ABSTRACTS

Hieracium s.str. speciation and evolution

Judith Fehrer, Karol Krak & Jindřich Chrtek

Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, 25243 Průhonice, Czech Republic

The large number of species and the presumed extensive past hybridization combined with an abundant occurrence of polyploidy and apomictic reproduction have presented enormous difficulties for the taxonomy of *Hieracium* s.str. Thus, the study of their relationships was greatly hampered by species circumscription. Our first molecular analysis of this group that focused on diploids and polyploid basic species revealed that the problems go even deeper than anticipated, but also helped to understand the basic processes involved in *Hieracium* speciation. Only a small part of the basic species did *not* have hybrid origin. These formed two groups coinciding with Western or Eastern European origin who probably survived in different glacial refugia. The 'Western' group comprised *H. humile*, *H. tomentosum*, *H. pictum*, *H. stelligerum*, *H. lawsonii*, *H. ramondii*, and *H. recoderi*, which have their main distribution in the Western Alps and in the Pyrenees, but also the widespread or Central European species *H. bifidum*, *H. murorum*, and *H. schmidtii*. The only species not fitting geographically into the 'Western' group was *H. transylvanicum*. Hybrid origin within the 'Western' group was found for *H. lucidum* (NW Sicily) and *H. cordifolium*, *H. gymnocerinthe*, *H. candidum* and *H. cerinthoides* (Pyrenees). Apart from a distinguishable subgroup of Pyrenean species, almost no genetic diversity was found among 'Western' species. To the 'Eastern' group belonged (i) all species from the Balkans (*H. petrovae*, *H. kittanae*, *H. naegelianum*, *H. pannosum* and *H. sparsum* – the latter had apparently hybrid origin), which were most basal, (ii) *H. alpinum* with a lineage on its own, (iii) a group consisting of *H. porrifolium*, *H. bupleuroides*, *H. villosum* and *H. pilosum*, and (iv) a group comprising *H. umbellatum*, *H. eriophorum*, *H. virosum*, and *H. canadense*. Besides, *H. pojoritense* and one accession of *H. bupleuroides* had hybrid origin involving 'Eastern' parents. Despite these clearly distinguishable species groups, the low genetic variation also occurring among 'Eastern' species suggests a rapid recent divergence of the different lineages. Most taxa with hybrid origin comprised species from both groups and may have mainly formed as a result of secondary contact after the retreat of glaciers. They comprised *H. prenanthoides*, *H. amplexicaule*, *H. gouani*, *H. mixtum*, *H. caesium*, *H. racemosum*, *H. sabaudum*, *H. bracteolatum*, *H. lachenalii*, *H. laevigatum*, *H. heterogynum*, *H. plumulosum* (*H. waldsteinii* s.l.), *H. gymnocephalum*, *H. olympicum*, *H. glaucum*, and single accessions of *H. villosum* and *H. pilosum*. Several genetic lineages occurred only in hybrids, but not in any extant 'pure' species. For example, *H. bracteolatum*, *H. racemosum*, *H. sabaudum*, *H. lucidum*, *H. olympicum* and one accession of each *H. prenanthoides*, *H. villosum*, *H. pilosum*, and possibly also *H. mixtum* had hybrid origin involving at least one extinct parent; *two* extinct lineages contributed to *H. gymnocephalum*, *H. plumulosum* and *H. heterogynum*. In the latter two taxa and in two accessions of *H. prenanthoides*, contributions from 3-4 different parents were detected. A large number of meanwhile extinct taxa involved in the hybrid origin of basic (even diploid) species helps to better understand the taxonomic difficulties in *Hieracium* s.str. (paper available at http://www.biomedcentral.com/1471-2148/9/239)

Three new low-copy nuclear markers for low level systematic studies in the Asteraceae – development and preliminary results of the phylogenetic analysis of *Hieracium* subgen. *Hieracium* and *Hieracium* subgen. *Chionoracium*

Karol Krak, Judith Fehrer, Petra Caklová & Jindřich Chrtek

Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, 25243 Průhonice, Czech Republic

In molecular systematics of plants, low-copy nuclear genes are the tools of choice in cases when cpDNA and nrDNA give only restricted resolution and/or conflicting phylogenetic signals. Such results are relatively frequent in studies at species level (or below), especially when polyploidization, hybridization and introgression are the driving forces of the evolutionary processes.

Here we present three newly developed low-copy nuclear markers for the Asteraceae: Gamma-glutamylcysteine synthetase (GSH1), Squalene synthase (SQS) and Glycine hydroxymethyltransferase (SHMT). All three markers amplified successfully in the representatives of 7 out of 8 tested tribes of the family. To assess their potential utility for low taxonomic level phylogenies, the phylogenetic signal and the level of variation for each of these markers was estimated on a small number of accessions from the Lactuceae subtribe *Hieraciinae*. All three markers have considerably higher variation (1.9–4.5 times) than ITS. However, incongruence in tree topologies was observed among the results of the different phylogenetic analyses.

Given that all accessions used for the pilot study were confirmed diploids and that no more than two sequence types per accession were obtained despite the high number of sequenced clones, we consider both intraindividual variability and topological incongruence to be results of allelic variation connected with extensive hybridization processes rather than paralogy caused by gene duplication.

Two of these markers, SQS and SHMT were analyzed in 70 species of *Hieracium* subgen. *Hieracium* and *Hieracium* subgen. *Chionoracium* represented by 80 accessions. The preliminary analyses of these datasets resulted in huge topological incongruences when the low copy nuclear gene phylogenies were compared to each other or to previously published results (based on nrDNA ETS and cpDNA). Likewise in morphology, frequent multi-directional hybridization processes affected the evolutionary pathway of these subgenera into a maze, complex and obscure from the molecular point of view as well.

Patterns of molecular variation in Swedish *Hieracium* s. str.

Torbjörn Tyler

With cooperation from Mikael Hedrén, Jane Jönsson & Maren Wellenreuther

Department of Biology, Botanical museum, Lund University, Ö. Vallgatan 20, SE-223 62 Lund, Sweden, email: torbjorn.tyler@botmus.lu.se

To initiate the first studies of molecular variation in Fennoscandian *Hieracium* s. str, we wanted to know the ploidy level of as many micro-species as possible, and the overall pattern of variation in the (biparentally inherited) nuclear and (maternally inherited) chloroplast genomes, and to compare this data with patterns of morphometric variation and taxonomic classifications. To achieve this we have 1) analysed 679 accessions representing 242 (micro-)species with flow cytometry (ploidy-level analysis), 2) developed 10 novel primer pairs (using a modified FIASCO-protocol) that amplify polymorphic nuclear microsatellite loci ± universally within the genus, 3) analysed 530 accessions (mainly Swedish members of sect. *Hieracium*, *Bifida*, *Vulgata*, *Tridentata* and *Oreadea*, but also some representatives of other sections from other geographic regions) with respect to these 10 microsatellite loci, 4) screened c. 20 chloroplast regions for sequence variation (using 10 representatives of the abovementioned sections), and based on that screening we have 5) sequenced the *atp*B–*rbc*L intergeneric spacer for c. 500 accessions representing c. 200 (micro-)species. The ploidy level analysis revealed that, contrary to what has previously been assumed, triploids and tetraploids are approximately equally common among Swedish members of *H.* sect. *Hieracium*, *Bifida*, *Vulgata*, *Tridentata* and *Oreadea*. In addition, one pentaploid microspecies was also revealed. Triploids and tetraploids occured in all four sections, but tetraploids were found to be more common among species from northern Scandinavia, and, more importantly, among species morphologically intermediate between the sections. With very few exceptions, (micro-)species were found to be homogeneous with respect to the ploidy level. The nuclear microsatellites revealed extensive variation, and a preliminary analysis of this data will be presented at the workshop. As shown by previous work, the chloroplast genome of *Hieracium* was found to contain only low levels of variation, but based on the *atp*B–*rbc*L sequences, two major and relatively distantly related haplotypes (with rather equal frequencies in the material analysed) and a number of subtypes of these could be identified. A preliminary analysis where this haplotype variation is related to morphology and taxonomy will be presented at the workshop.

Variation in apomictic *Pilosella* species: do we know how and where it is expressed?

František Krahulec, Anna Krahulcová, Ivana Plačková & Radka Rosenbaumová

Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Průhonice, Czech Republic

The present taxonomic concept of apomictic *Pilosella* species is a broad one; most of the species are represented by many genotypes. This seems very reasonable with respect to the relatively high level of residual sexuality.

The infraspecific variation is expressed at several levels: chromosomal (different ploidy levels), genotype and chloroplast DNA haplotype level. Variation in ploidy levels is the best known, because during more than 50 years considerable knowledge has been collected. But even the most known species as *P. officinarum* (both sexual and apomictic) were only fragmentarily studied in some parts of the distribution area (in this and most other species, especially in the south-eastern part of Europe).

Variation at the genotype level is known extremely unevenly. Whereas in some geographic areas that have been studied extensively in the past, the knowledge is deep, in most areas, nothing is known. Most of the basic species have a low level of variation in some parts of the distribution area, while high variation was recorded in some smaller areas. The most variable seems to be *P. piloselloides* subsp. *bauhinii*, which has many genotypes at the locality level.

The situation of intermediate species is different. Their variation evidently depends on the frequency of their origin: sometimes variation is expressed at the locality level, sometimes on larger geographic scale. There are differences in particular regions, similar to basic species.

We have only fragmentary knowledge about the distribution of chloroplast haplotypes. The existing knowledge allowed us to study the origin of some hybridogenous taxa in particular regions, but phylogeographic studies are not possible so far.

Evolutionary significance of polyploidy and hybridization in the *Pilosella alpicola* group – what we know and what we would like to know

Barbora Šingliarová[1], Patrik Mráz[1,2], Iva Hodálová[1] & Jindřich Chrtek[3,4]

[1] Institute of Botany, Slovak Academy of Sciences, Dúbravská cesta 14, SK-845 23 Bratislava, Slovakia, e-mail: barbora.singliarova@savba.sk
[2] Department of Biology, University of Fribourg, CH-1700 Fribourg, Switzerland
[3] Department of Botany, Charles University, Benátská 2, CZ-128 01 Praha 2
[4] Institute of Botany, Academy of Sciences of the Czech Republic, CZ-25243 Průhonice, Czech Republic

According to our biosystematic study, the *Pilosella alpicola* group comprises four morphologically well-differentiated and geographically vicariant species with contrasting cytotype pattern. *Pilosella alpicola* s.str. is a polyploid (4*x* and 5*x*) taxon distributed in the Swiss and Italian Alps. *Pilosella ullepitschii* is a diploid, self-incompatible taxon endemic to the Carpathians. Four ploidy levels (2*x*–5*x*) with a complex cytogeographic pattern and a high frequency of mixed-ploidy populations were recorded in *P. rhodopea*. *Pilosella serbica* is a diploid sexual taxon confirmed only from Serbia and Montenegro. In spite of a clear morphological separation, molecular analyses suggest a rather recent and monophyletic origin of the group, with the exception of *P. alpicola* s.str. While *P. rhodopea* polyploids originated by autopolyploidization, *P. alpicola* s.str. is an allopolyploid species that originated from hybridization between Balkan *P. rhodopea* and Alpine *P. glaciale*. The reproduction mode of the polyploids is linked to their origin. All cytotypes of *P. rhodopea* reproduce sexually, while polyploids of *P. alpicola* s.str. are apomictic. Allozymic differentiation among morphologically well-defined species was rather low, but reflecting the mode of reproduction and the type of polyploidy.

Genetic markers used so far (allozymes, cpDNA and ITS sequences) failed to show evolutionary relationships between the closely related *P. rhodopea*, *P. ullepitchii* and *P. serbica*. Therefore, we plan to use more discriminative markers such as AFLPs. Hybridogeneous origin of *P. alpicola* s.str. will be studied by FISH and GISH techniques to determine how many copies of each parental genome were involved in the tetra- and pentaploid cytotype of *P. alpicola* s.str.

Our further research will be focused on the fine-spatial cytotype structure and on microhabitat preferences of the cytotypes of *P. rhodopea* in mixed-ploidy populations, on gene flow between cytotypes and on differences in fitness under natural and experimental conditions. Morphological characters discriminating particular taxa of the *P. alpicola* group are mainly those considered as important phenotypic traits involved in evapotranspiration (density and colour of indumentum). Similarly, cline variation from North (Carpathians) to South (Balkan) was observed in genome size and some ecophysiological traits related to water-use efficiency. It seems that adaptive vicariant speciation after range fragmentation played an important role in the evolutionary history of the group. Preliminary results based on plants collected in the field should be verified in a common garden experiment.

Natural hybridization occurs, when *P. rhodopea* and *P. serbica* co-occur with *P. macrantha* or *P. pseudopilosella* as evidenced by intermediate morphology and additive patterns of ITS sequences. However, we do not know anything about the frequency and direction of the interspecific gene-flow.

Geographic pattern of cytotype variation in *Pilosella echioides* (Asteraceae) in Europe

Pavel Trávníček[1,2], Jindřich Chrtek[1,2], Zuzana Dočkalová[1], Tomáš Peckert[3], Radka Rosenbaumová[4] & Jana Rauchová[4]

[1] Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 53 Průhonice, Czech Republic
[2] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01 Praha, Czech Republic
[3] Administration of Český les Protected Landscape Area, Náměstí republiky 110, CZ-348 61 Přimda, Czech Republic
[4] Department of Botany, National Museum in Prague, Cirkusová 1740, 193 00 Praha, Czech Republic

Pilosella echioides is a perennial, predominantly sexual and self-incompatible herbaceous plant distributed in Central and Eastern Europe and in steppe regions of Asia. Six ploidy levels have been previously discovered in Central Europe, namely 2*x*, 3*x*, 4*x*, 5*x* and 6*x*, and several mixed-ploidy populations have been found. However, a more general geographical pattern of cytotypes and the frequency of particular cytotypes in mixed-ploidy populations remained a puzzle. Principal objectives of the present study were to elucidate the distribution of particular cytotypes (i) in Europe (large-scale pattern), and (ii) in a model area with previously detected mixed-ploidy populations in SW Moravia and an adjacent part of Lower Austria (small-scale pattern). Diploid populations seem to prevail throughout Europe; mixed ploidy populations with two to four intermingled sexual cytotypes (2*x*, 3*x*, 4*x*, 5*x*) occur in the Czech Republic and in Lower Austria. Populations consisting exclusively of tetraploid apomictic plants were discovered in northern Hungary and southern Slovakia (Danube Basin), their taxonomic position is still unclear. Besides these, sexual tetraploids were found in NW Hungary. The extremely complicated variation in Hungary is underlined by the occurrence of intermediate types between *P. echioides* and *P. rothiana*. A detailed survey of mixed-ploidy populations at the Havraníky heathland (SW Moravia, Czech Republic) and adjacent areas revealed a common co-occurrence of up to four cytotypes (2*x*, 3*x*, 4*x* and 5*x*) even at a fine spatial scale (several square centimetres). Triploids markedly prevailed (more than 75% of the plants), considerably lower frequencies were detected for diploids, tetraploids, and pentaploids. The predominance of the triploid (sexual) cytotype is with no doubt mysterious, especially if we consider that only sexual (allogamy) reproduction was confirmed for all cytotypes and that crosses between two triploids never resulted in triploid progeny as showed by hand pollination. Elucidation of the spatial pattern at the Havraníky heathland falls in a complex project aimed at the population dynamics of mixed ploidy populations. Detailed analysis of cytotype diversity in the progeny of di-, tri- and tetraploid plants from subpopulations with contrasting frequencies of cytotypes and consequent comparison with spatial pattern of adult plants is in progress.

Floral scent investigations and their taxonomical use in *Hieracium*

Martin Feulner

Department of Plant Systematics, University of Bayreuth, Universitätsstr. 30, 95440 Bayreuth, Germany

The main question of floral scent investigation regards interactions of flowering plants and their pollinators. Profiles of floral scent compounds were rarely used for phylogenetic analyses and taxonomy. Nevertheless, in some cases tree topologies generated by non-coding DNA markers are in some cases congruent with those based on floral scent data. Species-rich *Hieracium* is well-known for a high degree of endemism and infra-specific differentiation including many subspecies ("microspecies") of very restricted distribution. In *Hieracium* subgen. *Pilosella* floral scents of 27 predominantly Bavarian species, mostly of *Hieracium calodon*, *H. zizianum* and *H. densiflorum* are investigated. In *Hieracium* s. str. 37 taxa were investigated so far. Floral scent compositions were studied by GC-MS analysis of dynamic headspace samples. Altogether, about 60 floral scent compounds were identified in both subgenera, mainly benzenoids, fatty acid derivatives, monoterpenes, homoterpenes and sesquiterpenes. Only few subgenus-specific substances could be identified. The chemical patterns were found to be species-specific and are thus of taxonomical value in both subgenera. The data support some rearrangements at subspecific level, such as the inclusion of *H. bauhini* subsp. *hispidissimum* in *H. densiflorum*. In *Hieracium* s. str. a close relationship between sections comprising high growing plants as sections *Tridentata*, *Drepanoidea* and *Umbellata*, as well as between sections comprising plants with zero to one stem leaf as sections *Hieracium*, *Bifida* or *Oreadea* was found.

The taxonomy and distribution of *Hieracium* and *Pilosella* (Asteraceae) in Mongolia and China

Alexander N. Sennikov

Botanical Museum, Finnish Museum of Natural History, P. O. Box 7, FI-00014
University of Helsinki, Finland, email: alexander.sennikov@helsinki.fi

The diversity of *Hieracium* L. and *Pilosella* Hill in Eurasia is much declining eastwards, with rather few species registered from Mongolia and China. The need for a new revision of these taxa appeared in a compilation of V. I. Grubov (ed.), *Plants of Central Asia* (vol. 14b, *Compositae*: *Cichorieae* published in 1 Feb 2009). The basis of this revision were the herbarium collections of LE and MW.

Ten species are recorded in the genus *Hieracium*. The most widely distributed species belong to the groups *Virosum – Umbellatum*, represented by *H. virosum* Pall. (syn. *H. czamyjashense* Tupitzina), polymorphic *H. robustum* Fr. s. l. (syn. *H. robustum* subsp. *turanicum* Zahn, *H. sangilense* Tupitzina), and *H. umbellatum* L. s. l. Special Tian-Shanian taxa are *H. kirghisorum* Üksip s. l. and *H. regelianum* Zahn, whereas *H. krylovii* Nevski ex Schljakov is found in Tian-Shan and Altai. *Hieracium czadanense* Tupitzina (syn. *H. crocatum* Fr. subsp. *mongolicum* Bräutigam) is a narrowly distributed taxon of Tuva and Mongolia. The puzzling *H. subramosum* Lönnr. (syn. *H. ganeschinii* Zahn, *H. erythrocarpum* Peter subsp. *aryslynense* Zahn) and *H. kuusamoënse* Vainio (syn. *H. schischkinii* Üksip, *H. constringentiforme* Üksip) have almost no observable variation in Fennoscandia, East Europe, southern Siberia and Tian-Shan. The peculiar *H. korshinskyi* Zahn (syn. *H. tschamkorijense* Zahn subsp. *alatavicum* Zahn, *H. almaatense* B.Fedtsch. et Nevski, *H. aczelmanicum* Schischk. et Serg., *H. turkestanicum* (Zahn) Üksip) continues from Tian-Shan to the Himalayas. The difference in the *Hieracium* taxa between Tian-Shan and Altai is reflected mostly in the relevant poverty of the latter.

In the genus *Pilosella*, *P. echioides* (Lumn.) F.Schultz et Sch. Bip. (*P. asiatica* (Naeg. et Peter) Schljakov), *P. proceriformis* (Naeg. et Peter) Soják s. l. (syn. *P. tjumentzevii* (Serg. et Üksip) Tupitzina, *P. katunensis* Tupitzina), *P. dublitzkii* (B.Fedtsch. et Nevski) Sennikov (syn. *P. altaica* (Naeg. et Peter) Schljakov), *P. sabinopsis* (Ganesch. et Zahn) Tupitzina and a hitherto undescribed species are recognised. Among these five taxa, *P. sabinopsis* has a narrow distribution in S Siberia and N Mongolia, and the taxa of *P.* sect. *Echinina* show a prominent difference between lowland steppe and montane taxa, and between Tian-Shan and Mongolian Altai.

After publication of the aforementioned account, a new revised and detailed treatment is in progress, with descriptions, dot distribution maps, typifications and discussions, to be finished in 2010.

Case study of *Pilosella officinarum* in Central Europe: ploidy and breeding systems and their correlation with morphology

Tomáš Urfus

Department of Botany, Faculty of Science, Charles University in Prague, Benátska 2, Prague, Czech Republic

Pilosella officinarum F. W. Schultz et Sch. Bip. (syn. Hieracium *pilosella* L.; Asteraceae) belongs to a complex group of partly apomictic plants. The enormous variability is caused especially by: polyploidization, a combination of the sexual and apomictic breeding mode, widespread hybridization, and vegetative reproduction (Krahulcová et al. 2000).

DNA ploidy level (determined by Flow Cytometry) or chromosome counts were determined for 768 plants of *Pilosella officinarum* from 216 localities from all over the area.

Three ploidy levels were recorded within the area of Central Europe. The most widespread cytotype was the tetraploid one ($2n$=36, 65%), the second most common was pentaploid ($2n$=45, 18%), and the least common was the hexaploid level ($2n$=54, 17%). Breeding systems of most of the plants were determined. They showed that tetraploids were sexual and pentaploids were apomictic, but hexaploid plants were according to breeding mode separated in two distinct groups (apomicts and sexuals), which are also geographically devided.

Morphometric analysis was carried out for individual plants of particular cytotypes ($4x$, $5x$, $6x$). Principal Component Analysis, Discriminant Analysis and Nonparametrical Classification Analysis detected that tetraploid and hexaploid plants are distinctly morphologically separated, while pentaploids share morphological features of both. Nevertheless, the pentaploid cytotype keeps special characters that slightly distinguish pentaploid plants from the others. Even the sexual and apomictic hexaploids can be distinguished on the basis of morphological features. Such results indicate that both groups of hexaploids might be unrelated. Sexual hexaploids are considered to be of relict origin whereas apomictic hexaploids appear to be connected to a possible hexaploid cytotype distribution centre in the Carpathian Mts. as well as the majority of the pentaploid cytotype. Such results may reflect reticulate evolution of the group and they are reflecting the complexity of the traditional taxonomic view.

Structure of selected *Pilosella* populations in Bulgaria and their comparison with central Europe

Anna Krahulcová[1], Vladimir Vladimirov[2], František Krahulec[1] & Siegfried Bräutigam[3]

[1] Institute of Botany, Academy of Sciences of the Czech Republic, 252 43 Průhonice, Czech Republic, e-mail: krahulcova@ibot.cas.cz, krahulec@ibot.cas.cz
[2] Institute of Botany, Bulgarian Academy of Sciences, Acad. Georgi Bonchev St., bl. 23, 1113 Sofia, Bulgaria, e-mail: vdvlad@bio.bas.bg
[3] Senckenberg Museum für Naturkunde Görlitz, Postfach D-300154, 02806 Görlitz, Germany

During the last years we paid attention to the structure of *Pilosella* populations in Bulgaria. Chromosome numbers, breeding systems, and additionally chloroplast haplotypes and clonal structure were studied. In comparison with Central Europe, there are pronounced differences:

1. There are more diploid species, but it seems that they are isolated from the polyploid ones; their hybridization is comparatively very rare.
2. Polyploid sexual types are almost absent (except triploid *P. alpicola* and tetraploid *P. pavichii*).
3. Most of the polyploids are hexaploids, more rare are pentaploids, and tetraploids (*P. officinarum*, *P. pavichii*) are very rare.
4. We did not find any 2*n*+*n* hybrids, probably because of low viability of potential high polyploids.
5. Stabilized hybrids are probably very rare in Bulgaria; as far as we know, only *P. guthnickiana* is such a case.

First results of this project were published recently (Krahulcová A., Vladimirov V., Krahulec F. & Bräutigam S. (2009): The agamic complex of *Pilosella* (Asteraceae) in Bulgaria and SW Romania: variation in ploidy levels and breeding systems. – Phytologia Balcanica 15/3: 377-384.)

Populations of the *Pilosella* species in ruderal habitats within the city of Prague: chromosome numbers and reproductive mode

Veronika Křišťálová[1], Jindřich Chrtek[2,3], Anna Krahulcová[2] & František Krahulec[2]

[1] Department of Ecology, Faculty of Environmental Sciences, Czech University of Life Sciences, Kamýcká 129, CZ-165 21 Prague 6 - Suchdol, Czech Republic, email: vkostalova@gmail.com
[2] Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic, email: krahulec@ibot.cas.cz
[3] Department of Botany, Faculty of Science, Charles University in Prague, Benátská 2, CZ-128 01, Prague, Czech Republic, e-mail: jindrich.chrtek@ibot.cas.cz

Pilosella populations were studied on 49 selected localities situated along highways and railways within the city of Prague and its close vicinity. These habitats are especially suitable for the spread, formation and survival of these species, because competition-free habitats are formed. The study was done during a short period; altogether, four basic species and 12 hybridogenous ones (or hybrids) were recorded. The following species, hybridogenous species or hybrids (with ploidy levels/chromosome numbers and reproductive mode) were found: *P. aurantiaca*, *P. caespitosa* (4*x*, 5*x*), *P. officinarum* ($2n = 36$, sexual; $2n = 54$, sexual; $2n = 63$), *P. piloselloides* subsp. *bauhinii* ($2n = 45$, 54; both apomictic), *P. piloselloides* subsp. *praealta* (5*x*; apomictic), *P. brachiata* (4*x*; sterile), *P. densiflora* (4*x*), *P. flagellaris, P. floribunda*, *P. erythrochrista*, *P. glomerata* (4*x*, 5*x*; apomictic), *P. leptophyton* (5*x*; apomictic), *P. rothiana* (4*x*, apomictic), *P. setigera* (apomictic), *P. visiani* (4*x*; apomictic), *P. ziziana* (apomictic), and a so far undescribed hybridogenous type, *P. setigera* × *P. piloselloides*.

Pilosella visianii is reported from the Czech Republic for the first time. New habitats formed during highway construction seem to be suitable for *Pilosella* species. Many types previously known to be rare such as *P. rothiana* find here open space for further spreading, not only at the local scale, but throughout the whole country.
(The paper was submitted to Preslia.)

PROJECTS

Residual sexuality in facultatively apomictic plants: detection, rate and manifestation in the populations of *Pilosella*

Anna Krahulcová[1], Olga Rotreklová[2], František Krahulec[1] & Radka Rosenbaumová[1,3]

[1] *Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice*
[2] *Institute of Botany and Zoology, Faculty of Sciences, Masaryk University, Brno*
[3] *Department of Botany, National Museum, Prague*

Facultative apomixis combines usually dominant asexual seed production replicating the maternal genome, and occassional sexuality via either reduced or unreduced gametes. Part of the progeny can also be generated by haploid parthenogenesis, which recombines and halves the maternal genome. The currently finished project was aimed at the apomictic *P. piloselloides* (pentaploid and hexaploid), *P. aurantiaca* (tetraploid) and their hybrids which are capable of producing non-maternal (aberrant) progeny: 1) in natural hybrid swarms where the tetraploid sexual *P. officinarum* coexists and 2) in experimental crosses with this sexual pollen donor. The resulting progeny was screened using flow cytometry and/or specific morphological traits in cultivated seedlings. If the putative parental taxa in the field differed in their chloroplast DNA haplotypes, the maternal parent of coexisting recent hybrids was determined. The actual population structure was compared with the structure of the seed progeny generated by both facultatively apomictic and sexual mothers. The role of apomicts as paternal plants in crosses was also estimated, using the pollen stainability test.

The apomictic mothers gave rise to very diverse progeny, namely in advanced hybrid swarms. With respect to ploidy level and reproductive mode, they generated even more variation than did their sexual counterparts. Most of the progeny types formed by facultatively apomictic mothers as seeds were also detected among mature plants in the respective populations. Exceptions were both the polyhaploids (dihaploids and mostly also trihaploids) and the progeny exceeding the octoploid level, which usually originated from fertilization of unreduced female gametes. Such progeny types probably have a negligible viability in nature. Again, the high-polyploid progeny ($6x$ – $8x$) has an important role in hybridizing populations due to the versatile reproductive mode. Most apomicts in *Pilosella* can hybridize as maternal as well as paternal plants. Several apomictic pollen-less biotypes were detected.

The system consisting of the parental tetraploid species *P. aurantiaca* (strong apomictic reproduction), *P. officinarum* (sexual) and their hexaploid $2n + n$ hybrids was used to study the penetrance of apomixis. All hybrids contained a complete genome of the apomictic mother and a half genome of the sexual parent. Diverse expressivity of apomixis in hybrids corresponds to a multi-locus model of inheritance, which was recently proposed for *Poa pratensis*.

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Phylogeny of subtribe Hieraciinae (Asteraceae) – a model example of contrasting evolutionary strategies in closely related lineages

Jindřich Chrtek & Judith Fehrer

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

Evolutionary patterns in related lineages with contrasting ploidy and reproductive mode are crucial topics of contemporary biology. Our model system, subtribe *Hieraciinae* (Asteraceae), combines features that are unique in plants: two different kinds of apomixis and the presence of a sexual sister genus. A multigene phylogeny based on complete or (in apomictic groups) representative sampling will serve as a backbone for interpretations on 1) historical biogeography: localization of evolutionary and diversity centres of the subtribe (connections of Iberian and North African floras, Macaronesian colonization, migration routes between Eurasia and North America); 2) genome size: diversification and evolution, responsible mechanisms at the genomic level (diversity and quantity of transposable elements), relation to ecogeographical and biological factors, adaptive role; 3) karyotype evolution: localization of transposable elements, especially in connection with the Avoidance of Meiosis Locus (LOA), and their potential influence on reproductive isolation and the origin of apomixis.

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Phylogeography and cytotype structure of *Hieracium intybaceum* (Asteraceae)

Jaroslav Zahradníček[1] & Jindřich Chrtek Jr.[1,2]

[1] Department of Botany, Faculty of Science, Charles University in Prague, Benátska 2, Prague, Czech Republic, email: j.zahradnicek@gmail.com
[2] Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic

Effects of climate changes on the genetic structure of populations and changes in distribution areas (fragmentation, retreat to lower altitudes and recolonization, differentiation, surviving in refugia) in the recent history of alpine species belong to very timely research areas as they contribute to our knowledge of the phylogeny in large mountain ranges.

Hieracium intybaceum (Asteraceae) is a well defined, karyologically differentiated species (di-, tri- and tetraploids) that grows on silicate rocks in high altitudes in the Alps, Vosges and Schwarzwald Mts. Though traditionally recognised as a part of genus *Hieracium* (subgenus *Hieracium*), molecular data suggest an older isolated lineage; there are most likely some differences in reproduction, too.

The proposed combination of molecular and flow-cytometry methods along with morphometrics and reproduction experiments will help to elucidate the phylogeographical pattern within and among cytotypes and the origin of new polyploid cytotypes. It can also contribute to the knowledge of main refugia and migration routes during the recolonization of high altitudes.

Understanding the cytotype structure in the distribution area and ecological demands of particular ploidy levels along with the knowledge of reproduction can clarify the mechanisms helping the polyploid complexes to survive and evolve.

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POSTERS

Pilosella aurantiaca and *P. officinarum* hybridizing in the field: population structure of an exemplary hybrid swarm in North Rhine-Westphalia, Germany

Anna Krahulcová[1], Uwe Raabe[2] & František Krahulec[1]

[1] Institute of Botany, Academy of Sciences of the Czech Republic, Průhonice, Czech Republic
[2] Landesamt für Natur, Umwelt und Verbraucherschutz NRW, Recklinghausen, Germany

The population is comprised of two tetraploid, morphologically distinct parental species, an introduced facultatively apomictic *P. aurantiaca*, a native sexual *P. officinarum* and their recent hybrids, which are tetraploid or hexaploid. The hybrid swarm, first found in 1990, is growing on nutrient-poor fallow land, but the meadow was occassionally mown in the past. A wide spectrum of coexisting hybrid morphotypes has practically been unchanged during twenty years, involving types i) more close to *P. aurantiaca* (corresponding to *P. rubra*), ii) intermediate between the parental species (*P. stoloniflora*), and iii) several different types more or less close to *P. officinarum*. When comparing the present situation with that twenty years ago, the abundance of some morphotypes as well as the whole population size have changed. Recently, the population structure was studied with respect to ploidy level, genome size, breeding system and chloroplast-DNA haplotypes. Using isozyme phenotypes, the genotype structure of apomictic plants was identified.

The hybrid corresponding to *P. rubra* is hexaploid with a variable reproductive mode, producing a considerable amount of sexual and polyhaploid progeny besides true apomictic progeny. Its seed fertility is reduced. The genotype structure and DNA content in this hexaploid suggest a repeated origin via $2n + n$ hybridization of *P. aurantiaca* (maternal parent) and *P. officinarum*. The other coexisting hybrids are tetraploid and sexual. Two chloroplast-DNA haplotypes were found in *P. officinarum* at this locality, one of them shared with *P. aurantiaca*. This fact did not allow an unequivocal identification of the maternal parent in the hybrids. The different genome size (DNA content in the monoploid chromosome set) in the putative parental species, *P. aurantiaca* and *P. officinarum*, is reflected in their homoploid hybrids which have different proportions of parental genomes. Thus, multistep hybridization (backcross to *P. officinarum*) suggested by morphological characters of the tetraploid hybrids, was supported by genome size data.

Sexual reproduction as a source of ploidy level variation in the agamic complex of *Hieracium* subgenus *Pilosella* (*H. pilosella* and *H. bauhinii* as a model system)

Radka Rosenbaumová[1], Anna Krahulcová[2] & František Krahulec[2]

[1] *Department of Botany, National Museum, Cirkusová 1740, CZ-193 00 Prague, Czech Republic*
[2] *Institute of Botany, Academy of Sciences of the Czech Republic, Zámek 1, CZ-252 43 Průhonice, Czech Republic*

Apomixis (clonal reproduction through seeds) is not usually regarded as a source of genetic variation. Nevertheless, exceptional diversity in morphology, ploidy level, mode of reproduction, etc. emerges in agamic complexes, where apomixis coexists with sexual reproduction. A study of recent hybridizations in young agamic complexes, e.g. *Hieracium* subgen. *Pilosella*, can improve our understanding of the origin of such diversity.

A model population from the vicinity of Valov (NW Bohemia) consisted of 4*x* sexual *H. pilosella* and 6*x* apomictic *H. bauhinii* (parental species), and their 5*x*, 7*x*, and 8*x* hybrids. Crosses between the parental species were performed to quantify their potential to produce ploidy level variation. Flow cytometry revealed significant ploidy level variation in the progeny, showing that reduced as well as unreduced gametes of both parents participated in crosses. The progeny from the cross where *H. pilosella* served as a maternal parent (273 plants) consisted of 4*x* progeny resulting from autogamy (18.3%) and of 5*x* hybrids (81.7%). The progeny from the cross where *H. bauhinii* served as a maternal parent (821 plants) consisted of apomictically derived 6*x* progeny (93.2%), of three types of hybrids (5*x* - 4.8%, 7*x* - 0.1%, and 8*x* - 0.7%), and of 3*x* parthenogenetic progeny (1.2%). To quantify the ploidy level variation really formed in the field, the ploidy level was estimated in the progeny that was obtained from seeds collected from the parental species in the model population. The progeny of *H. pilosella* (317 plants) consisted of 4*x* progeny from auto/allogamy (98.7%) and of 5*x* hybrids (1.3%). The progeny of *H. bauhinii* (486 plants) consisted of apomictically derived 6*x* progeny (92.4%), of four types of hybrids (5*x* - 2.7%, 7*x* - 0.4%, 8*x* - 2.9%, and 10*x* - 0.4%), and of 3*x* parthenogenetic progeny (1.2%).

Hybridization between *H. pilosella* and *H. bauhinii* generated significant variation in ploidy level under both experimental and field conditions. Nevertheless, it seems that only a part of this variation can influence evolution of the population because neither 3*x*, nor 10*x* adults were detected in the field. Some selection disadvantage of these cytotypes can be suggested.

When regarded as maternal parents, sexual *H. pilosella* gave rise to significantly lower ploidy level variation than apomictic *H. bauhinii*, and the proportion of 5*x* hybrids produced by *H. pilosella* was much lower in the field than in the experiment. The apomictic species thus appears to be a better source of ploidy variation in the population.

Variability in morphology, karyology and reproductive behaviour in a hybrid swarm of hawkweeds from Rašovice village (South Moravia)

Olga Rotreklová[1] & Anna Krahulcová[2]

[1] Department of Botany and Zoology, Masaryk University, CZ-611 37 Brno, Czech Republic; e-mail: orotrekl@sci.muni.cz
[2] Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 53 Průhonice, Czech Republic

Hieracium subgen. *Pilosella* has a high taxonomic diversity including hybridogenous types. Population studies on co-occuring sexual and facultative apomictic taxa are necessary to understand the selective processes during the evolution of new types. In a hybrid swarm situated on a steppe slope, the variability of plants in morphology, ploidy level, reproductive systems and progeny origins was studied. On the locality co-occurred two parental taxa, *Hieracium pilosella* (6*x*, facultatively apomictic), *H. densiflorum* (4*x*, sexual), and their hybrids (4*x*, 5*x*, 6*x*, and 8*x*; sexual, apomictic or sterile). Hybrids were classified as *H. fallacinum* and *H. pilosellinum*, besides plants closely resembling *H. pilosella.* We found that:

1. Progeny of sexual *H. densiflorum*-mothers was uniform and formed exclusively sexually, via fusion of reduced gametes (*n*+*n* progeny).
2. Progeny of facultatively apomictic *H. pilosella*-mothers originated via four different pathways: apomixis (somatic parthenogenesis, 2*n*+0 progeny, the dominant breeding system), sexual mating via fusion of either reduced (*n*+*n* progeny) or unreduced gametes (2*n*+*n* progeny), and haploid parthenogenesis (*n*+0, polyhaploids).
3. The reproductive behavior of the hybrids was more diverse: Pentaploids were sterile or semisterile, poor seed-set was produced exclusively sexually (*n*+*n* progeny). Hexaploids were either semisterile or produced seeds sexually (*n*+*n* progeny prevailed while that of 2*n*+*n* origin was rare). Part of the octoploids (recent 2*n*+*n* hybrids derived from a pentaploid mother) were semisterile plants, which formed poor seed-set sexually (frequently *n*+*n* progeny, rarely 2*n*+*n* progeny). The other octoploids, derived from an octoploid apomictic mother, produced progeny apomictically (2*n*+0 progeny), almost half of their progeny were produced via haploid parthenogenesis (*n*+0 polyhaploid progeny), and only a small part of their seeds were produced sexually (either *n*+*n* or 2*n*+*n* progeny).
4. Tetraploid progeny, derived from apomictic octoploid mothers via haploid parthenogenesis, again produced mainly tetraploid progeny via apomixis, and rarely *n*+*n* or 2*n*+*n* progeny of sexual origin.
5. The maternal breeding system was conserved in the majority of the respective progenies.

Our study demonstrates the versatility of reproductive pathways operating in polyploid facultative apomicts in the field and the importance of their residual sexuality as we found in a former study on other hybrid swarms of *Hieracium* subgen. *Pilosella*.

Ploidy level and reproductive behaviour in facultatively apomictic high-polyploid *Hieracium* subgen. *Pilosella*

Olga Rotreklová[1] & Anna Krahulcová[2]

[1] *Department of Botany and Zoology, Masaryk University, CZ-611 37 Brno, Czech Republic; e-mail: orotrekl@sci.muni.cz*
[2] *Institute of Botany, Academy of Sciences of the Czech Republic, CZ-252 53 Průhonice, Czech Republic*

The reproductive behaviour and the capacity to generate variation in ploidy level was studied in hexaploid, heptaploid and octoploid mother-plants of *Hieracium* subgen. *Pilosella.* They originated in the field from hybridizations between facultatively apomictic and sexual species (via fertilization of unreduced gametes) in eight hybrid swarms: seven in the Czech Republic and one in Germany. In all of them, *Hieracium pilosella* (tetraploid, pentaploid or hexaploid, sexual or apomictic) was one parent, hybridizing either with *H. bauhinii* (pentaploid or hexaploid, apomictic), *H. aurantiacum* (tetraploid, apomictic) or with *H. densiflorum* (tetraploid, sexual). Seeds were collected both from open-pollinated plants in the field and from open-pollinated/emasculated plants in the garden. The method of Flow Cytometric Seed Screen (FCSS) was used to detect ploidy level and reproductive origin of embryos within particular maternal arrays; chromosome counts and flow cytometric detection of DNA-ploidy level were used to detect the variation in ploidy level within the cultivated seedlings.

Three ways in shaping the ploidy level variation were found:

1. Mating via unreduced gametes ($2n + n$, $n + 2n$) even further increased the maternal ploidy level in their progeny, reaching up to $8x – 12x$.

2. Mating via reduced gametes ($n + n$) or true apomixis ($2n + 0$) resulted in the cytotypes commonly occurring at the respective localities ($4x – 6x$), or it conserved the maternal ploidy level ($6x$, $7x$, $8x$), respectively.

3. Haploid parthenogenesis ($n + 0$) reduced the maternal ploidy level, ranging from $3x$ to $4x$ in the polyhaploid progeny.

The vigour observed in embryos and seedlings of the same origin was different. As many well-developed seeds had detectable embryo and endosperm using the FCSS method, only a small part of the seed-set collected from the same plants were able to germinate. Comparing the variation within the seeds and seedlings, a lack of progeny cytotypes with extreme ploidy levels (lower than $4x$ and those ranging between $10x – 12x$) was found in seedlings. The extremely high ploidy levels are yet more suppressed in the field, as no plants exceeding the octoploid level were found. Nevertheless, the facultatively apomictic high-polyploid hybrids may increase the total ploidy variation in populations. Due to relevant sexual reproduction, they can produce new biotypes with favourable combinations of characters.

***Hieracium alpinum* group in Slovenia**

Branko Vreš[1], Igor Dakskobler[2] & Brane Anderle[3]

[1] Jovan Hadži Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Novi trg 2, SI-1000 Ljubljana, Slovenia; e-mail: branevr@zrc-sazu.si
[2] Regional unit Tolmin, Jovan Hadži Institute of Biology, Scientific Research Centre of the Slovenian Academy of Sciences and Arts, Brunov drevored 13, SI-5220 Tolmin, Slovenia; e-mail: dakskobler@zrc-sazu.si
[3] Hraše 34, SI-4248 Hraše, Slovenia

During our investigations of the flora of the Slovenian Alps in the last decade, some new localities for the *Hieracium alpinum* group were found. In the past, only two localities for *H. alpinum* were known from Slovenia, both from the Julian Alps: Mt. Mangart (9547/4) and Kriški podi (9548/4). During the investigations in 2005 and 2006 the presence of *H. alpinum* on Mt. Mangart (9547/4) was confirmed. A new locality was found on the Italian side (close to the border) in 2009 and the present vegetation type was characterized. In 2007, *H. alpinum* was registered for the first time in the Kamnik Alps on the Korošica plateau (9653/2). In 2009, it was found on the ridge from Kreda to Slatna above the Fužina pasturelands (9648/4) in the Julian Alps and two new localities were reported also from the Peca (9454/4, 9554/2 - near the Austrian border in the Karavanke range). The vegetation type was also studied. On the latter localities *H. alpina* is confined to primary alpine or subalpine meadows with acidic soils in communities with *Avenula versicolor*. The presence of *H. halleri* in Slovenia was also confirmed. This species grows on two localities: on Mt. Mangart (9547/4) in the Julian Alps and on Mt. Belščica (9550/2) in the Karavanke range. Both species mentioned are proposed to be included in the Red data list of Slovenian vascular flora as rare (R) species.