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Genetic Variation in the *Hieracium rohacsense* Group (*Hieracium* sect. *Alpina*)

By

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With 2 Figures

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Summary

MRÁZ P., CHRTEK J. jun. & KIRSCHNER J. 2001. Genetic variation in the *Hieracium rohacsense* group (*Hieracium* sect. *Alpina*). – *Phyton* (Horn, Austria) 41 (2): 269–276, 2 figures. – English with German summary.

Five isozyme systems (AAT, ADH, LAP, PGM, SKD) were studied in two tetraploid apomictic taxa of the *Hieracium rohacsense* group (*Hieracium* sect. *Alpina*). No intra- and inter-population variation was found in *H. rohacsense* Krt., endemic to the West Carpathians, which is in accordance with its narrow morphological variation. In contrast, a probably still unnamed tetraploid taxon from Mt. Pop Ivan (Ukrainian East Carpathians) belonging to the *H. rohacsense* group was represented by three phenotypes detected in one population. The role of diploid sexual taxa occurring in the alpine and subalpine belts of the Ukrainian East Carpathians in maintaining genetic variability is discussed. Apart from morphological characters the two closely related taxa included in this study can be separated also by their patterns of Pgm-1 locus.

Zusammenfassung

MRÁZ P., CHRTEK J. Jun. & KIRSCHNER J. 2001. Genetische Variation in der *Hieracium rohacsense*-Gruppe (*Hieracium* sect. *Alpina*). – *Phyton* (Horn, Austria) 41(2): 269–276, 2 Abbildungen. – Englisch mit deutscher Zusammenfassung.

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Fünf Isozymsysteme (AAT, ADH, LAP, PGM, SKD) wurden in zwei tetraploiden, apomiktischen Taxa der *Hieracium rohacsense*-Gruppe (*Hieracium* sect. *Alpina*) untersucht. In *H. rohacsense* KIT., welches in den West-Karpaten endemisch ist, wurde Variabilität weder in, noch zwischen den Populationen festgestellt; dies steht mit der geringen morphologischen Variabilität im Einklang. Eine möglicherweise noch unbenannte, tetraploide Population vom Berg Pop Ivan (Ukrainische Ost-Karpaten), die zur *H. rohacsense*-Gruppe gehört, hatte dagegen drei Phänotypen in einer Population. Die Rolle diploider sexueller Taxa in der alpinen und subalpinen Stufe der Ukrainischen Ost-Karpaten für die Erhaltung der genetischen Vielfalt wird diskutiert. Die beiden hier behandelten, nahe verwandten, tetraploiden Taxa können, außer durch morphologische Merkmale (Behaarung), auch durch ihr Muster am Pgm-1-Lokus unterschieden werden.

Introduction

The genus *Hieracium* s.str. (hawkweed) belongs to a group of genera in which agamospermy (seed apomixis) plays a major role (GUSTAFSSON 1946–1947, NOGLER 1984, ASKER & JERLING 1992). Seeds develop by diplospory of the *Antennaria* type (embryo sac originates from megaspore mother cell directly by mitosis; incomplete meiosis resulting in unreduced gametes has also been reported, see BERGMAN 1941) coupled with autonomous endospermy, i.e. endosperm development is independent on fertilization of the polar nuclei (BERGMAN 1941, GENTCHEFF & GUSTAFSSON 1940, ROSENBERG 1927, RUTISHAUSER 1967, SKAWIŃSKA 1963). Agamospermy is closely associated with polyploidy; the great majority of *Hieracium* (s.str.) taxa are either triploid ($2n = 27$) or tetraploid ($2n = 36$) apomicts. Sexuality is extremely rare and is confined to a few diploid species (e.g. MERXMÜLLER 1975, SCHUHWERK 1996).

Agamospermy together with hybridization processes in the past have given rise to a very large number of constant variants, identifiable in nature and distinguishable from each other that have been described as either subspecies, as has traditionally been the case in Central Europe (cf. ZAHN 1921–1923), or at the rank of species (Great Britain, Scandinavia).

Hieracium rohacsense KIT. has been described from the Western Carpathians (Slovakia) whereas a related taxon from the Alps has been described as *H. rauzense* MURR. ZAHN 1936 treated them as a single species (*H. rohacsense*), which he divided into 39 subspecies. The distribution in Central Europe includes the Alps, the Carpathians and the Sudeten Mts. The present project focused on (i) *H. rohacsense* s.str., which is considered by the present authors to be endemic to the high mountain ranges of the Western Carpathians (MRÁZ & MARHOLD 1999), (ii) a distinct population found in the glacial cirque of Mt. Pop Ivan (Marmarosh Mts., Ukrainian Eastern Carpathians), which differs from any hitherto described infra-specific taxon of *H. rohacsense*.

The most important character that might be used to separate the "Pop Ivan" plants from *H. rohacsense* s. str. is the type of indumentum – they have more numerous glandular hairs and less numerous simple eglandular and stellate hairs on involucral bracts (MRÁZ unpubl.). Both *H. rohacsense* s.str. and "Pop Ivan" plants were proved to be tetraploid and agamospermous (emasculatation experiments, MRÁZ 2001).

Isozyme analysis has been successfully used for assessing the pattern of genetic variation in both agamospermous and sexual *Hieracium* species (populations) (SHI & al. 1996, STACE & al. 1997, ŠTORCHOVÁ & al. 2001). The aims of present study were: (i) to evaluate the amount and pattern of genetic variation in *Hieracium rohacsense* s.str. and in the closely related but morphologically distinct "Pop Ivan" population, and (ii) to investigate whether the isozymes could be useful as discriminatory taxonomic markers between both studied taxa.

Materials and Methods

Living plants were collected in 1996 by the first author, and then cultivated under field conditions in the experimental field at the Institute of Botany, Slovak Academy of Sciences in Bratislava. The origin of collections is given in Table 1. Selected plants were brought to the Institute of Botany, Academy of Sciences of Czech Republic, Průhonice, for isozyme analysis. Voucher specimens are deposited in the Herbarium of the Institute of Botany in Bratislava (SAV).

Young fresh leaf tissue was used for isozyme analysis. Plant material was ground at 4 °C in the 0.1 M Tris-HCl extraction buffer (100 ml): 1.211 g Tris, 548 µl of

Table 1.
Analysed material (abbreviation used: S-Slovakia, U-Ukraine).

Taxon	Locality data with numbers of analysed plants in brackets
<i>H. rohacsense</i> Kt.	S: Nízke Tatry Mts., saddle between Mt. Kráľička and Mt. Lajštroch, 1500–1600 m a.s.l., 48°55'11" N, 19°42'25" E, 16. VII. 1996 (5)
	S: Západné Tatry Mts., Roháčska dolina valley, near the former Ťatliakova chata chalet, 1350 m a.s.l., 49°12'53" N, 19°44'58" E, 9. VIII. 1996 (11)
	S: Západné Tatry Mts., below the Zábrad' saddle to the Látná dolina valley, 1550–1600 m a.s.l., 49°13'32" N, 19°45'05" E, 9. VIII. 1996 (15)
	S: Západné Tatry Mts., Račková dolina valley, ca. 400 m above the crossroads with Gáborova dolina valley, 1500 m a.s.l., 49°11'12" N, 19°49'03" E, 12. VIII. 1996 (23)
population "Pop Ivan"	U: Marmarosh Mts., Mt. Pop Ivan, slopes of main glacial cirque, E exposition, 1850–1900 m a.s.l., 47°56' N, 24°20' E, 30. VII. 1996 (15)

Table 2. A survey of enzyme systems analysed. Monomeric enzymes are denoted by M and dimeric enzymes by D.

Enzyme (E.C. number)	Locus abbrev.	No. of alleles per locus	Subunit structure
Alcohol dehydrogenase (1.1.1.1)	Adh-1	1	D
Aspartate aminotransferase (2.6.1.1)	Aat-2	2	D
Leucine aminopeptidase (3.4.11.1)	Lap-1	1	M
Phosphoglucomutase (5.4.2.2)	Pgm-1 Pgm-2	2	M —
Shikimate dehydrogenase (1.1.1.25)	Skd	3	M

70 mM 2-mercaptoethanol, 0.494 g of 26 mM sodium metabisulfit, 0.194 g of 11 mM L-ascorbic acid, 4 g of soluble PVP, pH adjusted to 8.0 with 1 N HCl, Dowex Cl- was added (ŠTĚPÁNEK & KOTTOVÁ, unpubl.). Crude homogenates were centrifuged at 4 °C for 10 minutes at 15,000 rpm. The clear supernatant was immediately applied to the gel or stored deep frozen at -70 °C. The analysed isozyme systems are given with details in the Table 2. As a separating gel buffer was used 1.82 M Tris-HCl, pH 8.9; as a spacer gel buffer 0.069 M Tris-HCl, pH 6.9; the electrode buffer was Tris-glycine, 0.02 M Tris and 0.24 M glycine, pH 8.3. The staining procedures followed VALLEJOS 1983 with some exceptions (cf. KIRSCHNER & al. 1994). For AAT the amount of aspartic acid and α -ketoglutaric acid was changed to 60 and 30 mg respectively. The LAP buffer was replaced by 0.2 M Tris-maleat pH 6.0. PGM was stained by the agar overlay method. The gels were scored in the framework of the larger group of taxa from sect. *Alpina*, including other material than *H. rohacsense* and plants from Mt. Pop Ivan (see also ŠTORCHOVÁ & al. 2001). The loci were annotated according to the mobility of their respective isozymes (1, 2 ...), the alleles by lowercase letters (a, b ...) according to the mobility of the corresponding allozymes. The relative mobility of the bands was calculated as the ratio of the distance of band from the start to total distance of the gel (from the start to the end of gel).

Results

Five enzyme systems representing 6 isozyme loci were analysed electrophoretically. Observed isozyme phenotypes are shown in Fig. 1. Because it is not possible to test the inheritance of isozyme bands in apomicts, analysis of zymograms in terms of loci and alleles was based on the expected conserved numbers of isozymes, their quaternary structure and subcellular compartmentalisation (WEEDEN & WENDEL 1989). Our results have also been compared with those of diploid sexual plants of *H. alpinum* from the Eastern Carpathians (CHRTEK & al. unpubl.).

The allelic interpretations of phenotypes in the studied taxa are given in Fig. 1 and in Tab. 3. At the locus Pgm-2 only band patterns were scored, because of the complexity of obtained zymograms (see Fig. 1. and Fig. 2.).

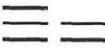
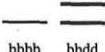
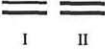
Locus	Zymogram and the interpretation	Relative mobility of bands
		
AAT-2	acc	0.39, 0.36, 0.33
		
ADH-1	aaa	0.42
		
SKD	aa	0.45, 0.38, 0.34
		
PGM-1	bb	0.21, 0.19
		
PGM-2	I II	0.41, 0.39, 0.35
		
LAP-1	ccc	0.21

Fig. 1. Observed isozyme patterns and allelic interpretation of zymograms. In the case of Pgm-2 no genetic interpretation of phenotypes was attempted.

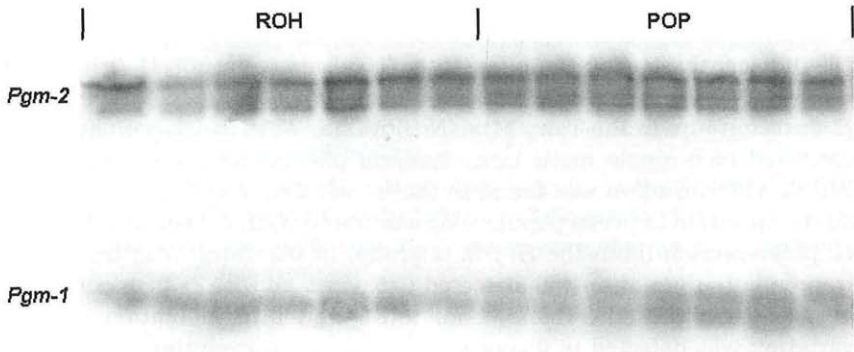


Fig. 2. Zymogram of Pgm system. Abbreviations: POP – “population Pop Ivan”, ROH – *H. rohacsense*.

Heterozygosity was detected in Aat-2 and Skd in both taxa, in Pgm-1 on the plants from Mt. Pop Ivan. Both taxa could be separated from each other in Pgm-1 and Pgm-2 patterns (Fig. 1, Fig. 2). However, one plant from population “Pop Ivan” shared the same phenotype at Pgm-2 as all plants of *H. rohacsense*.

Table 3.
Multilocus phenotypes found in the present study.

Taxon / multilocus phenotypes	Locus and allelic interpretation (number of analysed plants in brackets), for Pgm-2 only numbers of the type of band pattern is given.					
	Aat-2	Adh-1	Lap-1	Pgm-1	Pgm-2	Skd
<i>H. rohacsense</i> 1.	accc (25)	aaaa (28)	cccc (51)	bbbb (51)	I (51)	aabc (48)
"Pop Ivan" 1.	accc (2)	aaaa (12)	cccc (12)	bbdd (12)	II (12)	aabc (12)
2.	–	aaaa (2)	cccc (2)	bbdd (2)	II (2)	aaab (2)
3.	–	–	cccc (1)	bbdd (1)	I (1)	aaab (1)

The 51 studied plants from four populations of *H. rohacsense* were not variable in any locus studied. In contrast the isozyme study of the 15 plants of the population "Pop Ivan" (E Carpathians) revealed three different phenotypes in one population. Apart from one plant having another isozyme pattern at Pgm-2 and Skd, two differs at Skd, and the rest of population shared a unique phenotype. The deviating individuals were not re-analysed because of the lack of material.

Discussion

Similarly to *H. rohacsense*, no isozyme variation has been found in 25 agamospecies of *Hieracium* sect. *Alpina* from the British Isles. However, 12 of these agamospecies were studied in one population only (STACE & al. 1997). Recently studied *H. halleri* VILL. (*H. alpinum* group), *H. krivanense* (WOEL. & ZAHN) SCHLYAKOV, and *H. slovacum* CHRTEK jun. (both of the *H. fritzei* group) in the Tatry Mts. (N Slovakia, Western Carpathians) also consisted of a single multi-locus isozyme phenotype; furthermore, very little RAPD variation was found in the former two taxa (ŠTORCHOVÁ & al. 2001). Variation between populations was found in *H. crassipedipilum* and *H. pinetophilum* (both the *H. fritzei* group) in the Tatry Mts., but no intrapopulation variation was detected (isozymes and RAPD; ŠTORCHOVÁ & al. 2001). On the other hand, both intra- and interpopulation isozyme variation was detected in 8 species of sect. *Alpina* from the British Isles, and in three other species from the same area only interpopulation variation was found (STACE & al. 1997). Considerable variation and geographic structure of genetic differentiation between populations was found in *Hieracium alpinum* in the Tatry Mts. (ŠTORCHOVÁ & al. 2001). A significant correlation between geographic and Euclidean distances was found in the case of allozyme markers ($r = 0,639$, $P = 0,041$) and nearly significant in the case of the RAPD data set ($r = 0,031$, $P = 0,074$).

Our findings are in general agreement with first results of studies focused on breeding systems and variation pattern in the genus *Hieracium* in

the Western and Eastern Carpathians. While three diploid sexual species *H. alpinum* (incl. *H. alpinum* subsp. *augusti-bayeri* ZLATNÍK), *H. conicum* ARV.-TOUV., and *H. transsilvanicum* HEUFFE (PASHUK 1987; CHRTEK 1996, 1997) have been discovered in the Ukrainian Eastern Carpathians, all West Carpathian taxa, growing in the subalpine belt, so far tested have been triploid or tetraploid apomicts (MÁJOVSKÝ & al. 1987, MRÁZ 2001, ŠTORCHOVÁ & al. 2001). Recent hybridization events between diploid sexual species or between pollen producing tetraploids and sexual diploids might therefore have occurred in the Eastern Carpathians. Intrapopulation variation in "Pop Ivan" population can be explained by residual sexuality (although it was not observed in analysed plants, it cannot be excluded), mutational changes, or by polytopic origin of particular genotypes by repeated hybridization events. The uniclonal structure of *H. rohacsense* coincides with the expected lack of sexuality in this species and with the narrow range of morphological variation (MRÁZ, unpubl.).

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