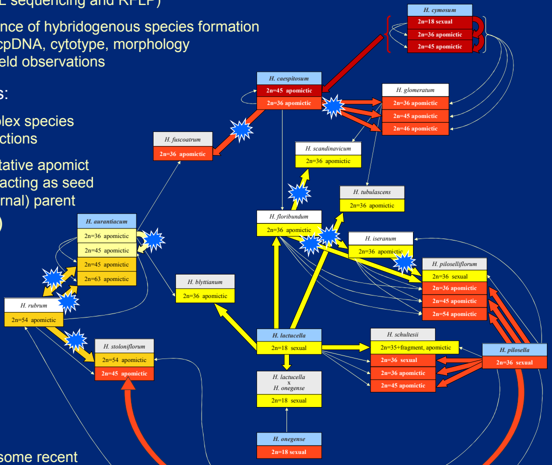
Hybridization in subgenus *Pilosella*

Approach:

- Sampling of species across Central European mountain ranges (Czech Republic, Germany, Poland)
- Determination of chromosome numbers and mode of reproduction
- Determination of chloroplast DNA (cpDNA) haplotype (*trn*T-L sequencing and RFLP)
- Inference of hybridogenous species formation from cpDNA, cytotype, morphology and field observations

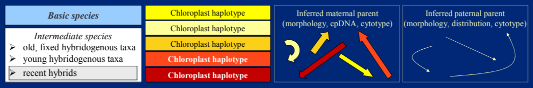
Results:

- Complex species interactions
- Facultative apomict often acting as seed (maternal) parent (✷)



- Only some recent hybrids show both major haplotype groups (red and yellow), others show only one despite recurrent formation

Caption:



References:

Krahulec, F., Krahulcová, A., Fehrer, J., Bräutigam, S., Plačková, I., Chrtek, J. (2004) The sudetic group of *Hieracium* subg. *Pilosella* in the Krkonoše Mts: a synthetic view. Preslia 76: 223–243.

Fehrer, J., Šimek, R., Krahulcová, A., Krahulec, F., Chrtek. J., Bräutigam, E., Bräutigam, S. (2005) Evolution, hybridization, and clonal distribution of apo- and amphimictic species of *Hieracium* subgen. *Pilosella* (Asteraceae: Lactuceae) in a Central European mountain range. In: Bakker, F.T., Chatrou, L.W., Gravendeel, B., Pelser, P.B. (Eds.): Plant Species-Level Systematics: Patterns, Processes and New Applications. Regnum Vegetabile 143, Koeltz, Königstein, pp. 175–201.

Natural hybridization in *Hieracium* subgenus *Pilosella* plays an ongoing role in the recurrent formation of polyploid intermediate taxa, some of which form stable populations and occupy large distribution areas.

Hybridization in subgenus *Hieracium*

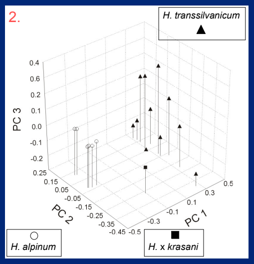
Approach:

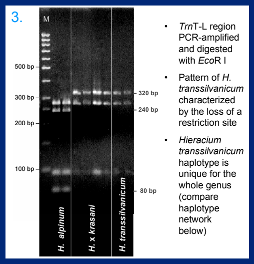
- Study of rare recent diploid hybrids in disturbed habitats where parental species exceptionally came into secondary contact (Romanian and Ukrainian Carpathians)
- Determination of chromosome numbers, mode of reproduction, pollen viability
- Morphological analysis and experimental crosses of parental species
- Isozyme analysis of putative hybrids and parental species
- Determination of chloroplast haplotypes (*trn*T-L sequencing and RFLP)
- Confirmation of hybrid origin by morphology, isozyme analysis, cpDNA, experimental crosses and field observations

Results:

1. Morphology intermediate (e.g., branching pattern, number of heads), somewhat closer to *H. transsilvanicum*
2. Isozyme patterns indicate hybrid origin (6 enzyme systems, 11 loci; either additive patterns or isozyme phenotypes shared with either parent; Principal Component Analysis)
3. Chloroplast DNA reveals *H. transsilvanicum* as maternal parent in all hybrids
4. Pollen fertility of hybrids comparable to parents, hybrids completely seed sterile
5. Phenotype reproducible in experimental reciprocal crosses of parental taxa

H. alpinum *H. x krasani* *H. transsilvanicum*





- *Trn*T-L region PCR-amplified and digested with *Eco*R I
- Pattern of *H. transsilvanicum* characterized by the loss of a restriction site
- *Hieracium transsilvanicum* haplotype is unique for the whole genus (compare haplotype network below)

Reference:

Mráz, P., Chrtek, J. jun., Fehrer, J., Plačková, I. (2005) Rare recent natural hybridization in *Hieracium* s.str. – Evidence from morphology, allozymes and chloroplast DNA. Plant Syst. Evol. (in press), published online 8 August 2005 (http://dx.doi.org/10.1007/s00606-005-0329-1).

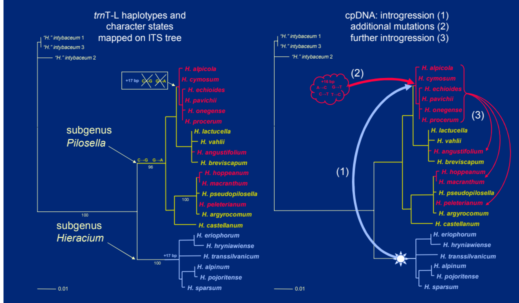
Natural hybridization in *Hieracium* subgenus *Hieracium* occurs very rarely and does not play a considerable role in speciation nowadays, but must have done so in the past (500–10,000 polyploid species).

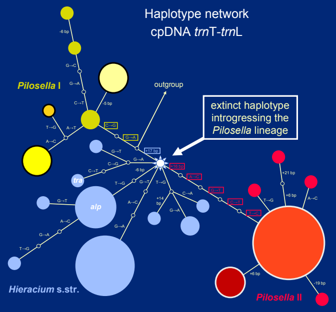
Ancient hybridization between subgenera *Pilosella* and *Hieracium*

Approach:

- Sampling of all subgenus *Pilosella* basic species and of a representative selection of subgenus *Hieracium* basic species
- Sequencing of the chloroplast *trn*T-*trn*L intergenic spacer
- Representation of mutational changes in a cpDNA haplotype network
- Sequencing of the internal transcribed spacer (ITS) for diploid species
- Mapping of the major cpDNA haplotypes on the nuclear ITS tree
- Interpretation of incongruences and character state evolution

Maximum likelihood tree of nuclear ribosomal ITS reflecting the species phylogeny





filled bullets = cpDNA haplotypes size of bullets = number of species
red and yellow bullets encircled black or white = previous haplotypes
tra* / *alp in blue bullets = haplotypes of ***H. transsilvanicum* / *H. alpinum***
T→G /-5 bp...= mutational changes open circles = extinct haplotypes
mutational changes in squares = character states mapped on ITS tree

Results:

- Separation of the hybridogenous complex of subgenus *Pilosella* into two divergent chloroplast haplotype groups
- No correlation between cpDNA haplotypes and other evidence
- Nuclear ITS tree corresponding to presumed relationships (e.g., both subgenera monophyletic, other reasonable clades)
- *Pilosella* I and II haplotype groups not derived from each other (i.e., not nested, incongruent character distribution on ITS tree)
- Mediating position of subgenus *Hieracium* cpDNA
- Character distribution suggests introgression of subgenus *Hieracium* ancestral haplotype (✷) into a *Pilosella* lineage
- Evolution of ancestral, still most widespread *Pilosella* II chloroplast haplotype (five synapomorphic mutations)
- Introgressed *Pilosella* lineage speciated rapidly (many species, including polyploids) in near absence of nuclear DNA variation
- Geographic isolation during *Pilosella* II haplotype development and speciation is probable (extinct intermediate haplotypes, species distribution consistent with different glacial refugia)
- Introgression of most widespread *Pilosella* II haplotype into other *Pilosella* lineages after secondary contact

Historical hybridization between subgenera *Pilosella* and *Hieracium* might have been responsible for an accelerated rate of cpDNA evolution and speciation as well as for an increased ecological amplitude of species in the introgressed lineage.