Experimental hybridization in the genus *Hieracium* s. str.: crosses between diploid taxa

Experimentálna hybridizácia v rode *Hieracium* s. str.: kríženie vybraných diploidných druhov

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The few attempts to produce artificial hybrids in the genus *Hieracium* s. str. have usually failed due to the use of polyploid parental taxa reproducing via agamospermy. Presented here for the first time are data on artificial hybridization in *Hieracium* s. str. which may help in understanding the microevolutionary processes resulting in the great morphological and genetic diversity in this genus. Diploid, sexually reproducing species (*H. alpinum*, *H. pojoritense, H. transsilvanicum* and two stable morphological types of *H. umbellatum* – of a low altitude and a high mountain type) were used as parent plants in experimental crosses. In most cases true hybrids, with intermediate morphology, were obtained. All the hybrids tested were diploid and produced a high amount of stainable pollen (65–92%). Hybrid progeny resulting from one cross exhibited a large range of morphological variation due to the combination of alleles from unrelated parental species. The percentage of well-developed achenes per capitulum, in capitula with at least one well-developed achene, in hybrids, ranged from 1.9 to 12.5% after free or controlled pollination, with an average of 4–5% per capitulum. Similar results (1.9–12.1%) were obtained from triple-cross hybrids. However, most of the capitula of hybrid progeny (either F1 or triple) were completely sterile after free or controlled pollination. Sterility is probably caused by genome incompatibility of unrelated parental taxa belonging to different sections. In two crosses, where strictly allogamous diploid plants of *H. umbellatum* (both morphotypes) were used as mother plants and F1 hybrids as pollen donors, some matroclinal progeny were obtained. This is a further example of the previously reported mentor effect. Diploid hybrids may be involved as pollen donors in gene flow as they produce uniformly sized and viable pollen. They are probably substantially less important as seed parents.

Keywords: apomixis, *Compositae*, homoploid hybridization, hybrid fitness, *Lactuceae*, sterility

Introduction

Interspecific hybridization is considered to be important in plant speciation (Arnold 1992, Rieseberg 1997). Information on natural hybridization and its rate is crucial for understanding the evolution of species. However, one or more hybrids have been recorded only for ca 6–16% of plant genera (Ellstrand et al. 1996). Apart from the data on spontaneous hybridization in the field, experimental crosses have contributed much to the identification of microevolutionary processes like species compatibility, pre- and postzygotic isolation barriers, pollen competition, reproductive capacity of hybrid progeny, selection and the role of introgression (Nieto Feliner et al. 1996, Nieto Feliner 1997, Rieseberg & Carney

1998, Khalaf & Stace 2000, Lihová et al. 2000, Burke & Arnold 2001, Mooring 2002, Lexer et al. 2003, Jarolímová 2005).

The holarctic genus *Hieracium* L. s. str. is taxonomically one of the most complicated and at the same time most species-rich genera in the plant kingdom (see the number of entries in Index Kewensis, Royal Botanical Gardens Kew, 1993). The complexity of the genus is reflected in the reticulate pattern of morphological variation (Zahn 1921–1923), widespread polyploidy and agamospermic formation of seeds in polyploid taxa. Most of the karyologically studied taxa are tri- or tetraploids ($x = 9$, $2n = 3x = 27$, $2n = 4x = 36$, respectively) (see Schuhwerk 1996 and references therein). There are few chromosome numbers above the tetraploid level (Stace et al. 1995, Chrtek 1996, Pul'kina & Tupitsyna 2000) and aneuploids are extremely rare (for references see Schuhwerk 1996). Polyploidy is connected with gametophytic apomixis, the diplospory of *Antennaria*-type (Gustafsson 1946). So far, there is no evidence of facultative apomixis in the genus and therefore obligate apomixis is usually considered as the sole mode of reproduction in *Hieracium* polyploids. In contrast, there is a high number of polyploids, but sexually reproducing diploids ($2n = 2x = 18$) are rare and confined mostly to the southern part of the distributional range of the genus (mainly S Europe, for actual list of diploids see Chrtek et al. 2004). For some diploid taxa strict allogamy (self-incompatibility) was proven by isolation experiments (Turesson 1922, Chrtek 1997, Mráz 2003). However, in a series of different interspecific crosses, an induced autogamy in diploids (so called "mentor effect") was recorded (Mráz 2003).

It seems that interspecific hybridization along with introgression were the main evolutionary processes resulting in the great diversity in this genus. The following facts support a hybrid origin of polyploid *Hieracium* taxa: (i) Polyploids are morphological intermediates among themselves as well as among several diploid species (Zahn 1921–1923). (ii) Molecular studies have shown fixed heterozygosity in polyploid taxa (Stace et al. 1997, Mráz et al. 2001, Štorchová et al. 2002, Chrtek & Plačková 2005; high level of heterozygozity was found also in polyploids of the closely related genus *Pilosella*, Peckert et al. 2005). Generally, fixed heterozygosity is considered sound support for an allopolyploid origin (e.g. Asker & Jerling 1992, Brochmann et al. 2004). (iii) Abnormal microsporogenesis is recorded in several *Hieracium* polyploid taxa. Various numbers of uni-, bi-, tri- or tetravalents show uneven chromosome pairing due to chromosome heterology (Rosenberg 1927, Gentcheff & Gustafsson 1940, Aparicio 1994) resulting in the production of variable sized pollen or even full male sterility in polyploids. This feature may be as a good indicator of polyploidy in this genus (Mráz et al. 2002). (iv) Many plant hybrids of recent origin do not produce seed. From this point of view, apomixis may represent an elegant mechanism for "escaping from sterility" (Asker & Jerling 1992).

Nowadays interspecific hybridization in the genus *Hieracium* is probably highly restricted. There are a few records of recent natural hybrids between diploid taxa (Mráz et al. 2005; and some other not yet published records of Chrtek & Mráz, see Material and Methods). Similarly, interspecific hybridization among diploid taxa within closely related genera *Stenotheca* Monn. (Guppy 1978) and *Pilosella* Hill. (Turesson 1972) may occur, but the hybrids are seldom found in nature. On the other hand in the genus *Pilosella* natural hybridization between diploid and polyploid taxa and among polyploids is relatively common (reviewed in Krahulcová et al. 2000)

Considering that *Hieracium* s. str. belongs to the most species-rich genera in the world it is surprising that there are almost no data on experimental crosses in this genus. This is in strong contrast to *Pilosella*, where several tens of papers on this topic have been published so far (see Krahulcová et al. 2000 and references therein).

After his successful experiments with *Pisum* L., Johann Gregor Mendel was among the first scientists to produce artificial hybrids of *Hieracium* s.l. (Krahulcová et al. 2000). While in the case of the closely related genus *Pilosella* the crossing ability was high (Mendel 1870), Mendel did not obtain interpretable and publishable data from crossing *Hieracium* s. str. (see some short notes in his letters to Carl Nägeli in Results and discussion) (Mendel 1950). Mendel obtained few hybrids from crosses in which *H. umbellatum* L. was used as the pollen donor and an unnamed species, later classified as *H. racemosum* Willd., as well as from crosses between *H. umbellatum* and *H. vulgatum* Fr. (s.l.). Mendel also obtained progeny from experimental hybridization of *H. murorum* L. and *H. umbellatum*, but they did not look like hybrids and clearly resembled the maternal plant. Their origin was explained by self-fertilization (Mendel 1950: 2), although it was likely agamospermy, an unknown phenomenon at the time Mendel was doing his experiments. Similarly, all efforts of Ostenfeld to produce hybrids via artificial hybridization were unsuccessful. However, he noted that the offspring from unprotected plants of the sexual species *H. virgicaule* Nägeli et Peter were very heterogeneous in the terms of morphology (Ostenfeld 1921). Later, Zlatník (1938: 41) outlined the results of artificial reciprocal crosses between *H. alpinum* L. and *H. murorum*. He obtained matroclinal progeny that reproduced apomictically ("... apogamishe Bastarde ... sind matroklin ..."), but considered them to be hybrids.

In order to study microevolution within the genus *Hieracium*, the first author of the present paper, later on with help of his diploma students, experimentally hybridized selected diploid, and diploid and polyploid taxa. Among the interesting results was the first record of autogamy in crosses among diploid and between diploid and polyploid taxa (Mráz 2003).

In this paper, the results of crosses between several diploid taxa, focusing on the characterization of hybrid progeny by means of morphology, pollen quality, chromosome number and reproductive capacity, are presented.

Material and methods

Parental taxa used in crosses

Parent plants were collected during the years 1996–2001 at various sites in Slovakia, Romania and Ukraine (for exact localities see Appendix 1 in Mráz 2003). After transplantation, plants were cultivated and cross-pollinated in a lowland experimental garden under field conditions and in some cases in unheated semi-open greenhouse in the Botanical Garden of P. J. Šafárik University of Košice. The following taxa were used as mother and / or pollen donor plants: *Hieracium alpinum, H. pojoritense* Woł., *H. transsilvanicum* Heuff. and two morphologically distinct types of *H. umbellatum* (Figs 1–5; see Electronic Appendix 1 (http://www.ibot.cas.cz/preslia) for colour images of Figs 1–12). High mountain type of *H. umbellatum* from the Eastern Carpathians differs from the morphotype of low altitude having small number of capitula (usually 3–5; low altitude type usually 20–50), distinctly bigger capitula than the type of low altitude, low number of stem leaves (up to 15; the most usually above 20 in the type of low altitude), broadly elliptic to ovate stem leaves (type of low altitude has lanceolate, oblong lanceolate to oblong elliptic stem leaves). Moreover, the plants of high mountain type are smaller (up to 30–40 cm) then *H. umbellatum* coming from the low altitude (usually above 50 cm). The differences between both types are stable also in cultivation (3 years of observations). For high mountain populations of *H. umbellatum* from the Ukrainian Carpathians two names have been recently used in the literature: *H. conicum* Arv.-Touv. (Chrtek 1996) and *H. hryniawiense* Woł. (Mráz 2003). The first taxon was originally described from the French Alps and probably does not reach the territory of the Carpathians (in a narrow sense). The latter one, described from the Ukraine, with high probability represents another taxon closely related *H. sabaudum* s.l. (based on study of scanned original material of *Hieracium hryniawiense* Woł. kindly sent to us by J. Chrtek jun.). In this respect, the use of both names for these high mountain populations seems to be incorrect. For these reasons we treated these high mountain populations within hypervariable *H. umbellatum*.

Each parent was examined karyologically and all proved to be diploids (Mráz 2003). The hybridization scheme is given in Table 1. All parent taxa were easily recognized by means of their morphology and all belong to different sections sensu Stace (1998). In addition to the difference in morphology each particular taxon differs in ecology and altitudinal preferences. The diploid cytotype of *H. alpinum* (*H*. sect. *Alpina*) is confined to the alpine and subalpine belts of the Eastern and Southern Carpathians (Chrtek 1997, Mráz & Szeląg 2004). The second species, *H. pojoritense* (*H*. sect. *Italica*), is a stenoendemic taxon of calcareous relict rocks of the montane and submontane belt in NE Romania (Ştefureac & Tăcină 1979). *Hieracium transsilvanicum* (*H*. sect. *Vulgata*) is a typical species of spruce and fir-beech forests in the Eastern and Southern Carpathians and Balkan Peninsula. While the high mountain populations of *H. umbellatum* usually grow on rocky slopes in glacial cirques or on subalpine meadows, a form of low altitude prefers abandoned meadows and forest margins in most parts of Eurasia (Zahn 1921–1923, both morphological types of *H. umbellatum* belong to the *H*. sect. *Hieracioides*). The above-mentioned taxa usually do not co-occur. However, they were rarely observed in close proximity at some localities (e.g. *H. alpinum* and high mountain type of *H. umbellatum*, *H. alpinum* and *H. transsilvanicum* or *H. pojoritense* and *H. transsilvanicum*; personal observations of the first author). In two cases, spontaneous hybridization in the field was observed between *H. alpinum* and *H. transsilvanicum* (Mráz et al. 2005 at two sites, J. Chrtek & P. Mráz, unpubl., at one site), and *H. alpinum* and high mountain *H. umbellatum* (J. Chrtek et al., in prep., at two localities).

Artificial hybridization

The inflorescences of plants used in the experiments were enclosed in nylon bags until anthesis to prevent cross-pollination. The crosses were made at the stage of stigma receptivity by softly rubbing the capitula together, to facilitate transfer of pollen to the stigmas, 1–2 times per day for 2–5 consecutive days, if possible. Apart from the primary crosses between the above-mentioned taxa, the progeny of these crosses (F1 hybrids) were subjected to further artificial (triple) hybridization with diploid plants, which flowered at the same time as the F1 hybrids.

Fig. 1. – Habitus of parental taxon *Hieracium alpinum* L., scale bar = 10 cm. See Electronic Appendix 1 for colour images of Figs 1–12.

Fig. 2. – Habitus of parental taxon *Hieracium pojoritense* Woł., scale bar = 10 cm.

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Flora Ucranie

Hieracium transsilvanicum Heuff.

Eastern Carpathians, Chorna Hora Mts.
Polonina Breskulska, border of spruce
forest, 1390 m a.s.l.
23/07/2003, P. Mráz & J. Chrtek
N 48°08' 35.0", E 24°28' 56.7'

Fig. 3. – Habitus of parental taxon *Hieracium transsilvanicum* Heuff., scale bar = 10 cm.

Fig. 4. – Habitus of parental taxon *Hieracium umbellatum* L. (high mountain morphotype). This plant (no. X22/3) arose from selfing in the cross no. X22 (see text), scale bar = 10 cm.

Fig. 5. – Habitus of parental taxon *Hieracium umbellatum* L. (morphotype of low altitude), scale bar = 10 cm.

Fig. 6. – Hybrid progeny from cross no. X1: *Hieracium transsilvanicum* × *H. umbellatum* (morphotype of low altitude), scale bar = 10 cm.

Table 1. – Experimental interspecific hybridization involving some diploid species of the genus *Hieracium* L. The first parent in a cross is always the mother plant, the second the pollen donor. Numbers below species names are the cultivation numbers of the parental taxa. (L) – morphotype of low altitude, (H) – high mountain morphotype, R – reciprocal cross.

Cross no.	Parents	Well-developed fruit (%)	Germination (%)	No. of F1 progeny		
				Evaluated	Hybrids	Selfs
	H. transsilvanicum × *H. umbellatum* (L)					
X1	1067 × 736	100	53	8	8	0
	H. transsilvanicum × *H. alpinum*					
X2	1064 × 649	68	32	10	10	0
X5	R	72	40	21	17	4
	H. umbellatum (H) × *H. alpinum*					
X9	699 × 639	100	72	54	54	0
	H. transsilvanicum × *H. pojoritense*					
X10	1067 × 776	100	42	13	13	0

Estimates of fruit quality

Full mature achenes were collected and classified as well-developed or poorly developed. While the well-developed fruits were stiff, plump, well-pigmented, black, brown or dark red, poorly developed achenes were narrow, flimsy and weakly pigmented or even white. Well-developed fruit may or may not contain an embryo; poorly developed fruit always lack embryos (confirmed by stereomicroscopy). The fruit-set was determined as the percentage of well-developed achenes among the fruit within a capitulum of those capitula with at least one well-developed fruit. If the capitulum did not produce any well-developed achenes, just the number of sterile capitula and the number of plants with no well-developed fruit were recorded.

Germination

After a 6–8 month cold stratification, the well-developed achenes were germinated in Petri dishes on wet filter paper. Germination was measured as the percentage of the well-developed achenes that germinated.

Evaluation of progeny resulting from artificial hybridization

The progeny from cross-pollination were evaluated by means of morphology (shape, size, number and position of the leaves on the stem; shape and size of phyllaries; indumentum of the leaves, stems, peduncles and phyllaries; style colours; stem branching pattern; number of capitula). Parental species involved in the crosses were easily distinguished by their morphological characters, which were also apparent in the progeny.

Estimate of pollen viability

Striking differences in the production of pollen by diploid and polyploid taxa of *Hieracium* s. str. have been recorded. The diploids produce an abundance of uniformly sized pollen, while polyploids produce either a little pollen of variable size or no pollen (Aparicio 1994, Mráz et al. 2002, Kovalčiková 2004). As F1 diploid hybrids produce uniformly sized pollen, we focused on estmating pollen viability. Male fertility of hybrid progeny was estimated by staining the pollen with acetocarmine in glycerol jelly (Marks 1954). In order to remove the pollen three unopened tubular flowers per capitulum were dissected with a razor blade in a drop of acetocarmine jelly. One-hundred pollen grains per individual were evaluated, and both viable (well-stained) and non-viable (unstained) grains were scored.

Chromosome number

The chromosomes were counted in mitotic metaphases in the root tips of the hybrid progeny cultivated in pots. For details of the method see Mráz in Chrtek et al. (2004). The number of karyologically analyzed plants are given in Tables 2 and 4.

Reproductive capacity of hybrid progeny

In order to determine the reproductive capacity, the number of well-developed fruit produced by F1 and triple hybrids from three types of experiments were scored: (i) F1 and triple hybrids were crossed with diploid *Hieracium* taxa, (ii) were reciprocally crossed or (iii) they were kept unisolated and exposed to free pollination. The source of pollen for free pollination, in addition to the hybrids and their parents, may have been from ca 200 plants of *Hieracium*, belonging to different taxa and ploidy levels, cultivated together with the hybrid progeny. Some capitula of F1 or triple hybrids were isolated during the whole flowering period to test the self-incompatibility system (SI).

Results and discussion

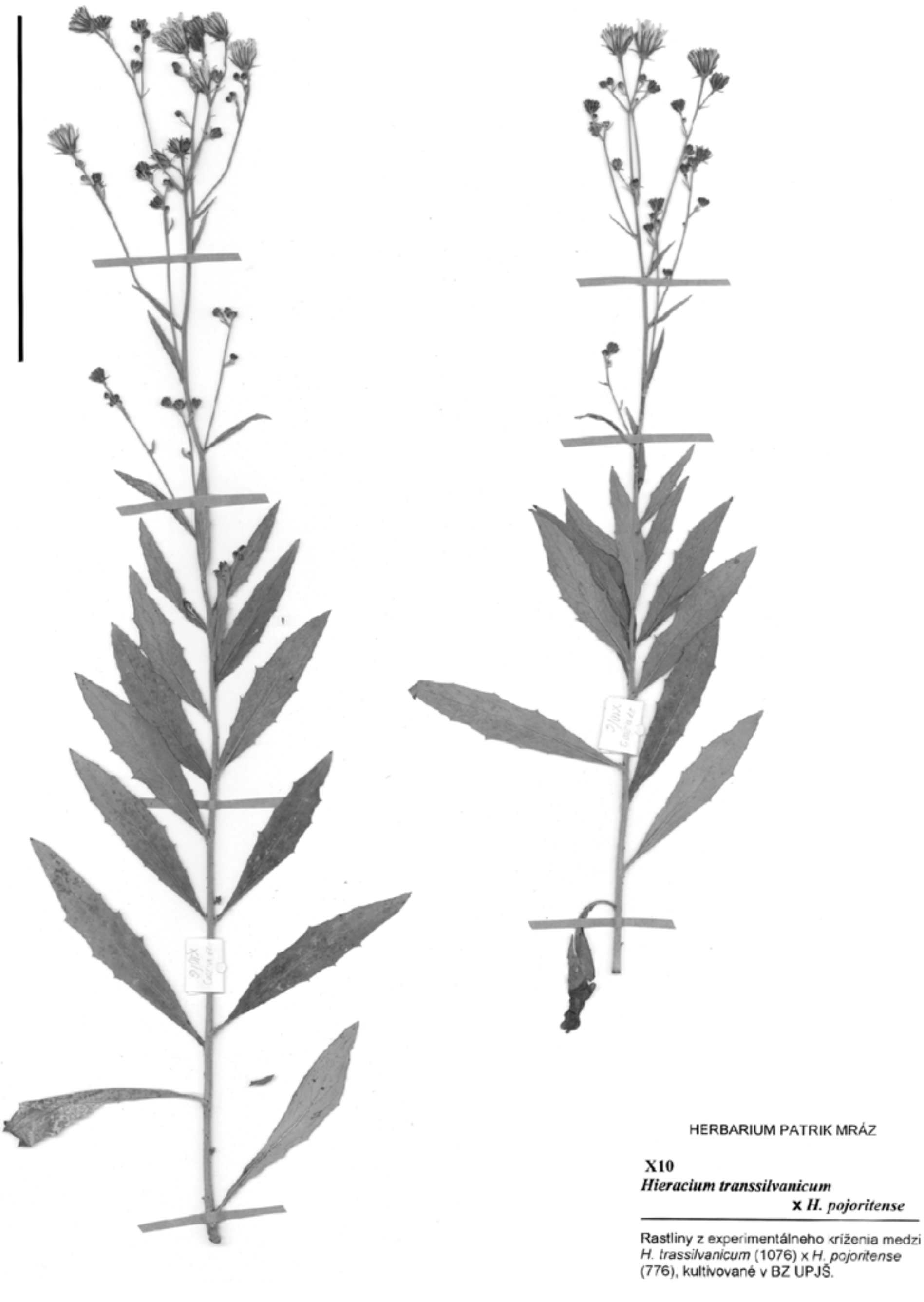
Hybridity

The proportion of well-developed fruit produced by the primary crosses between diploid taxa was relatively high. Mean fruit set per cross ranged between 68–100% with an average of 88% (Table 1). The mean germination of the achenes that originated from these crosses was 48%. Almost all F1 progenies exhibited clear hybrid origin. Only 4 plants (19%) in cross no. X5 arose via autogamy, the rest of the offspring were true hybrids. This is the first detected occurrence of autogamy in the otherwise self-incompatible genus *Hieracium* (Mráz 2003), although a total failure of the self-incompatibility system is recorded for several diploid – triploid crosses (see Mráz 2003, Mráz & Tomčíková 2004). From the above results it is obvious that hybridization at the diploid level is possible. The results of the very rare attempts at artificial hybridization in *Hieracium* s. str., which were unsuccessful or at least not clearly interpretable (cf. experiments of Mendel, Ostenfeld and Zlatník mentioned in Introduction), can now be acounted for. Mendel had no idea about

Rastliny z experimentálneho kríženia medzi *H. alpinum* (649) x *H. trassilvanicum* (1064), kultivované v BZ UPJŠ.

Fig. 7. – Hybrid progeny from cross no. X5: *Hieracium alpinum* × *H. transsilvanicum*, scale bar = 10 cm.

Fig. 8. – Hybrid progeny from cross no. X9: *Hieracium umbellatum* (high mountain morphotype) × *H. alpinum*, scale bar = 10 cm.



Rastliny z experimentálneho kríženia medzi *H. trassilvanicum* (1076) x *H. pojoritense* (776), kultivované v BZ UPJŠ.

Fig. 9. – Hybrid progeny from cross no. X10: *Hieracium transsilvanicum* × *H. pojoritense*, scale bar = 10 cm.

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X10
Hieracium transsilvanicum
× *H. pojoritense*

Rastliny z experimentálneho kríženia medzi *H. trassilvanicum* (1076) x *H. pojoritense* (776), kultivované v BZ UPJŠ.

Fig. 10. – Hybrid progeny from cross no. X10: *Hieracium transsilvanicum* × *H. pojoritense*, scale bar = 10 cm.

Fig. 11. – Triple hybrids from cross no. X21: (*Hieracium transsilvanicum* × *H. umbellatum* – morphotype of low altitude) × *H. umbellatum* – high mountain morphotype, scale bar = 10 cm.

Fig. 12. – Triple hybrids from cross no. X22: *H. umbellatum* – high mountain morphotype × (*Hieracium transsilvanicum* × *H. umbellatum* – morphotype of low altitude), scale bar = 10 cm.

the existence of apomixis in *Hieracium* the first embryological evidence for which was reported early in the 20th century (Rosenberg 1917). Most of the taxa used by Mendel were polyploids, which form seed apomictically. His notes in letters to C. Nägeli (Mendel 1950: 2, 21, 26): "..., I fear that in spite of all precautions, self-fertilization did occur"; "I have not yet succeeded in producing hybrids of Archieracia, ..."; "In the Archieracia it is very difficult to prevent self-fertlization"; "Thus far only two hybrids have been obtained", clearly show that almost all the progeny Mendel obtained were of apomictic origin. The important feature of this diplospory of *Antennaria* type is precocious embryony, where the unreduced egg cell develops into an embryo before the flowers open so that fertilization is impossible (Bergman 1941: 29). Thus, the possibility of hybridization, with *Hieracium* polyploids serving as mother plants, is highly limited. For this reason, Zlatník's record of obtaining apomictically reproducing hybrids from reciprocal crosses between *H. alpinum* and *H. murorum* (Zlatník 1938: 41), is rather surprising. Both taxa are usually triploid apomicts. On the other hand, Zlatník might have used a diploid cytotype of *H. alpinum* from the Eastern Carpathians in the Ukraine, obtained on one of his many botanical field trips there in the first half of 20th century. Unfortunately, he did not mention the exact provenance of the plant material used in his experiments.

Morphology of hybrid progeny

The F1 hybrids are usually intermediate in most morphological characters. However, in the reciprocal crosses (X2 and X5) between *Hieracium alpinum* and *H. transsilvanicum* and in the cross between high mountain type of *H. umbellatum* and *H. alpinum* (X9), the hybrids usually more closely resemble the mother plant than the pollen donor (mainly in the size and number of capitula, or in general habit/branching pattern in the latter case; Fig. 8). This is also the case of the rare recent natural hybrids between *H. transsilvanicum* and *H. alpinum* (Mráz et al. 2005). Although coming from two different crosses (X1: *H. transsilvanicum* × *H. umbellatum* – a type of low altitude and X10: *H. transsilvanicum* × *H. pojoritense*), the F1 hybrids are more or less similar to each other in general habit (Figs 6 and 9), number of stem leaves, position of stem leaves, and character of indumentum.

Hybrid progeny of one cross exhibit high levels of morphological variation (number and size of capitula, character of indumentum, shape and position of the stem leaves, branching pattern, etc.; Figs 6–12) due to the different allelic combinations of the unrelated parental taxa. The characteristic trait of the hybrids from the different crosses is a distinctive complex branching pattern, unusual in the parental taxa. Hybrids usually form numerous lateral branches often along the whole stem.

Interestingly, intermediacy in phenology was recorded in the hybrid plants from the cross (X1) between two phenologically different taxa *H. transsilvanicum* and *H. umbellatum*. While the former species usually flowers in cultivation from mid-May to the beginning of June, the latter blooms from mid-July to the mid-September. The hybrids start to bloom from the end of June.

Chromosome numbers

The diploid chromosome number ($2n = 18$) was recorded in all the hybrids (Table 2). This means that the homoploid artificial hybridization yielded hybrids of the same ploidy level

Table 2. – Characterization of F1 hybrid plants obtained from primary crosses (details given in Table 1.). Percentage of well-developed fruit as counted only for those capitula that produced at least one well developed achene. Abbreviations: Ncap – number of capitula, Nfr – total number of fruit, Npl – number of plants, Nwellfr – number of well-developed fruit, x – mean. Note: Only well-developed fruit was germinated. The percentage germination is scored as germinated fruit/well-developed fruit × 100.

Cross no.	Chromosome number		Stainable pollen		Well-developed fruits in free pollination (only successful pollination scored)				Unsuccessful free pollination	Germination (%)
	2n	Npl	range (%)	x (%)	Npl	range (%)	x (%)	Npl / Ncap / Nfr / Nwellfr	Npl/Ncap	
X1	18	4	80–87	85	3	3.2–5.4	4.2	3 / 5 / 184 / 8	4 / 24	50
X2	18	4	–	91	1	–	–	–	1 / 3	–
X5	18	7	–	92	1	1.9–12.5	5.5	1 / 3 / 148 / 8	1 / 3	12.5
X9	18	5	75–85	80	3	2.8–7.2	4.9	3 / 4 / 263 / 13	3 / 6	50
X10	18	8	65–85	77	3	3.0–6.1	4.5	3 / 8 / 243 / 11	11 / 115	33.3

Table 3. – Control crosses in which the F1 hybrids were the mother plants or pollen donors. The first parent in a cross is the mother plant, the second the father plant. Abbreviations: Ncap – number of capitula, Nfr – total number of fruit, Npl – number of plants, NT – not tested, Nwellfr – number of well-developed fruit, x – mean, s.n. – sine numero. Abbreviations of species names: ALP – *Hieracium alpinum*, POJ – *H. pojoritense*, TRANS – *H. transsilvanicum*, UML – *H. umbellatum,* morphotype of low altitude, UMH – *H. umbellatum,* high mountain morphotype. Note: Only well-developed fruit was germinated. The percentage germination is scored as germinated fruit/well-developed fruit × 100.

Cross no.	Parents	Well-developed fruits (only successful pollination scored)			Unsuccessful pollination	Germination	No. of triple progeny		
		range (%)	x (%)	Ncap / Nfr / Nwellfr	Ncap	(%)	Evaluated	Hybrids	Selfs
	(TRANS × UML) × UMH								
X21	X1/1 × 700	3.2–5.7	4.8	5 / 187 / 9	0	55.6	3	3	0
X22	R	13.8–59.4	37.9	4 / 259 / 98	0	60.2	16	7	9
	(TRANS × UML) × UMH								
s.n.	X1/7 × 700	–	3.7	1 / 27 / 1	0	NT	–	–	–
s.n.	R	27.0–28.9	27.9	2 / 75 / 21	0	NT			
	ALP × TRANS								
s.n.	X5/9 × X2/18	–	1.7	1 / 57 / 1	1	100	–	–	–
s.n.	R	–	–	–	4	–	–	–	–
	(TRANS × POJ) × UML								
s.n	X10/15 × 12JP	–	4.2	1 /24 / 1	6	100	–	–	–
X26	R	8.6–28.8	18.7	2 / 94 / 20	2	65	5	4	1

as the parental taxa, with the participation of both male and female reduced gametes. Rare recent hybrids between different diploid parental taxa are also diploid (Mráz et al. 2005, J. Chrtek et al., in prep.). Merxmüller (1975: 193) records an allegedly spontaneous diploid hybrid *H. leiocephalum* Bartl. ex Griseb. which arose from an interspecific cross between two unprotected diploid taxa, *H. porrifolium* L. and *H. umbellatum* L., cultivated in Munich botanical garden.

Pollen viability

The percentage of pollen of the hybrids that stained was in the range 65–92% (Table 2), which is quite similar to that of some diploid taxa and diploid natural hybrids (Mráz et al. 2005). High stainability and production of uniformly sized pollen (although not measured here) clearly indicate regular microsporogenesis in our F1 interspecific progeny. No abnormality in pollen size of different diploid *Hieracium* taxa is recorded (Kovalčiková 2004). Also no abnormalities in chromosome pairing during microsporogenesis are recorded in diploid interspecific hybrids within a closely related genus *Stenotheca* (syn. *Hieracium* subgen. *Chionoracium* Sch. Bip.), although some hybrids have a very low percentage of stainable pollen (Guppy 1978).

Reproductive capacity of artificial interspecific hybrids

The reproductive capacity of hybrid progeny was evaluated in terms of the percentage of well-developed fruit produced when flowers were isolated, freely pollinated and by controlled crosses. No well-developed achenes were produced when flowers of hybrid plants of crosses X1 (1 plant) and X5 (1 plant) were isolated.

The proportion of well developed fruit in capitula of freely pollinated plants, with at least one achene, was 1.9–12.5% in F1 hybrids. However, many of the capitula of several hybrids were completely sterile (Table 2). Although, some well-developed achenes were obtained from artificially produced hybrids, only a small percentage germinated. Moreover, some of the seedlings were abnormal morphologically, e.g. the seedling that resulted from the free pollination of hybrid plant no. X5/8 had an undeveloped radicula and died early. Abnormalities (lack of chlorophyll, unusually cut leaf lamina of cotyledons) were observed also in seedlings from primary crosses (Mráz 2003).

Five hybrid plants were crossed either reciprocally (X2 and X5) or with two different morphotypes of *H. umbellatum* (see Table 3), which were flowering at the same time as the hybrids. Only one well-developed achene (from 281 fruits in total) was obtained from the cross between two hybrid plants, which originated from the cross between the same parental species of *H. alpinum* and *H. transsilvanicum* (crosses no. X2 and X5), but in a different direction. Although in cultivation the plant reached the rosette leaf stage, it died before flowering. According to the shape and indumentum of the leaves this plant was clearly a hybrid, but it was not clear if this plant was matroclinal or arose via hybridization. Three plants of the natural hybrid *H.* ×*krasani* (*H. alpinum* × *H. transsilvanicum*) were fully sterile as mother plants when freely pollinated, or back- or reciprocal cross (Mráz et al. 2005).

In the three crosses where the hybrid plants served as the mother plants the percentage of well-developed achenes was very low (3.2–5.7%, see Table 3). On the other hand, in the reverse cross (diploid species as maternal plant, diploid hybrid as pollen donor) the pro-

Table 4. – Characterization of triple hybrid plants. Percentage of well-developed fruit as counted only for those capitula that produced at least one well developed achene. Abbreviations used: Ncap – number of capitula, Nfr – total number of fruit, Npl – number of plants, Nwellfr – number of well-developed fruit, x – mean.

Cross no.	Chromosome number		Well-developed fruits in free pollination (only successful pollination scored)			Unsuccessful free pollination
	2n	Nplants	range (%)	x (%)	Npl / Ncap / Nfr / Nwellfr	Npl / Ncap
X21	18	1	–	1.8	1 / 1 / 57 / 1	1 / 8
X22	18	6	1.9–4.0	2.6	1 / 3 / 201 / 5	1 / 2
X26	18	1	2.8–12.1	6.5	2 / 8 / 302 / 19	2 / 10

duction of well-developed fruits was significantly higher (8.6–59.4%). Interestingly, this reverse cross (crosses no. X22 and X26) resulted in some matroclinal offspring. This is further confirmation of previously reported induced autogamy (so-called mentor effect, Mráz 2003). Characterization of the triple hybrids that originated from the above mentioned control crosses is given in Table 4. The average percentage of well-developed achenes produced by triple hybrids, in capitula with at least one well-developed fruit, when freely or control pollinated was similar to that produced by the F1 plants (1.8–6.5%).

Sterility (or almost sterility) was observed in both experimental and natural *Hieracium* hybrids (cf. Mráz et al. 2005) and is probably caused by chromosomal and genic incompatibilities between parental taxa. Currently known diploids taxa are morphologically not related and belong to the diferent sections (Stace 1998). All crosses presented in this paper are in fact intersectional (see Material and Methods). Full sterility or low production of well-developed achenes were recorded also in triple hybrids, where the two parental taxa of the total of three were closely related (high mountain type and type of low altitude of *H. umbellatum*) (Table 3, cross X21).

Considering the genus *Pilosella*, there are little data on homoploid hybridization at the diploid level, but many results of crosses between diploid and polyploid taxa as well as among various polyploids (Krahulcová et al. 2000). Gadella (1992) found sexually reproducing interspecific diploid hybrids from crosses between two closely related diploids; *P. hoppeana* (Schult.) F. W. Schultz et Sch. Bip. and *P. peleteriana* (Mérat) F. W. Schultz, and two distinct species belonging to the different sections – *P. lactucella* (Wallr.) P. D. Sell et C. West and *P. hoppeana*. On the other hand, the hybrid *P. lactucella* × *P. peleteriana* (intersectional cross found in nature as a sterile diploid, cf. Turesson 1972) could not be resynthezid experimentaly (Gadella 1992).

Evolutionary considerations

Most of the recent *Hieracium* polyploid taxa probably arose via interspecific hybridization, although formation of meiotic trivalents in triploids may indicate an autopolyploid origin in some cases (Bergman 1935, Guppy 1978). It seems that recent hybridization is rare. Some factors (internal and external), which inhibit recent natural hybridization in this genus, were discussed by Mráz et al. (2005). Different diploid taxa are usually geographically and/or ecologically separated and moreover, some internal factors, like preference for conspecific pollen or an induced autogamy, may contribute to the rarity of natural interspecific hybridization in nature. In the present paper a mentor effect (an induced autogamy) is confirmed, which may contribute to the low rate of hybridization, although most of the progeny were true interspecific hybrids. The rare occurrence of natural diploid hybrids may also be linked with their very low reproductive capacity via achenes. Further, if natural selection acts on germination and on seedlings, which was not considered here, the propagation of hybrids seems to be limited. Consequently, the participation of diploid hybrids in gene flow as pollen donors in nature is probably more frequent than their role as mother plants.

Only diploid × diploid natural hybrids have been recorded (Mráz et al. 2005, J. Chrtek et al., in prep.), although gene flow between pollen producing polyploids and diploids is likely as illustrated by experimental crosses (Paule 2004, Mráz & Tomčíková 2004 and unpubl. data). If we assume that the diploid ploidy level is the most primitive, the polyploids should evolve from the diploid state. Interspecific hybridization followed by an increase in ploidy level of the diploid hybrids could be a possible pathway of polyploid formation. Another possibility is the production of unreduced gametes (2n). Certain meiotic abnormalities may occasionally occur and result in a proportion of gametes with the somatic chromosome number (for role of unreduced gametes in polyploidization see e.g. Thompson & Lumaret 1992, Bretagnolle & Thompson 1995, Ramsey & Schemske 1998). Although this phenomenon is unknown in *Hieracium* diploids, it is recorded in diploid *Pilosella peleteriana* (syn. *Hieracium peleterianum* Mérat) (Gadella 1988).

See http://www.ibot.cas.cz/preslia for Electronic appendix 1.

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Súhrn

V práci sú po prvýkrát prezentované ucelenejšie výsledky experimentálnych krížení v rode *Hieracium* s. str. Doteraz publikované informácie o umelom krížení sú veľmi kusé a len ťažko interpretovateľné. Viaceré pokusy J. G. Mendela boli neúspešné z dôvodu použitia apomikticky sa rozmnožujúcich rodičovských rastlín, o čom však samotný Mendel pochopiteľne nevedel. Agamospermia v rode bola objavená až na začiatku 20. storočia. V pokusoch sme použili ako rodičovské rastliny nasledujúce diploidné, sexuálne sa rozmnožujúce druhy: *H. alpinum*, *H. pojoritense, H. transsilvanicum* a dva morfologicky odlišné typy *H. umbellatum* (vysokohorský typ a typ nižších polôh) patriace do odlišných sekcií. V drvivej väčšine prípadov sme získali hybridné potomstvo. Rastliny z jedného kríženia boli navzájom značne morfologicky premenlivé. Všetky karyologicky analyzované rastliny mali diploidný počet chromozómov (2n = 18) rovnako ako rodičovské rastliny. Hybridy sa vyznačovali značnou produkciou veľkostne homogénneho peľu a vysokým zastúpením životaschopných peľových zŕn (65–92 %) podobne ako rodičovské diploidné druhy. Percento dobre vyvinutých nažiek v úboroch, v ktorých bola prítomná aspoň jedna dobrá nažka bolo v rozmedzí 1.9–12.5 % (priemerne 4–5 %) na úbor po voľnom alebo kontrolovanom opelení. Podobné výsledky sme získali aj u trojnásobných (“triple”) hybridov. Avšak väčšina úborov hybridných rastlín (F1 aj trojnásobných hybridov) netvorila žiaden dobre vyvinutý plod. Medzidruhové hybridy tak možno považovať za takmer sterilné (ako materské rastliny). Nakoľko boli v pokusoch zahrnuté morfologicky veľmi odlišné rodičovské druhy patriace do rôznych sekcií, nekompatibilita na úrovni chromozómov, resp. celého genómu rodičovských druhov pravdepodobne spôsobuje značné poruchy v tvorbe zárodočných mieškov primárných aj trojnásobných hybridov. V dvoch prípadoch recipročných krížení, kde materské rastliny boli *H. umbellatum* a darcovia peľu hybridy, sme zistili indukovanú autogamiu (tzv. mentor efekt). Diploidné medzidruhové hybridy sa v prírode vyskytujú len zriedkavo, čo je zrejme dané ekologickými, geografickými (diploidy sú zvyčajne alopatrické druhy

s rozdielnymi ekologickými nárokmi) ale aj vnútornými (pravdepodobne preferencia peľu iného jedinca toho istého druhu, indukovaná autogamia, strerilita hybridov na úrovni nažiek) faktormi. Pri zriedkavom výskyte sa však diploidné krížence môžu zapojiť do toku génov vďaka tvorbe dobre vyvinutého a životaschopného peľu, zriedkavejšie aj prostredníctvom nažiek ako materské rastliny.

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