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Residual sexuality of the apomict *Pilosella rubra* under natural conditions in the Krkonoše Mts

Zbytková sexualita apomiktického druhu *Pilosella rubra* v modelové populaci v Krkonoších

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Because of residual sexuality, a maternal facultatively apomictic plant is able to produce more than just apomictic progeny. The production of such non-apomictic progeny has been studied in open-pollinated *Pilosella rubra*, a hexaploid species of hybridogenous origin. The mixed-species population was studied in montane grasslands in the Krkonoše Mts (the Sudetes). Progeny from achenes that were collected in the field were grown (2800 plants) and their DNA ploidy level was determined. Based on maternal/progeny comparisons regarding ploidy level and morphology, most of the progeny was formed apomictically (91.0%), while the remainder (9.0%) consisted of trihaploids and various hybrids. In previous garden crossing experiments, the residual sexuality of *P. rubra* was 11.9%, not significantly different from the value found in the present study. Most of the hybrid progeny grown from seeds originated from a conjugation of parental reduced gametes (n+n hybrids) except for the octoploid hybrid of *P. rubra* with any of the tetraploid taxa (2n+n hybrid). The range of variation in DNA content within particular progeny categories/cytotypes reflected the origins of the progeny. Examination of the species/cytotype composition of the population from which the achenes of *P. rubra* were collected, revealed hybrids of *P. rubra* established in the field and identified the co-occurring *Pilosella* species, some of which were the putative paternal parents of these hybrids. Based on a combination of morphology and ploidy level, hybrids of hexaploid *P. rubra* with the following *Pilosella* species were identified there: the tetraploid *P. officinarum* (hybrid *P.* ×*stoloniflora*, pentaploid, hexaploid and octoploid plants, one of the hybrid plants was a somatic mosaic (4x+8x)), the diploid *P. lactucella* (tetraploid hybrid), the tetraploid *P. floribunda* (pentaploid hybrid) and the tetraploid *P. iserana* (pentaploid hybrid). The facultatively apomictic *P. rubra* readily hybridizes as a maternal parent under field conditions, especially in species-rich populations. The progeny arising from these interspecific crosses increases the population diversity and may affect the evolution of such populations.

Keywords: *Pilosella rubra*, residual sexuality, facultative apomixis, hybridization, haploid parthenogenesis

Introduction

Apomixis in plants is an asexual production of seed that occurs without both meiosis and fertilization (Czapik 1997). If any of those processes are involved, we are talking about

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some form of sexual process (see the terminological remark in Krahulcová et al. 2014). Apomictic reproduction ensures the genetic identity of progeny is that of the maternal plant (Hand et al. 2015). Some other authors use a different approach considering that sexuality must include only cases of gamete fusion. Haploid parthenogenesis (involving the meiosis but avoiding the fusion of reduced gametes) is therefore included in apomictic reproduction (Maheswari 1950, Johri & Srivastava 2001), although it does not generate the maternal-like progeny.

Apomixis can either be obligate, i.e. entire progeny originates without meiotic reduction of parental gametes and without gamete fusion, or facultative (some part of the progeny originates sexually). This facultative sexuality, named residual sexuality, is to a certain extent common in otherwise apomictic plants (Asker & Jerling 1992).

The specific rates of non-apomictically derived progeny from a maternal facultative apomict have usually been determined by means of controlled crosses: for example, in *Potentilla puberula* Krašan (Dobeš et al. 2018), *Panicum maximum* Jacq. (Kaushal et al. 2018) or *Taraxacum* sect. *Erythrosperma* (Mártonfiová et al. 2010). The corresponding studies on the role of the residual sexuality in apomicts in the field are not common. Outside of the genus *Pilosella* with autonomous apomixis (for previous findings in *Pilosella* see below), residual sexuality has mostly been examined in the field in pseudogamous taxa – for example, in the *Ranunculus auricomus* complex (Hörandl & Temsch 2009), in *Rubus* subgen. *Rubus* (Šarhanová et al. 2012), in *Sorbus* (Hajrudinović et al. 2015, Lepší et al. 2019) and in *Cotoneaster* (Macková et al. 2020).

Crossing experiments revealed the effect of the residual sexuality in *Pilosella rubra* (Peter) Soják (Fig. 1) – the hexaploid maternal *P. rubra* has been pollinated by tetraploid *P. officinarum* (Krahulcová et al. 2004). With respect to the fact that *P. rubra* is a stabilized hybridogenous species (2n+n hybrid between *P. aurantiaca* and *P. officinarum*, see below), this crossing experiment was in fact a back-cross. This combination of parental cytotypes allowed us to distinguish the following origins of the progeny: apomixis (hexaploid progeny which included a small proportion potentially originating from autogamy), haploid parthenogenesis (triploid progeny originating from a parthenogenetic development of a reduced (meiotic) female gamete), and hybridization: pentaploid progeny (n+n hybrids), octoploid progeny (2n+n hybrids), and heptaploid progeny (n+2n hybrids; the terminology follows Harlan & deWet 1975). The achenes obtained from cross were divided into two parts. The first part was sown and the proportions of particular cytotypes were determined from cultivated seedlings (Krahulcová et al. 2004). The second half was analysed using the Flow Cytometric Seed Screening method (FCSS, Matzk et al. 2000), to find the proportions of particular cytotypes among the embryos (Krahulec et al. 2006). Comparison of the cytotype proportions in seeds and seedlings allowed us to conclude, which progeny cytotypes were likely to be eliminated during seed germination and early stages of development of the seedlings. Irrespective of the different cytotype composition, the residual sexuality in the crossed maternal plants of *P. rubra* was found to be about 10%, when analysing both the progeny embryos and progeny seedlings, respectively (Krahulcová et al. 2004, Krahulec et al. 2006). The questions of the present study were: (i) Is the degree of the residual sexuality that was previously detected in *P. rubra* experimentally, comparable with the residual sexuality of this species in the field conditions, and (ii) do the products of this hybridization also occur in the field?

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No.
Pilosella rubra (Peter) Soják

Locality: Czech Republic, Krkonoše Mts. Pec pod Sněžkou: Velká Úpa, Pěnkavčí vrch settlement, 982 m a.s.l., 50°41'45.1"N, 15°47'19.5"E.

Habitat: this voucher comes from garden cultivation in Průhonice (collecetd 2. 6. 2016)

Collector: F. Krahulec and J. Doležal

Date: 24. 6. 2015

Number: 2191

Notes: $2n = 6x$ (FCM), apomictic.

Fig. 1. – *Pilosella rubra,* a hexaploid hybridogenous species described from the Krkonoše Mts.

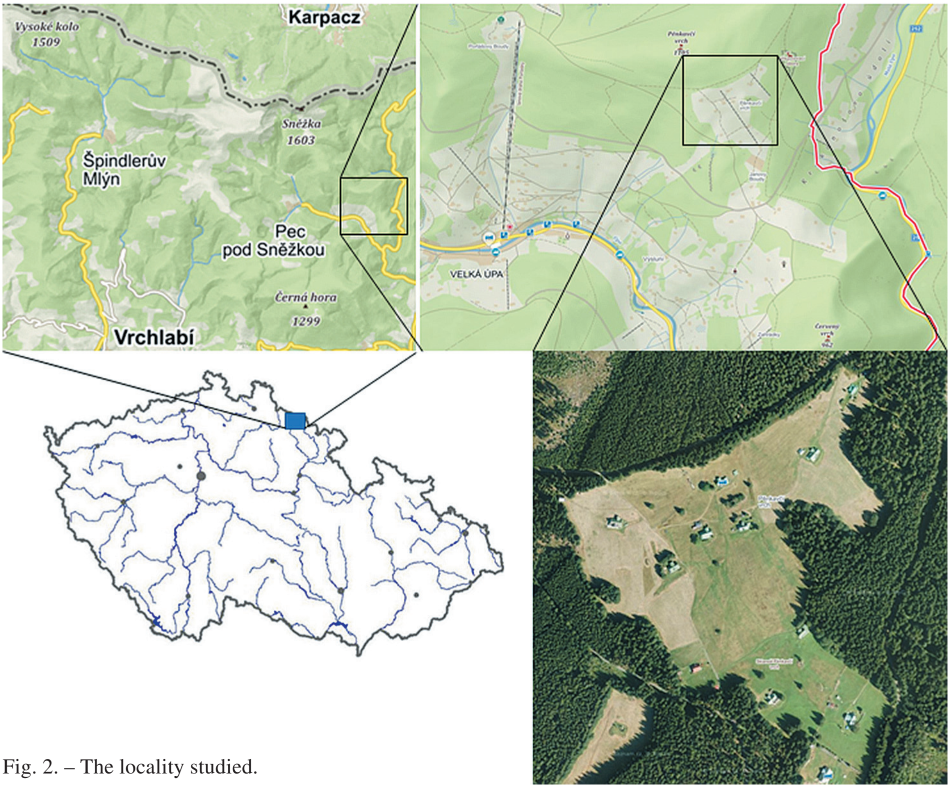

Fig. 2. – The locality studied.

Material and methods

Locality studied

Meadow complex at Pěnkavčí Vrch settlement in the eastern part of the Krkonoše Mts (Karkonosze, Riesengebirge). The locality is situated NE of Velká Úpa, at the altitude 975–1040 m, between the points: 50°41'44.1"N, 15°47'15.8"E; 50°41'54.8"N, 15°47'06.6"E; 50°41'57.3"N, 15°47'20.6"E; and 50°41'48.2"N, 15°47'25.3"E (Fig. 2). Grasslands in the area studied belong mostly to the alliance *Nardo-Agrostion tenuis*, and within it to the association *Sileno-Nardetum strictae*; more fertile parts harbour communities of the alliance *Polygono bistortae-Trisetion flavescentis*, more specifically to the association *Melandrio rubri-Phleetum alpini* (Chytrý 2007).

Plant material

Eighteen plants representing three species of *Pilosella* and four hybrids were collected at the locality during the years 2012–2020 (Table 1). The plants collected in 2012–2014 were transferred to the experimental garden of the Institute of Botany in Průhonice; the plants collected later were transferred to a private garden in Průhonice. The chromosome number and/or the DNA ploidy level were determined in these cultivated plants. The reproduction system was determined in five out of 18 plant accessions (Table 1). The karyological and reproductive attributes of all *Pilosella* taxa that co-occurred at the locality, were also available in our previously published studies on *Pilosella* in the Krkonoše Mts (Table 2)[1].

Achenes of *Pilosella rubra* were collected in the field on 10 July, 2014. Later they were checked to remove empty and damaged achenes and on 1 August they were sown in a mixture of garden soil, compost and sand (2:2:1). Young seedlings were replanted into small pots on 20 August, and on 8 September young plants were replanted to pots 7 × 7 × 8 cm. These pots were placed in the private garden of Jan Doležal in Týniště nad Orlicí (Dukelská str., 50°09'21.7"N, 16°05'03.3"E, alt. 259 m). Altogether 2800 plants survived to the following spring; once they started to flower, these progeny plants were presorted into three groups. The first, the largest group were plants morphologically corresponding to maternal *P. rubra*. The second group were putative trihaploids, which were easily determinable according to morphological characters (Krahulec et al. 2011). The remaining plants, the hybrids, comprised the third group.

Names of the plant taxa follow Chrtek in Kaplan et al. (2019). Specimens of analysed plants from the field are stored in herbarium of the Institute of Botany (PRA), those of plants cultivated from achenes are stored in the private herbarium of Jan Doležal.

In this paper we follow the taxonomic concept that is generally used for the genus *Pilosella* in Central Europe: we distinguish the basic and the intermediate species, the latter being of hybridogenous origin (Bräutigam & Greuter 2007). The intermediate species are usually stabilized by apomixis (Fehrer et al. 2007). We include in this category only stabilized hybridogenous species, which behave as independent entities. We also distinguish recent hybrids, which are indicated by "×". The hybrid formula characterizing the parental combination of a hybrid taxon, indicates either equal proportions of parental morphological characters or the characters of either parent that prevail. Specifically, *P. rubra* is a hybridogenous hexaploid species, with a combination of the genomes of *P. aurantiaca* (4x) and *P. officinarum* (2x). It was described from the Krkonoše Mts by Peter (1881). It has a high penetrance of apomixis and behaves independently of its parents in the field. Morphologically and genomically identical plants were found as recent hybrids in many areas commonly with their parents (for a list see Krahulec et al. 2020); we use the name *P.* ×*rubra*. Such plants have low penetrance of apomixis and grow only with their parents.

Determination of chromosome number, DNA ploidy level and reproductive system

Chromosomes were counted in root-tip meristems of pot-grown plants. The squashed meristems were stained with lacto-propionic orcein (Dyer 1963); for a detailed description of the procedure, see Krahulcová & Krahulec (1999). Using flow cytometry (FCM), we determined the DNA ploidy level (Suda et al. 2006) both in some of the cultivated plants originating from the field (Table 1) and in the progeny seedlings that were grown from achenes of *P. rubra* (Table 3). The seedlings were presorted according to morphology into three groups (see above). The DAPI staining method (Doležel et al. 2007) was applied using the nuclei-extracting buffer (Otto I) and the staining buffer (Otto II) supplemented

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[1] The hexaploid cytotype of *P. stoloniflora* published by Krahulcová & Krahulec (1999) was in fact based on the poorly developed plants of *P. rubra* (*Pilosella rubra* was later studied in detail at the same locality).

Table 1. – Individual plants that were collected in the population at Pěnkavčí Vrch (the Krkonoše Mts) and analysed in this study. These cultivated plants are characterized by accession label and ploidy level, the reproduction system (RS) was determined in five accessions. Grouping of two accessions in the column means that the two respective morphologically identical plants were collected in a very close proximity in the field and therefore they are considered to be monoclonal (the spread of *Pilosella* plants is not limited to propagation by seeds, but includes also the clonal growth by means of stolons). To complete the overview of all taxa that co-occurred in the population, the previously published data are added in Table 2, referring to plants from other localities in the Krkonoše Mts. Methods used for determination of RS (for description see the Materials and methods section): A – presence of seeds in emasculated versus in open-pollinated capitula; B – screening of progeny origins using the FCSS method. No. – number of individuals analysed. Origin of the seeds: OPG – sampled from open pollinated plants in the garden; OPL – sampled from open pollinated plants at the locality.

Taxon	Accession	DNA ploidy level, chromosome number	RS	Method	No.	Ploidy of embryos/ progeny seedlings	Origin of seeds	Remark
P. rubra × *P. floribunda*	2146	$2n \approx 5x \approx 45$ $2n = 5x = 45$	mainly sexual, residually apomictic	A, B	32	3x to 7x–8x	OPG	reduced seed-set, origin of embryos: n+n, n+2n, 2n+0, n+0
P. rubra × *P. iserana*	2188	$2n \approx 5x \approx 45$						
P. rubra × *P. iserana*	2189A 2189B	$2n \approx 5x \approx 45$						
P. rubra × *P. lactucella*	FK2020/7	$2n \approx 4x \approx 36$						
P. ×*stoloniflora*	2192	$2n \approx 5x \approx 45$						
	FK2020/1	$2n \approx 5x \approx 45$						
	FK2020/6	$2n \approx 5x \approx 45$						
P. ×*stoloniflora*	2182A 2182B	$2n \approx 8x \approx 72$ $2n = 8x = 72$	mainly haploid parthenogenesis and sexual mating, residually apomictic	A, B	22	4x to ~10x	OPG	reduced seed-set, origin of embryos: n+0, n+n, 2n+n, 2n+0
	FK2020/2	$2n \approx 8x \approx 72$						
P. ×*stoloniflora*	2193	$2n \approx 6x \approx 54$						
P. ×*stoloniflora*	FK2020/3	$2n \approx 4x \approx 36$ + $2n \approx 8x \approx 72$						
P. rubra	2191	$2n \approx 6x \approx 54$	apomictic	B	14	3x, 6x	OPL	origin of embryos: 2n+0, n+0
P. aurantiaca	2194 2196	$2n \approx 4x \approx 36$	apomictic	B	22	4x	OPL	origin of embryos: 2n+0
	FK2020/4	$2n \approx 4x \approx 36$						
P. floribunda	2147	$2n \approx 4x \approx 36$						

Table 2. – Previously published data on plants occurring on the studied locality (Pěnkavčí Vrch) in the Krkonoše Mts. Asterisk indicates the data from nearby localities Janovy boudy (Braunboden) and slopes to Velká Úpa. RS – reproduction system. [1]This record is related to poorly developed plants of *P. rubra* (see the remark at p. 407).

Taxon	Chromosome number, DNA ploidy level	RS	Reference
P. onegensis	$2n = 2x = 18$	sexual	Krahulcová et al. 2001*; Krahulec et al. 2004*; Fehrer et al. 2005*
P. lactucella	$2n = 2x = 18$, $2n \approx 2x \approx 18$	sexual	Krahulcová and Krahulec 1999*; Krahulcová et al. 2001; Krahulcová et al. 2013; Krahulec et al. 2004*; Fehrer et al. 2005*
P. officinarum	$2n = 4x = 36$, $2n \approx 4x \approx 36$	sexual	Krahulcová and Krahulec 1999*; Krahulcová et al. 2001; Krahulcová et al. 2013; Krahulec et al. 2004*; Fehrer et al. 2005*
P. aurantiaca	$2n = 4x = 36$, $2n \approx 4x \approx 36$	apomictic	Krahulcová and Krahulec 1999*; Krahulcová et al. 2001; Krahulcová et al. 2013*
P. aurantiaca	$2n = 5x = 45$, $2n \approx 5x \approx 45$	apomictic	Krahulcová et al. 2001; Krahulcová et al. 2013
P. caespitosa	$2n = 4x = 36$	apomictic	Krahulcová & Krahulec 1999; Krahulcová et al. 2001; Krahulcová et al. 2013*
P. caespitosa	$2n = 5x = 45$	apomictic	Krahulcová et al. 2013
P. iserana	$2n = 4x = 36$, $2n \approx 4x \approx 36$	apomictic	Krahulcová & Krahulec 1999*; Krahulcová et al. 2013*; Krahulec et al. 2004*
P. iserana	$2n = 5x = 45$ (a single accession)	apomictic	Krahulcová et al. 2001*
P. floribunda	$2n = 4x = 36$	apomictic	Krahulcová & Krahulec 1999*; Krahulcová et al. 2001*; Krahulcová et al. 2013; Krahulec et al. 2004*
P. rubra	$2n = 6x = 54$	apomictic	Krahulcová & Krahulec 1999; Krahulcová et al. 2001*; Krahulcová et al. 2013
P. stoloniflora	$2n = 5x = 45$		Krahulcová et al. 2001
P. stoloniflora	$2n = 6x = 54$	apomictic	Krahulcová & Krahulec 1999*[1]
P. piloselliflora (incl. *H. apatelium*)	$2n = 4x = 36$	sexual, apomictic	Krahulcová & Krahulec 1999*; Krahulcová et al. 2001*; Krahulcová et al. 2013; Krahulec et al. 2004*; Fehrer et al. 2005*
P. piloselliflora (*incl. H. apatelium*)	$2n = 5x = 45$	apomictic	Krahulcová & Krahulec 1999*; Krahulcová et al. 2001; Krahulcová et al. 2013; Krahulec et al. 2004*
P. piloselliflora (incl. *H. apatelium*)	$2n = 6x = 54$	apomictic	Krahulcová et al. 2001

with mercaptoethanol (2 µl ml^{-1}) as an antioxidant. Fluorescence intensity was determined using either the Ploidy Analyser PA II or the CyFlow cytometer (both instruments produced by Partec GmbH, Münster, Germany), both equipped with an HBO high-pressure mercury lamp for UV excitation. Relative DNA content was estimated using the diploid *Pilosella lactucella* as an internal standard (DNA content 4.07 pg/2C, Suda et al. 2007). Evaluating the FCM analyses, only those histograms, in which the coefficient of variance of peaks did not exceed 3%, were accepted. A total of 3000 nuclei were scored for each sample. Table 4 gives equivalents of DAPI measured DNA content of *P. lactucella* (internal standard, 2C=4.07 pg DNA) for individual categories distinguished within *P. rubra* progeny.

Table 3. – Number of progenies of *Pilosella rubra* belonging to particular ploidy levels. Hexaploid plants (marked as *), which were morphologically identical with mother plants, were evidently of apomictic origin. Chimeric plants were of several types: combination 6x + 12x (three seedlings), 6x + 8x (one seedling), 5x + 7x (three seedlings), 4x + 8x (one seedling).

Ploidy level	No. of progeny (%)	Ploidy level	No. of progeny (%)
3x	65 (2.3)	7x	14 (0.5)
4x	34 (1.2)	8x	30 (1.1)
5x	71 (2.5)	12x	8 (0.3)
6x*	2545 (90.9)	chimera	8 (0.3)
6x hybrid	25 (0.9)		

Table 4. – DAPI genome size equivalent of *Pilosella lactucella* (2C = 4.07 pg) for particular ploidies from progeny of *P. rubra* and sample of *P. rubra* from the field. Abbreviations: 6x(r-f) – the four samples measured represented 4 groups of 20 plants of *P. rubra* from three different localities; 6x(r-p) – hexaploid plants of progeny considered as *P. rubra*; 6x(h) – hexaploid plants of different morphology than *P. rubra* considered as n+n hybrids; S.D. – standard deviation; n – number of plants analysed; CV – coefficient of variation.

Ploidy	Mean	S.D.	n	CV (%)
3x	1.391	0.098	65	7.0
4x	1.983	0.015	10	0.7
5x	2.413	0.094	73	3.9
6x(r-f)	2.856	0.017	4	0.6
6x(r-p)	2.864	0.022	10	0.8
6x(h)	2.868	0.074	44	2.6
7x	3.488	0.145	5	4.2
8x	3.871	0.126	44	3.6
12x	5.700	0.032	8	0.6

Two methods were used for the determination of the reproductive system, i.e. sexual versus apomictic reproduction mode: (A) the comparison of the seed-set in the emasculated versus open-pollinated capitula. This method detects parthenogenetic seed development and is routinely used in *Compositae* with autonomous apomixis (Gadella 1984, Richards 1986). The fertilization-independent seed formation is predominantly coupled with apomeiosis in wild-type apomicts within *Pilosella* (Bicknell & Koltunow 2004) and therefore the emasculation test is an approximation for plants reproducing apomictically (Hand et al. 2015). The presence of well-developed (filled) achenes in each of three emasculated capitula per plant was sufficient for determination of an apomictic reproduction mode. Those plants that were scored as sexual, formed at least some seeds in open pollinated capitula, whereas the emasculated capitula did not form any. (B) The Flow Cytometric Screen of Seeds was used for the determination of reproductive origins of seeds (FCSS method, Matzk et al. 2000), especially in those hybrids that formed only low amounts of seeds in open-pollinated capitula. The seed doublets were analysed together with *Bellis perennis*, the diploid *Pilosella lactucella*, or the triploid accession of *Pilosella aurantiaca* as an internal standard in each sample; the standard used was chosen according to expected ploidy levels in the respective embryos and endosperm tissue in analysed seeds. For details of the FCSS procedure modified for *Pilosella*, see the previous studies (for example Krahulcová et al. 2014, 2018). The use of the respective method for determination of the reproduction system in individual plants originating from the field is given in Table 1.

Results

The cultivated progeny of P. rubra and its residual sexuality

In total 2800 individuals were grown from achenes collected at the locality. This progeny comprised three categories of plants (Table 3). The largest group (90.9%) were hexaploid, and morphologically corresponded to the maternal parent *P. rubra*; these plants were evidently of apomictic origin. The second group were easily distinguishable triploid (trihaploid) plants with weak growth (Fig. 3), indicating haploid parthenogenesis (2.3%). The remaining group was conspicuously heterogeneous in morphology and was formed of hybrids with a diverse range of ploidy level (4x, 5x, 6x, 7x, 8x, 12x). To compare variation of genome size within particular ploidy categories, we studied the genome size (estimated as equivalents of DAPI measured genome size of diploid *P. lactucella* used as an internal standard; Fig. 4, Table 4).

Residual sexuality in the field and garden experiment

In the garden experiment where *P. rubra* (6x) had been pollinated by *P. officinarum* (4x) (Krahulec et al. 2006) the residual sexuality was 11.9%. Residual sexuality in the progeny cultivated from achenes originating in the field was 9.1%. The comparable progeny categories from both studies (i.e. trihaploids, hexaploids and hybrids) are given in Table 5. In spite of the fact that the present study is based on one order higher number of plants, the results of both experiments are not statistically different: in both, we can consider the level of the residual sexuality in *P. rubra* to be about 10%.

Table 5. – Comparison of progeny categories of *Pilosella rubra* from the garden pollination experiment, based on data by Krahulec et al. (2006), and on achenes collected at Pěnkavčí vrch in the Krkonoše Mts. The distribution of progeny across categories in the garden experiment and in the field was not significantly different ($\chi^2 = 4.176$, P > 0.1).

Progeny category	3x		6x		Hybrids		Sum
	no.	%	no.	%	no.	%	no.
Garden experiment	14	4.0	312	88.1	28	7.9	354
Progeny of achenes from field	65	2.3	2545	90.9	190	6.8	2800

Fig. 3. – Particular types of trihaploids from *P. rubra*

Taxa present at the locality as potential pollen donors

The following species were found on the studied locality (for their karyological and reproductive attributes see Table 1, 2) *P. aurantiaca, P. caespitosa*, *P. lactucella*, *P. officinarum, P. onegensis* (all basic species) and the hybridogenous species *P. floribunda*, *P. iserana, P. piloselliflora, P. schultesii, P. rubra* and *P. stoloniflora*.

Hybrids of Pilosella rubra present at the locality

P. rubra × *P. floribunda* (Fig. 5). – This previously unknown heteroploid hybrid had already been found in 2012. It is intermediate between the parental taxa, both in morphology and in ploidy/chromosome number (2n = 5x = 45, Table 1). Although both putative parents are apomictic, their hybrid had a very poor seed set in open-pollinated capitula. Most of these progeny embryos were of sexual origin, only a single embryo originated apomictically (Table 1).

P. rubra × *P. iserana* (Fig. 6). – This is also an undescribed heteroploid hybrid. Morphologically, it is intermediate between parents; its leaves have a low number of stellate hairs beneath; it has stolons, their leaves are more similar to *P. iserana*. Ploidy level is pentaploid corresponding to a cross between tetraploid and hexaploid putative parents.

P. rubra × *P. lactucella* (Fig. 7). – The hybrid with this parentage is tetraploid, and corresponds to n+n origin (likely triploid gamete of *P. rubra* and haploid gamete of *P. lactucella*). It has small leaves reminiscent of *P. lactucella*, orange flowers and leaves with many stellate hairs beneath – a *P. rubra* character.

P. rubra × *P. officinarum.* – This heteroploid hybrid has been found as four cytotypes. The first one is a pentaploid, morphologically corresponding to *P. stoloniflora* (Fig. 8, Table 1). Comparing the ploidy level of the putative parents (hexaploid × tetraploid), this hybrid had evidently originated from a cross via reduced parental gametes (n+n hybrid).

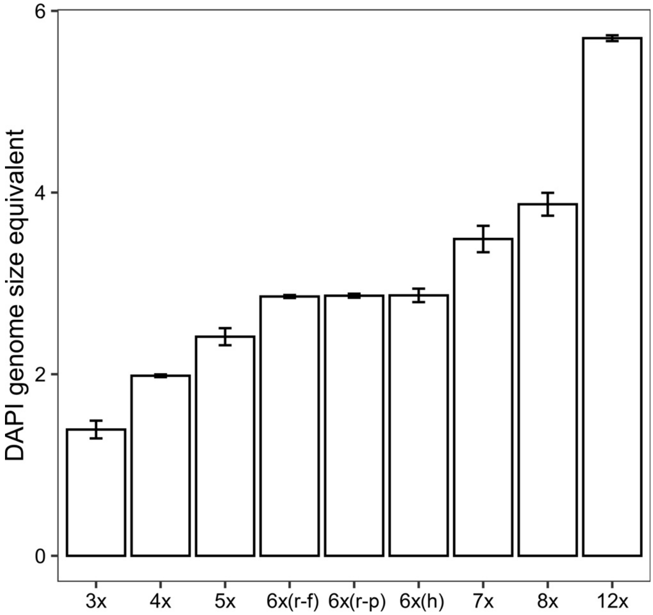

Fig. 4. – DAPI genome size equivalent of *P. lactucella* (diploid, used as internal standard) for individual categories of progeny of *P. rubra*. For abbreviations see Table 4.

This hybrid is relatively common at the locality, the colour of flowering capitula is variable between that of the parents: some plants are intermediate, orange, some others more yellow with orange ligulate flowers on the periphery of the capitulum. The second type was hexaploid and morphologically was more like *P. stoloniflora* than *P. rubra* (Fig. 9). It probably originated as product of a back cross of pentaploid *P. stoloniflora* and *P. rubra* as their n+n hybrid (3x + 3x). The third hybrid was morphologically different from the previous ones because it had only one big red capitulum (Fig. 10). Such a plant was described as *P. rubripilosella* by Schneider (1888-1895). The estimation of ploidy level by flow cytometry corresponded to the octoploid level and the follow-up chromosome counts confirmed this (2n = 8x =72, Table 1). Because we had earlier found morphologically identical plants among the octoploid experimental hybrids between *P. rubra* and *P. officinarum* (see Discussion), we are sure that this hybrid found in the field must have arisen from an unreduced gamete of *P. rubra* (6x) and a reduced gamete of *P. officinarum* (2x). Ploidy level of another plant with one red capitulum was, surprisingly, a mosaic of two ploidy values, which corresponded to tetraploid and octoploid ploidy levels.

Discussion

Identity of progeny formed by open-pollinated Pilosella rubra in the field

The majority of the progeny was hexaploid, morphologically identical to the maternal parent and therefore highly probably of apomictic origin. Triploid plants had evidently originated from a parthenogenetic development of a reduced female gamete of *P. rubra*. These trihaploids were small-sized, some of them with poor growth. Their leaves were

Det. S. Bräutigam 28. 8. and 29. 8. 2013

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No.

Pilosella

Locality: Czech Republic, Krkonoše Mts, Pec pod Sněžkou: Velká Úpa, Pěnkavčí settlement, 1003 m a.s.l., 50°41'50.5"N, 15°47'18.5"E.

Habitat: managed meadow

Collector: F. Krahulec, R. Prausová and V. Srnková

Date: 4. 7. 2013

Number: 2146

Notes: 2n = 5x (FCM). Putative hybrid P. rubra × P. floribunda.

Fig. 5. – Pentaploid hybrid of *P. floribunda* and *P. rubra*

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No.

Locality: Czech Republic, Krkonoše Mts, Pec pod Sněžkou: Velká Úpa, Pěnkavčí vrch, 992 m a.s.l., 50°41'48.1"N, 15°47'19.0"E.

Habitat: originally managed meadow, this voucher comes from garden cultivation (collected 29. 5. 2016)

Collector: F. Krahulec

Date: 16. 6. 2015 Number: 2188 RU×PI

Notes: 2n = 5x (FCM).

Det. F. Krahulec 5. 9. 2019

Fig. 6. – Pentaploid hybrid of *P. iserana* and *P. rubra*

HERBARIUM INSTITUTI BOTANICI ACADEMIAE SCIENTIARUM PRŮHONICE (PRA)

Collection of ....

Loc.: Krkonoše Mts, Pec pod Sněžkou: Velká Úpa, Pěnkavčí vrch settlement, 50°41'47.8"N, 15°47'14.4"E - 50°41'46.689"N, 15°47'20.667"E, alt. 980-1000 m

Hab.: managed meadow, Sileno-Nardetum

Coll. F. Krahulec

Date: 17.6. 2020

Note: ŽK 2020/7, DNA ploidy 4x

Fig. 7. – Tetraploid hybrid of *P. lactucella* and *P. rubra*

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No.
Pilosella rubra (Peter) Soják × P. officinarum Vaill.

Locality: Czech Republic, Krkonoše Mts, Pec pod Sněžkou: Velká Úpa, Pěnkavčí vrch settlement, 982 m a.s.l., 50°41'45.1"N, 15°47'19.5"E.

Habitat: this voucher comes from garden cultivation in Průhonice (collected 29. 5. 2016)

Collector: F. Krahulec and J. Doležal

Date: 24. 6. 2015

Number: 2192

Notes: 2n = 5x (FCM).

Fig. 8. – Pentaploid hybrid *P.* ×*stoloniflora* (*P. officinarum* × *P. rubra*)

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No.
Pilosella stoloniflora (Waldst. & Kit.) F.W. Schultz & Sch. Bip.

Locality: Czech Republic, Krkonoše Mts, Pec pod Sněžkou: Velká Úpa, Pěnkavčí vrch settlement, 982 m a.s.l., 50°41'45.1"N, 15°47'19.5"E.

Habitat: this voucher comes from garden cultivation in Průhonice (collecetd 26. 5. 2016)

Collector: F. Krahulec and J. Doležal

Date: 24. 6. 2015

Number: 2193

Notes: 2n = 6x (FCM).

Fig. 9. – Hexaploid hybrid *P.* ×*stoloniflora* (*P. officinarum* × *P. rubra*)

small, narrow, and often yellow-green; and often had higher numbers of leaves than other seedlings (Fig. 2).

The third group of spontaneous hybrids was heterogenous. Those hybrids with ploidy levels of 4x, 5x and 6x had most likely originated from a conjugation of parental reduced gametes. An unknown, but probably very small part of the hexaploids in this “hybrid” category, which were morphologically different from the mother-like hexaploid progeny of apomictic origin, could be the products of autogamy because *P. rubra* is a hybrido-

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No.
Pilosella rubra (Peter) Soják × P. officinarum Vaill.

Locality: Czech Republic, Krkonoše Mts, Pec pod Sněžkou: Velká Úpa, Pěnkavčí vrch settlement, 992 m a.s.l., 50°41'47.1"N, 15°47'18.7"E.

Habitat: this voucher comes from garden cultivation in Průhonice (collecetd 28. 5. 2015)

Collector: F. Krahulec

Date: 17. 6. 2014

Number: 2182 plant A

Notes: 2n = 8x (FCM). 2n = 8x = 72 (chromosome count). The plant generates predominantly either sexual or polyhaploid (n+0) progeny, in addition to rare apomictic (2n+0) progeny

Fig. 10. – Octoploid hybrid *P.* ×*stoloniflora* (*P. officinarum* × *P. rubra*)

genous species thus segregating after selfing. However, the level of the residual sexuality in the crossed *Pilosella rubra* was only about ten percent (Krahulcová et al. 2004), and therefore the fraction of the selfed progeny that might potentially originate in the field by means of the heterospecific pollen (mentor effect) is probably negligible (Krahulcová et al. 1999).

Considering the ploidal diversity among the potential paternal *Pilosella* species that comprised the hybridising population (Table 1, 2), the heptaploid hybrids could originate either from an unreduced female gamete from *P. rubra* and a reduced male gamete (6x+1x) or from a reduced female gamete and an unreduced male gamete (3x + 4x). Similarly, an ambiguous origin can be inferred for the octoploid hybrids: either from an unreduced female gamete and a reduced male gamete (6x + 2x) or from a reduced female gamete and an unreduced male gamete (3x + 5x). The dodecaploid plants originated most probably from the doubling of chromosome sets of the parental plants (6x + 6x).

The chimeric plants could originate by chromosome doubling in part of the proembryo or embryo (combinations 6x + 12x and 4x + 8x), but two other chimeric plants (5x + 7x, 6x + 8x) suggest some disturbances during mitosis in early developmental phases connected with doubling of chromosomes (Table 3).

Variation in genome size detected within progeny cytotypes

Variation in genome size reflects the ways that particular cytotypes originated (Fig. 4, Table 4). It is evident that the genome size of the progeny classified as maternal (*P. rubra*) has little variation and is of the same size as plants of this species from three different localities in the Krkonoše Mts (altogether 20 plants grouped into four sets). On the other hand, hexaploid plants considered as hybrids have larger variation as well as trihaploids. The trihaploid plant genome size reflects the fact that *P. rubra* is a stabilized hybridogenous plant combining four genomes of *P. aurantiaca* and two genomes of *P. officinarum*; since their genome size is different (Suda et al. 2007) and their segregation might be irregular. The variation in genome size could also be influenced by aneuploidy caused by irregular meiosis. Low variation was also observed at tetraploids. For tetraploids, the pollen donor ought to be a diploid. *Pilosella lactucella* was the probable pollen donor, the second diploid species, *P. onegensis*, grows at a greater distance and has different morphology. High variation in the genome size of octoploids evidently reflects their two possible origins (2n+n, n+2n). Low variation was found in the dodecaploids (12x); they originated most probably by doubling of a hexaploid genome in the early phases of embryo development.

Residual sexuality in Pilosella rubra

Pilosella rubra has served as a model species in earlier studies on the residual sexuality in *Pilosella* (Krahulcová et al. 2004, 2011, Krahulec et al. 2006, 2011, Rosenbaumová et al. 2012). Evaluating the progeny classes among the cultivated progeny seedlings, Krahulec et al. (2006) estimated the level of the residual sexuality in the crossed *P. rubra* as 11.9%. In the present study, evaluating the progeny classes of the maternal *P. rubra* that was open-pollinated in the species/cytotype-rich population in the field, the corresponding level of the residual sexuality was a bit lower (9.1%). The comparable progeny categories from both studies (i.e. trihaploids, hexaploids and hybrids) are given in Table 5. In spite of the fact that the present study is based on a number of plants one order of magnitude higher, the results of both experiments are not statistically different; in both of them, we can consider the level of the residual sexuality in *P. rubra* about 10%, despite both experiments differed in many parameters. The earlier experiment (Krahulec et al. 2006) was based on artificial crossing (hand-pollination) of *P. rubra* with only one clone (genotype) of the pollen donor of *P. officinarum*. This cross was carried out under optimal weather conditions at an altitude of 300 m. Considering the hybridogenous origin of the maternal *P. rubra* itself (the hybrid formula *P. aurantiaca* > *P. officinarum*; Bräutigam 2017), the cross of *P. rubra* with *P. officinarum* was a back-cross. Therefore, the parental genomes could be comparatively closer to each other than in the case of the spontaneous open-pollination of *P. rubra* by diverse potential paternal taxa/cytotypes in the field. The spontaneous pollination in the field could also involve various pollinators acting under unknown weather conditions at the time of flowering. The source population of the seeds of *P. rubra* in the Krkonoše Mts is at an altitude of ~1000 m which is mountain climate and it is possible that some capitula remained unpollinated because of rainy or foggy weather. In spite of the differences between the two experiments, the results did not differ significantly. Of course, with respect to the range of ploidy levels in the numerous potential paternal plants in the field (Table 2), the spectrum of the spontaneous hybrid progeny from the field was wider than that found those from the experimental crossing.

Importantly, in spite of different environmental conditions and different amounts of progeny analysed and compared, no significant difference was found between the level of the residual sexuality in *P. rubra* in the experimental cross, and those open-pollinated in the field. It is not necessary to cultivate and analyse such large numbers of progeny as was done in this study. In addition, it is not always necessary to consider the differences in temperature and other environmental parameters that might potentially influence the effect of the residual sexuality in the facultatively apomictic plants (Šarhanová et al. 2012, Karunarathne et al. 2020). An important condition facilitating the effect of the residual sexuality in the field must be pointed out. The studied locality in the Krkonoše Mts was extremely rich in species and cytotypes of *Pilosella*, thus providing a wide spectrum of potential pollen donors. The residual sexuality in *P. rubra* was estimated there as 9.1% (Table 2). In an earlier study of *P. rubra* in the field, the corresponding level of the residual sexuality was detected as only 3.8% (Krahulcová et al. 2011). However, these authors reported the mean value of residual sexuality evaluating the progeny that were produced by open-pollinated plants of *P. rubra* from other four populations in the Krkonoše Mts; unlike the presently studied population at Pěnkavčí Vrch, these populations of *P. rubra* had fewer other species of *Pilosella* and therefore there was less opportunity for hybridization.

Origin of Pilosella hybrids occurring at the locality

Hybrids occurring at Pěnkavčí Vrch certainly have *P. rubra* as the mother plant in two cases. The hybrid with *P. floribunda* had the cp haplotype of *P. rubra* (P. Vít, F. Krahulec, unpublished data); this is a clear indication of the mother plant. The octoploid hybrid of *P. rubra* and tetraploid *P. officinarum* is a 2n+n hybrid; an alternative combination would be heptaploid. In other hybrids (penta- and hexaploid *P.* ×*stoloniflora*, hybrid with *P. iserana*) we do not know with certainty the maternal plant. In any case, the hybrid with *P. iserana* (tetraploid, apomictic) is certainly a product of residual sexuality of an apomictic species. On the other hand, hybrids of *P. rubra* × *P. lactucella* and *P. rubra* × *P. officinarum* have an apomictic and a sexual parent.

The tetra- and octoploid mosaic plant is of unknown origin. One possible explanation is that it originated as tetraploid progeny of an octoploid plant (present on the locality), which partially doubled their chromosome numbers during pre-embryonal stage. It is probably the first mosaic *Pilosella* plant reported from the field. Similar doubling of complete genomes was found in the progeny of the dihaploid of *P. aurantiaca* and trihaploid of *P. rubra* (Krahulec et al. 2011).

Residual sexuality in other taxa of Pilosella

A data set comparable to the one of the present study was published by Rosenbaumová & Krahulec (2015) for the hexaploid facultatively apomictic *P. bauhini*. As in the present study, the progeny produced by experimental hybridization was compared with progeny cultivated from achenes of *P. bauhini* collected in the field. The level of the residual sexuality was 6.6% and 7.6% for the progeny from experimental hybridization and the progeny from open pollination in the field, respectively. Analysis of these results (Table 6) also showed that the residual sexuality in *P. bauhini* did not significantly differ between the two experiments. This finding also supports our conclusions from the present study on *P. rubra*. In addition, similarly to the present study, Rosenbaumová & Krahulec (2015) also detected a higher ploidy variation among the progeny that were grown from achenes of *P. bauhini* collected in the field, than among the progeny that had originated from an experimental cross of *P. bauhini* with *P. officinarum,* because of greater variation of potential pollen donors, which occurred there.

Table 6. – Comparison of progeny categories of *Pilosella bauhini* from garden pollination experiment and on achenes collected near Valov (both based on data by Rosenbaumová & Krahulec 2015). The distribution of progeny across categories in the garden experiment and in the field was not significantly different ($\chi^2 = 0.427$, $P > 0.5$).

Progeny category	3x		6x		Hybrids		Sum
	no.	%	no.	%	no.	%	no.
Garden experiment	12	1.1	988	93.4	59	5.5	1059
Progeny of achenes from field	6	1.3	436	92.4	30	6.3	472

Using the experimental crosses, Bicknell et al. (2003) detected the level of the residual sexuality as 3.20% and 2.95% for aneuploid *P. aurantiaca* and triploid *P. piloselloides*, respectively. The data on other facultatively apomictic *Pilosella* species/cytotypes were published by Fehrer et al. (2007). They found that the facultatively apomictic basic species and stabilized hybridogenous species had low values of residual sexuality (5.3–6.5%), whereas the recent high-polyploid hybrids from an apomictic and a sexual parent had considerably higher values of residual sexuality (71.0–96.6%). Thus, due to a low penetrance of apomixis (Hand et al. 2015), these hybrids have a reproduction mode that we term “variable”. The data on the residual sexuality in the basic species of *P. aurantiaca* and in the stabilized hybridogenous species of *P. rubra* are also given by Krahulcová et al. (2011). These authors also compared the level of residual sexuality in several populations of the hexaploid *P. rubra* from the Krkonoše Mts (the mean value of 3.8%) with that of four recent hexaploid hybrids of the same origin (*P. aurantiaca* × *P. officinarum*) in two other geographic regions (the value of 52.5–92.3%). This finding by Krahulcová et al. (2011) supports the statement given by Fehrer at al. (2007) about differences in the reproductive behaviour in either the basic species or stabilized hybridogenous species in comparison with the recent hybrids.

The role of residual sexuality in Pilosella

The sexual mating of facultatively apomictic taxa of *Pilosella* is manifested in two ways. Firstly, most have some degree the functional pollen allowing them to generate variation via the paternal lineage (Rotreklová & Krahulcová 2016). Secondly, probably all apomictic taxa maintain some degree of residual sexuality that generates variation via the maternal lineage. Fehrer et al. (2007) gave examples of the importance of residual sexuality in *Pilosella* populations. Specifically, studies of chloroplast haplotypes have shown that apomictic *Pilosella* species (both the basic and those of hybridogenous origin) commonly served as maternal parents giving the origin to either new hybridogenous species or the recent hybrids (Krahulec et al. 2004, 2008, Fehrer et al. 2005, 2007, Krahulcová et al. 2009a, 2012, 2014). For example, the apomictic *P. aurantiaca* has been detected as mother plant of the tetraploid *P.* ×*stoloniflora* and of the hexaploid *P.* ×*rubra* in the Šumava Mts (Krahulec et al. 2008).

The second fact supporting the importance of the residual sexuality in *Pilosella* is the existence of hybrids between two apomictic parental species, which is also demonstrated in the current study by the hybrid between *P. floribunda* and *P. rubra*. Other n+n hybrids of two apomictic parents from the Krkonoše Mts are: *P. fuscoatra* (*P. aurantiaca* × *P. caespitosa* – Krahulcová et al. 2001), *P.* ×*scandinavica* (*P. glomerata* × *P. floribunda*) and *P. aurantiaca* × *P. blyttiana* (corresponding to *P. fusca* – both Krahulcová et al. 2013). The hybridization between two facultatively apomictic taxa is common in those regions where such taxa prevail, e.g. in the Balkans. There are commonly hybrids between two polyploid and apomictic parents, *P. officinarum* and *P. bauhini*, in Bulgaria (Krahulcová et al. 2009b, 2016, 2018). Several hybrids of two apomictic taxa, both of n+n and 2n+n origin, were recently described from the area of Prague (Krahulec et al. 2020). Hybridization between the two apomicts, *H. pilosella* (= *P. officinarum*) and *H. praealtum* (= *P. bauhini*) was also described from New Zealand (Morgan-Richards et al. 2004).

A third important point is connected with previous one: a sexual hybrid may appear from hybridization of two apomicts, as, for example, the hybrid between *P. floribunda* and *P. rubra* presented here. This switch from the parental apomixis to sexuality in the progeny has already been described by Chapman et al. (2003), who found the tetraploid sexual *P. officinarum* had arisen from hybridization between two different pentaploid clones of this species. Krahulec et al. (2020) documented two similar cases, namely, the hybrids between *P. aurantiaca* and *P. piloselloides* (*P.* ×*derubella*), and *P. aurantiaca* and *P. caespitosa* (*P.* ×*fuscoatra*).

Krahulcová et al. (2009a, 2012, 2014) showed the effect of residual sexuality on the population diversity of the mixed populations consisting of apomictic and sexual species. It has been shown that the apomictic maternal plants generate in such populations progeny with higher ploidy variation than do the sexual maternal plants (Krahulcová et al. 2009a). This finding from the field has been confirmed in experiments involving the reciprocal crosses between the sexual *P. officinarum* and the apomictic *P. bauhini* (Rosenbaumová & Krahulec 2015). In spite of a low degree of the residual sexuality, the apomictic *P. bauhini* produced progeny with diverse ploidy levels, similar to *P. rubra* in the present paper.

The detailed population studies showed the existence of minority cytotypes, that were often the 2n+n hybrids: such hybrids might more easily originate from a facultatively apomictic maternal parent, owing to the common production of unreduced female gametes in apomictic plants. Such rare 2n+n hybrids were found as individual plants several times, for example, in the hybrid swarm of the apomictic *P. polymastix* × the sexual *P. officinarum* (Krahulec et al. 2014), or in the Krkonoše Mts, where the heptaploid hybrid between two apomictic parents, the tetraploid *P. aurantiaca* and the hexaploid *P. rubra*, was recorded (Krahulcová et al. 2013).

Until now polyhaploid plants were not thought to play any role in the variation of populations in the field. It is probable that the polyhaploids were found two times, specifically as triploid individuals in the populations that were comprised of tetraploids and higher polyploids (Krahulec et al. 2008, 2014). Because of the absence of unequivocal proof of the polyhaploid origin of these "strange" cytotypes, such cases could be more common. Polyhaploids were commonly found among the progeny embryos and progeny seedlings that were formed especially by recent polyploid hybrids (Krahulec et al. 2011). The ability of polyhaploids to double their chromosomes might lead to the formation of new genotypes involving only half of the sexual process (presence of meiosis but no fusion of gametes; Krahulec et al. 2011).

The genus *Pilosella* was considered to be part of the genus *Hieracium* (*Hieracium* subgen. *Pilosella*), especially in central Europe. With respect to reproduction, both taxa are completely different. In *Hieracium*, diploids are sexual and polyploids apomictic (Mráz & Zdvořák 2019, Mráz et al. 2019). Mráz & Zdvořák (2019) found that 0.4 % of polyploid seeds were formed sexually, providing the first evidence of functional facultative apomixis. Indications of residual sexuality were found by Hand et al. (2015) during megaspore mother cell development. Diploids in *Pilosella* are sexual, but polyploids can be sexual or apomictic and all apomicts studied so far have some degree of residual sexuality. For that reason, *Pilosella* has higher evolutionary potential.

Residual sexuality in other agamic complexes

The data relating to populations in the field suggest different impacts of residual sexuality in different apomictic taxa. Matzk et al. (2001) showed extremely diverse reproductive modes in *Hypericum perforatum*. This was both at an individual level as well as on differences between populations (Molins et al. 2014). Only a negligible effect of the residual sexuality on the generation of variation was recorded in those apomictic biotypes of *Ranunculus auricomus* complex that coexisted with sexuals in mixed populations (Hörandl & Temsch 2009). On the other hand, residual sexuality is considered to play an important role in *Rubus* subgen. *Rubus* and *Sorbus*. Specifically, variable proportions of residual sexuality were found in tetraploid taxa of *Rubus* subgen. *Rubus* (Šarhanová et al. 2012). In addition, an occasional fertilization of an unreduced female gamete was recorded in triploids. Sexual mating via unreduced female gametes in both triploids and tetraploids increases the ploidy level of the progeny, thus stimulating the formation of taxa with higher ploidy levels in the agamic complex *Rubus* subgen. *Rubus* (Šarhanová et al. 2012). In another member of the *Rosaceae*, *Sorbus*, diverse reproductive pathways have been found in half the polyploid apomictic biotypes examined in Europe (Lepší et al. 2019). Thus, residual sexuality in apomicts is considered to be one of the principal mechanisms for diversification of this genus.

The genus Pilosella and its importance for flora diversity in the Krkonoše Mts

Apomictic plants are a common and important part of the flora of Europe (Gregor 2013). Different habitats are occupied by different numbers of apomicts (Gregor 2013). Secondary meadows are typical habitats of *Taraxacum*, *Alchemilla*, and also of *Pilosella* and grasses such as *Poa pratensis* or *Nardus stricta*. The area of the Krkonoše Mts, which is also a national park, has been studied by many botanists since the 19th century (cf. Šourek 1969). The genus *Pilosella* was studied mainly by German specialists (Fiek 1881, Nägeli & Peter 1885, Schneider 1888–1895). Currently the genus *Pilosella* is considered to be a typical group of secondary meadows in this area. Previous papers devoted to this topic dealt with chromosome numbers and breeding systems of individual species (Krahulcová & Krahulec 1999, Krahulcová et al. 2001, 2013, Chrtek 2004, Krahulec et al. 2004, Fehrer et al. 2005). The present paper shows that evolution within this genus is still occurring, hybridization being an important part. It is important that hybridization involves facultative apomicts as a mother plant. Similar hybridization of *Pilosella* species connected with the spread of *P. aurantiaca* and *P. rothiana* in semi-ruderal grasslands in Prague was recently described by Krahulec et al. (2020).

Continuing research on apomicts shows their richness is still underexplored, with new discoveries in even the well-known flora of central Europe (Király et al. 2017, 2019, Trávníček et al. 2018, Kirschner et al. 2019).

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Souhrn

V této práci prezentujeme výsledky studia zbytkové sexuality *Pilosella rubra*, hybridogenního hexaploidního druhu. Ta byla studována analýzou ploidie potomstva (2800 rostlin), vypěstovaného z nažek sbíraných na lokalitě Pěnkavčí Vrch u Pece pod Sněžkou v Krkonoších. Většina potomstva (90,9 %) vznikla apomikticky; zbytková sexualita byla 9,1 %. Potomstvo, které nevzniklo apomikticky, bylo tvořeno trihaploidy (triploidní rostliny vzniklé partenogeneticky z vaječné buňky s polovičním počtem chromozomů) a různými typy hybridů. Stupeň zbytkové sexuality se významně nelišil od dřívějších výsledků zahradního experimentu, stejně jako u druhu *P. bauhini*, jenž byl experimentálně testován v minulosti. Odrazem zbytkové sexuality na studované lokalitě je existence řady hybridů s dalšími druhy rodu *Pilosella*: *P. officinarum* (*P. ×stoloniflora*, pentaploidní, hexaploidní a oktoploidní), *P. lactucella*, *P. floribunda* a *P. iserana*. Zbytková sexualita apomiktů tak výrazně přispívá k diverzitě rodu *Pilosella* v Krkonoších.

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