

Genetic variation in *Hieracium alpinum* (Asteraceae) in the Krkonoše Mts (West Sudeten Mts, Czech Republic)

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Abstract: Five enzyme systems (EST, LAP, PGM, SKD, 6PGDH) were studied in three populations of triploid ($2n = 27$) agamospermous *Hieracium alpinum* s. str. (*H. alpinum* subsp. *alpinum*) in the Krkonoše Mts (West Sudeten Mts, Czech Republic). Altogether, five different multilocus genotypes were found and both intra- and between-population variation were detected. Within-population variability was found in all the studied populations. Mean population diversity $G_{sp} = 0.52$, component of total variance attributed to variation among population $G_{st} = 0.09$). Chromosome number $2n = 27$ was confirmed in all plants used in enzyme studies. Putative origins of genetic variation are briefly discussed.

Key words: *Hieracium*, allozymes, apomixis, compositae, Krkonoše Mts, Czech Republic

Introduction

The genus *Hieracium* L. (Asteraceae) is well known as a group in which polyploidy ($x = 9$) and agamospermy (seed apomixis) are widespread (GUSTAFSSON, 1946–1947; NOGLER, 1984; ASKER & JERLING, 1992). According to the recently published chromosome counts, triploids ($2n = 27$) and tetraploids ($2n = 36$) distinctly prevail (e.g., SCHUHWERK, 1996). Sexuality is extremely rare and is confined to a few diploid species (e.g., MERXMÜLLER, 1975; SCHUHWERK, 1996; CHRTEK et al., 2004). Seed development in agamospermous plants follow diplospory of *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 embryony, i.e. endosperm development is independent of fertilization of the polar nuclei (e.g. NOGLER, 1984). Diplosporous agamosperms are supposed to be candidates for obligate agamospermy (e.g., RICHARDS, 1997); this also coincides with uniclonal structure of some *Hieracium* species (SHI et al., 1996; STACE et al., 1997; MRÁZ et al., 2001; ŠTORCHOVÁ et al., 2002).

Hieracium alpinum L. s.str. (*H. alpinum* L. subsp. *alpinum*, for taxonomic review see CHRTEK, 1997) belongs to so called “main species” (“species principales, Hauptarten” – term used in *Hieracium* for non-hybridogeneous species with unique set of morphological characters). Distribution area extends from Greenland, through Iceland, the British and Scandinavian mountains, to north-western Russia; in Cen-

tral Europe it includes the Alps, the Sudeten Mts and further eastwards the Carpathians. Some isolated exclaves are in the Vosges, Harz and at the Vranica Plateau (Bosna and Hercegovina); records exist also from the Apennino Centrale and the Apuane Mts in Italy (ZAHN, 1921–1923; HULTÉN & FRIES, 1986; GOTTSCHLICH, 1987; BRÄUTIGAM, 1992). It grows in open-canopy grassland, in dwarf shrub communities, on rock ledges, bare stone slopes, and in alpine *Nardus* grasslands. *Hieracium alpinum* comprises both diploid sexual and triploid agamospermous populations, agamospermy has been confirmed by both castration experiments (CHRTEK, 1997) and cytoembryological studies (SKAWIŃSKA, 1963). They are geographically vicariant (diploids in the Eastern and Southern Carpathians, triploids in the remaining parts of the distribution area), population with intermingled diploids and triploids has never been reported (CHRTEK, 1997; MRÁZ, 2001, 2003; MRÁZ & SZELĄG, 2004). From the Krkonoše Mts, triploid counts were published by MĚSÍČEK & JAROLÍMOVÁ (1992), and CHRTEK (1994). The ploidy levels cannot be distinguished by means of morphological characters.

Genetic variation and differentiation within the whole distribution range of *H. alpinum* remains largely unknown. SHI et al. (1996) and STACE et al. (1997) studied genetic variation in Great Britain and Switzerland using allozyme and RAPD markers and rDNA ITS2 sequences. ŠTORCHOVÁ et al. (2002) used allozymes and RAPD markers to assess the pattern of genetic variation in the Tatra Mts (N Slovakia, Western Carpathians).

Our aim is to evaluate within- and between population variation of *H. alpinum* on a local scale. The Krkonoše Mts represent suitable model area – they are an isolated island of high mountain flora and one of hot spots of *Hieracium* diversity. Hybridogeneous taxa derived from *H. alpinum* are common here and detailed study of genetic diversity of *H. alpinum* is thus of principal importance.

Material and methods

Study site

The Krkonoše Mts are situated astride the boundary between the Czech Republic and Poland and constitute the highest part of the Sudeten Mts (the highest peak Sněžka/Śnieżka, 1602 m a.s.l.). *Hieracium alpinum* is here rather common component of alpine areas. Plants were collected at three localities (numbered 1–3) representing the main stations of *H. alpinum* in the Krkonoše Mts, in the alpine grasslands classified in the *Juncion trifidi*. Distances between localities are as follows: 1 to 2: 11.5 km, 1 to 3: 1.8 km, 2 to 3: 9.8 km.

Locality 1: Krkonoše Mts, E part: Mt. Studniční hora, W slopes, 4.3 km NW of the village of Pec pod Sněžkou, 1540 m a.s.l., 15 July, 2002, leg. J. CHRTEK.

Locality 2: Krkonoše Mts, W part: Mt. Vysoké kolo, W of the summit, 6.5 km NW of the village of Špindlerův Mlýn, 1490 m a.s.l., 22 July, 2001, leg. J. CHRTEK.

Locality 3: Krkonoše Mts, E part: Mt. Luční hora, summit plateau, 5.5 km NW of the village of Pec pod Sněžkou, 1545 m a.s.l., 17 July, 2002, leg. J. CHRTEK.

Plants, sampling scheme

One plot 5 × 5 m was established at each locality. Randomly distributed points (using coordinates determined by random number generator) were used. The plants nearest to the points were sampled; if no plant was around the point new point was chosen (otherwise spatially isolated plants would be favoured). Total of 30 plants was collected at each locality. Plants were transferred to an experimental garden in Průhonice and cultivated in field conditions. All plants were proven to be triploid ($2n = 27$). Voucher specimens are deposited in the herbarium of the Institute of Botany, Průhonice (PRA).

Chromosome numbers

Chromosome counts were made by J. CH. for all (90) cultivated plants used for allozyme studies. Root tip cuttings of mature plants were used. The material was pre-treated with a saturated solution of *p*-dichlorobenzene, fixed in a mixture of ethanol and acetic acid (3:1) and stored in 70% ethanol. The squash method and staining by lacto-propionic orceine were used (DYER, 1963).

Allozyme analysis

Young leaves of cultivated plants were used. Plant material was ground in extraction buffer generally according to KATO (1987) with some modifications: 0.1M Tris-HCl (pH 8.0), 70 mM mercaptoethanol, 26 mM sodium metabisulphite, 11 mM L-ascorbic acid, 4% soluble PVP, pH adjusted after the addition of ascorbate. Crude homogenates were centrifuged for 10 min at 15 000 rpm. Clear supernatant

was stored in deep freeze at -75°C . The PAGE was carried out using separating gel (8.16%) with the buffer 1.82 M Tris-HCl, pH 8.9; the stacking gel (4.0%) with the buffer (0.069 M Tris-HCl, pH 6.9); the electrode buffer was 0.02 M Tris, 0.24 M glycine, pH 8.3. The following enzymes were analyzed: EST (Esterase, EC 3.1.1.-), LAP (leucine aminopeptidase, EC 3.4.11.1), PGM (phosphoglucumutase, EC 5.4.2.2), 6PGDH (6-phosphoglucumate dehydrogenase, EC 1.1.1.44), and SKD (shikimic acid dehydrogenase, EC 1.1.1.25).

The staining procedures followed VALLEJOS (1983) to visualize 6-PGDH and SKD, and WENDEL & WEEDEN (1989) for PGM and EST, with the following modifications: 6-PGDH (0.1 M tris-HCl pH 8.4, 30 mg 6-phosphoglucumic acid), SKD (0.1 M Tris-HCl pH 8.4), colorimetric EST (Na-phosphate buffer pH 6.45, 25 mg β -naphthyl phosphate, 50 mg Fast Blue BB), PGM (24 mg MgCl_2 , 50 mg glucose-1-phosphate, 10 mg NADP). Visualization of LAP was done using buffer 0.2 M Tris-maleate pH 6. The gel was rinsed with the buffer and then incubated for 10 min in solution of 30 mL of the buffer, 40 mg L-leucyl- β -naphthylamide, HCl (in 50% acetone) and 60 mg MgCl_2 . Then 25 mg Fast Black K Salt in 30 mL of the buffer was added. The gel was incubated in dark, until bands appeared.

The interpretations of allozyme patterns were based on the analysis of allozyme patterns in populations of diploid sexual *Hieracium alpinum* (CHRTEK & KIRSCHNER, unpubl.). No allozyme analysis of the offspring was performed in present material because of the agamospermy documented in the taxa under study.

Data analysis

The multilocus isozyme genotype of each individual plant was determined on the basis of its pattern after electrophoresis. Mean number of alleles per locus (A), percentage of polymorphic loci ($\%P$), and observed frequency of heterozygotes (H_o , calculated from all allelically interpreted loci) were calculated for each population. Genotype diversity measures were calculated after ELLSTRAND & ROSE (1987) and ECKERT & BARRETT (1993): for all three populations, number of unique multilocus genotypes (G_{uni}), proportion of distinct genotypes (G/N , number of genotypes/number of individuals), genotype diversity within population (D , for calculation see e.g. NOYES & SOLTIS, 1996), and genotype evenness (E , for calculation see e.g. NOYES & SOLTIS 1996, it reflects proportional representation of genotypes within a population sample). Genotype frequencies were calculated within each population and within the whole sample and were used for estimation of isozyme diversity at different hierarchical levels as $G_{(pop/sp)} = 1 - \sum g_i^2$ (using the formula as in STACE et al., 1997), where g_i is the frequency of i th genotype within a population/species. G_{sp} was calculated as a mean of G_{pop} values. The partitioning of isozyme variation among populations was estimated as $G_{st} = 1 - G_{sp}/G_{sp}$.

Results

Five individual loci were analyzed and interpreted genetically (*Skd*, *Lap-1*, *Lap-2*, *Pgm-1*, and *6-Pgdh*), two of them were found to be polymorphic (*Skd*, *Lap-1*, Fig. 1). The enzyme EST consistently produced clear banding pattern. However, it was not possible to interpret the

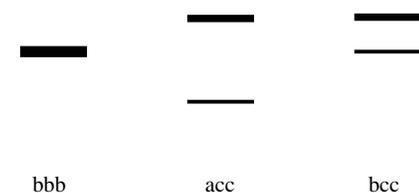
Table 1. Allozyme genotypes and their frequencies found in the examined populations of *Hieracium alpinum*. Genotype frequencies are calculated within the given population.

	Skd	Lap-1	Lap-2	Pgm-1	6-Pgdh	Genotype	Frequency of genotype (number of plants)
Popul. 1	aab	bbb	aab	bbc	abb	I	0.64 (19)
	aaa	acc	aab	bbc	abb	II	0.30 (9)
	aaa	bbb	aab	bbc	abb	III	0.03 (1)
	aab	acc	aab	bbc	abb	IV	0.03 (1)
Popul. 2	aab	bbb	aab	bbc	abb	I	0.70 (21)
	aaa	acc	aab	bbc	abb	II	0.27 (8)
	aaa	bbb	aab	bbc	abb	III	0.03 (1)
Popul. 3	aaa	acc	aab	bbc	abb	II	0.50 (15)
	aab	bbb	aab	bbc	abb	I	0.30 (9)
	aaa	bbb	aab	bbc	abb	III	0.13 (4)
	aaa	bcc	aab	bbc	abb	V	0.07 (2)

Table 2. Measures of allelic and genotypic diversity of *Hieracium alpinum* populations.

	<i>N</i>	<i>A</i>	<i>P</i>	<i>H_o</i>	<i>G</i>	<i>G_{uni}</i>	<i>G/N</i>	<i>D</i>	<i>E</i>	<i>G_{pop}</i>
Popul. 1	30	2.2	40	0.80	4	1	0.133	0.524	0.567	0.4986
Popul. 2	30	2.2	40	0.79	3	0	0.1	0.453	0.576	0.4362
Popul. 3	30	2.2	40	0.77	4	1	0.133	0.66	0.801	0.6382

N = number of plants, *A* = mean number of alleles per locus, *P* = percentage of polymorphic loci, *H_o* = observed frequency of heterozygotes, *G* = number of different multilocus genotypes, *G_{uni}* = number of multilocus genotypes unique to one population, *G/N* = “proportion distinguishable”, *D* = multilocus genotype diversity, *E* = multilocus genotype evenness, and *G_{pop}* = population genetic diversity.

A) *Lap-1*B) *Skd*

populations (1–3) are summarized in Table 2. Within-population variability was found in all studied populations (mean population diversity $G_{sp} = 0.52$, component of total variance attributed to variation among population $G_{st} = 0.09$).

Discussion

Genetic variation in Hieracium alpinum

Despite the apomictic mode of reproduction some genetic variation was revealed in populations from the Krkonoše Mts. Two polymorphic loci (*Skd*, *Lap-1*) were detected, slight differences in banding pattern were found in EST (allelic interpretation was not possible). Genotypic diversity for populations ranges from 0.45 (Popul. 2) to 0.66 (Popul. 3), genotypic evenness (*E*) is also variable ranging from 0.57 (Popul. 1) to 0.80 (Popul. 3). Our measures permit comparison with the results from studies of genetic variation in other parts of the distribution range. ŠTORCHOVÁ et al. (2002) reported genetic variation in the same cytotype (triploid agamosperous) from the Tatry Mts (N Slovakia, Western Carpathians). They discovered 4 multilocus genotypes in the area of ca 38×13 km, total of 40 plants. Comparing to our study, partly different loci were studied and a comparison of multilocus genotypes between the two areas (the Tatry Mts, the Krkonoše Mts) is rather limited. Based on three loci used in both studies (*Skd*, *Lap-1*, and *Pgm-1*), one of the genotypes (genotype II in the present study) is common for both

Fig. 1. Banding patterns and allelic interpretation of *Skd* and *Lap-1* loci.

pattern genetically. Altogether 5 different multilocus genotypes were found (Tab. 1). Measures of allelic variation (mean number of alleles per locus, percentage of polymorphic loci, frequency of observed heterozygotes), number of genotypes (*G*) and genotype diversity measures (G/N , G_{uni} , *D*, *E*, G_{pop}) for the studied

areas. However, no unique allele has been found in both Krkonoše Mts and Tatry Mts. The estimates of the set of plants from the Tatry Mts (mean estimates of genetic variation within populations) are as follows (our measures are given in brackets): $A = 1.47$ (2.2), $H_o = 0.47$ (0.79), $G_{sp} = 0.11$ (0.52), $G_{st} = 0.81$ (0.09). Thus, considerably higher heterozygosity and within-population diversity was detected in the Krkonoše Mts in comparison to the Tatry Mts. Distinct differences are in G_{sp} and G_{st} values. The component of total variance attributed to between-population variation was higher in the Tatry Mts. However, longer distances among populations in the Tatry Mts must be taken into account. We are also aware that the number of plants per population was lower in the samples from the Tatry Mts.

SHI et al. (1996) studied *H. alpinum* in the Swiss Alps and Scotland. They found differences within Swiss populations (2) involved variation in AAT and PGM genotypes. Furthermore, the Scottish plants displayed within-population variation in alpha-EST.

Origin of genetic variation

One common hypothesis for the origin of variation in agamosperms is based on occasional sexual reproduction among agamic clones and sexual plants (GUSTAFSSON, 1946–1947; GRANT, 1981; ASKER & JERLING, 1992). However, this seems to be recently an unlikely source of genetic variation in *Hieracium alpinum*. Although diploid sexual plants have been found, they are most likely confined to the Eastern and Southern Carpathians (Ukraine, Romania), and populations comprising sexual and agamospermous plants have not been observed (CHRTEK, 1997). Hybridization with other relative taxa can also be most likely ruled out, as no sexual species has been found to be sympatric with agamospermous cytotype of *H. alpinum*. On the other hand, recent genetic variation can be derived from the ancestral diploid populations; it might be suggested that triploid agamospermous plants originate from divergent elements (genotypes) of sexual *H. alpinum*. There are no alleles found in agamospermous plants in the Krkonoše Mts and they are missing in the diploid populations in the Eastern Carpathians (CHRTEK & KIRSCHNER, unpubl.). Another possibility how to explain the variation are mutations. Because of the rarity (or the lack) of recombination in diplosporous apomicts, mutations may become fixed in clonal lineages, and accumulate rapidly (MAYNARD SMITH, 1978; RICHARDS, 1997).

Further possible source of genetic variation in agamospermous *H. alpinum* can be crosses between agamospermous plants. Although meiosis has never been reliably documented in the course of embryo sac development, there are some indices that a disrupted meiosis may occur giving rise to probably (?) unviable macrospore tetrads (SKAWIŃSKA, 1963). Microsporogenesis is highly irregular, but limited number of viable pollen grains is likely produced.

Our finds bring further evidence of genetic differentiation in the agamospermous *Hieracium alpinum*.

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