Populations of *Pilosella* species in ruderal habitats in the city of Prague: consequences of the spread of *P. aurantiaca* and *P. rothiana*

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Consequences of *Pilosella aurantiaca* and *P. rothiana* (stabilized hybridogenous species *P. echiooides* > *P. officinarum*) spreading into three semi-ruderal localities in the city of Prague were studied. Numbers of chromosomes / DNA ploidy level and mode of reproduction are given for all the species and hybrids studied. Both *P. aurantiaca* and *P. rothiana* are apomictic and tetraploid with 2n = 4x = 36. *Pilosella rothiana* hybridizes with pentaploid *P. piloselloides* (*P. × heterodoxa*, 2n = 6x = 53/54) and tetraploid *P. officinarum* (*P. × bifurca*, 2n = 6x = 54). *Pilosella aurantiaca* hybridizes with tetraploid *P. caespitosa* (*P. × fuscoatra*, 2n = 4x = 36), *P. piloselloides* (*P. × derubella*, 2n = 5x = 45), *P. officinarum* (*P. × rubra*, 2n = 6x) - *P. rothiana* (2n = 6x = 54), *P. × bifurca* (with 2n = 5x = 45) and *P. visianii* (tetraploid, 2n = 4x = 36). Hybrids of *P. aurantiaca* with tetraploid *P. × leptophyton* were of two types, the tetraploid hybrid originating from parental reduced gametes and the hexaploid hybrid originating from a reduced and an unreduced parental gamete, respectively. Introgression from apomictic *P. bauhini* towards sexual *P. officinarum* was found in a hybrid swarm in one of the populations studied. Evolutionary potential of recent hybrids was evaluated with respect to their mode of reproduction; most of the recent hybrids were not apomictic. It seems impossible to predict the mode of reproduction from that of the parental species.

Keywords: *Pilosella aurantiaca*, *Pilosella rothiana*, hybridization, Czech Republic, hybrids, introgression, reproduction modes

Introduction

Apomictic plants, i.e. plants that produce seed asexually, are highly successful groups in the flora of Europe. Recently there has been a lot on research on these plants in many countries in Europe, which is often connected with the inventory of particular groups, for example, *Rubus* (Király et al. 2017, 2019, Trávníček et al. 2018), *Sorbus* (Lepší et al. 2015, Feulner et al. 2017). This research is reflected in several reviews dealing with the taxonomic treatment of particular taxonomic groups (Haveman 2013, Májeský et al. 2017, Hörandl 2018).

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The genus *Pilosella* is characterized by great diversity of taxa of different ranks and origins (Zahn 1922–1930). At the species level, three categories are distinguished, basic species, intermediate species of hybridogenous origin, and recent hybrids. In addition to this taxonomic diversity there is a high diversity in the ploidy levels and modes of reproduction, both of which influence taxonomic diversity and evolutionary potential. Ploidy levels of plants occurring in nature vary between diploid and octoploid. Diploids are invariably sexual, but higher ploidy level plants can be sexual or apomictic; heptaploids and octoploids are usually unstable and produce mostly hybrid or polyhaploid progeny. Hybridization is common, both homoploid and heteroploid, but not all species regularly hybridize. Hybrids are formed by combinations of reduced or unreduced gametes, reduced gametes often develop parthenogenetically and result in the production of polyhaploid progeny (progeny with half the number of chromosomes of the mother plant). Hybrids are often sterile or semi-sterile, but they can also be sexual, facultatively apomictic or produce all types of progeny including polyhaploids. Most species and hybrid *Pilosella* (with the exception of *P. piloselloides* and *P. echioides*) produce stolons, which enable them to multiply and spread locally.

Primary distribution of the species of *Pilosella* is Europe and western part of non-tropical Asia. Many species were introduced (intentionally or unintentionally) to other continents, often resulting in environmental and agronomic problems (Fehrer et al. 2007). Recently many taxa started spreading in ruderal and semi-ruderal habitats in western and central Europe; in this way species came into contact with other species, which resulted in new hybrid combinations. This is documented by many papers especially from western Europe, in particular Germany and Belgium (see below).

Recently we used a population-based approach to determine the processes that have resulted in the diversity of different morpho- and cytotypes in populations (Krahulcová et al. 2012, Krahulec et al. 2014). We found that almost every mixed-species population contains hybrids, the cytotypes of which correspond to combinations of the parents; in addition, some minority cytotypes occur. Most of them originated from unreduced gametes (e.g. Krahulcová et al. 2009, 2014). Having residual sexuality, apomicts play an important role in producing more variable progeny in terms of ploidy than sexual plants. They play a significant role in the production of high polyploids (e.g. Rosenbaumová & Krahulec 2015). Analysing the hybrid progeny from an apomictic maternal parent and a sexual pollen donor, we have also found that many of these new polyploids have a reduced penetrance of apomixis. That means, their progeny is variable, originating from apomixis, haploid parthenogenesis and sexual mating via both reduced and unreduced gametes (Hand et al. 2015). For that reason, such new high-polyploids with a hexaploid or a higher ploidy level usually do not reproduce as a stabilized apomictic lineage, which behave as an independent hybridogenous species. The combination of hybridizing biotypes and products of hybridization differ between regions (Krahulec et al. 2008) and even between localities (Krahulcová et al. 2014).

Several years ago, a paper on *Pilosella* taxa occurring in ruderal and semi-ruderal habitats within the city of Prague was published (Křišťálová et al. 2010). High diversity of both basic and hybridogenous species was reported and even some previously unknown hybrids were recorded, several of which were new for the Czech Republic. During subsequent years we occasionally collected data, which lead to the discovery of several localities, which we studied more systematically. This resulted in a more detailed understanding of
the processes occurring in populations of *Pilosella*. We also evaluated the importance of the spread of formerly rare species in evolutionary processes, which has occurred recently. The spread of formerly rare species of *Pilosella* is not confined to the Czech Republic, as the spread of some species and discovery of hybrids is reported recently in several other countries in central Europe: Germany (Gottschlich & Raabe 1991, Gottschlich et al. 2006, Krahulcová et al. 2012), Austria (Gottschlich & Raabe 1991, Schuhwerk & Fischer 2003, Brandstätter 2011), Belgium (Ronse & Gottschlich 2017) and Latvia (Krasnopolska 2018, 2019). It is evident that *Pilosella* is a group in which hybridization and formation of new hybrids is common and has recently resulted in speciation.

*Pilosella aurantiaca* is native of grasslands in mountains and *P. rothiana* occurs in xerothermic grasslands, rocky steppes and open forests. Currently, both species are spreading in ruderal habitats occupied by other species of *Pilosella* with which they hybridize. The aim of this paper is to report the recent hybridization involving these species and evaluate the evolutionary potential of the newly formed hybrids.

**Material and methods**

**Collection of plants**

We collected data for populations of *Pilosella* at three localities within the city of Prague (for description of localities and overview of taxa collected, see Results). We tried to collect all the morphologically distinguishable types, many of them repeatedly, to discover or reject cryptic variation.

Altogether 84 plants (47 plants of the basic species, 13 intermediate species and 24 recent hybrids) were collected and cultivated for subsequent analyses (Electronic Appendix 1). Collected plants were transplanted either to the experimental garden of the Institute of Botany or a private garden (plants collected in 2017, 2018, 2019). If available, we also collected seed that resulted from open pollination. All the plants studied are documented by herbarium specimens, which are deposited in the Herbarium of the Institute of Botany at Průhonice (PRA, cf. Thiers 2020). The specimens of some of these plants with undetermined ploidy levels are stored in private herbaria of Jan Doležal and Jaroslav Zámečník.

**Determination of chromosome number, DNA ploidy level, mode of reproduction and clonal identity**

The number of chromosomes in root-tip meristems of pot-grown plants were counted. The squashed meristems were stained with lacto-propionic orcein (Dyer 1963); for a detailed description of the procedure, see Krahulcová & Krahulec (1999).

DNA ploidy level (Suda et al. 2006) of cultivated plants was determined using flow cytometry (FCM). The DAPI staining method (Doležel et al. 2007) was applied using the nuclei-extracting buffer (Otto I) and staining buffer (Otto II) supplemented with mercaptoethanol (2 μl·ml⁻¹) as an antioxidant. Fluorescence intensity was determined using either the Ploidy Analyser PA II or a CyFlow cytometer (both instruments produced by Partec GmbH, Münster, Germany), both of which were equipped with an HBO
high-pressure mercury lamp for UV excitation. Relative DNA content was estimated using the diploid *Pilosella lactuca* as an internal standard (DNA content 4.07 pg/2C, Suda et al. 2007). The FCM procedure used for *Pilosella* is described in detail in Krahulcová et al. (2004).

The method used for determining the mode of reproduction of individual plants is given in Electronic Appendix 1. The mode of reproduction was confirmed for most plants during cultivation, based on the presence of well-developed (filled) seed in open-pollinated versus emasculated (cut) capitula (Gadella 1984, Krahulcová & Krahulec 1999). This method detects parthenogenetic seed development and is routinely used for autonomous apomictic *Asteraceae* (Richards 1986). Fertilization-independent seed formation is mainly associated with apomeiosis in wild-type apomictic *Pilosella* (Bicknell & Koltunow 2004) and therefore emasculation simulates apomictically reproducing plants (Hand et al. 2015). The seed-set was not quantified for the plants studied. The presence of well-developed (filled) achenes in each of three emasculated capitula per plant (checked by using tweezers to apply slight pressure to achenes viewed under a stereomicroscope) was sufficient for confirming apomictic reproduction. Those plants of which open pollinated capitula produced a normal seed-set, whereas those with emasculated capitula did not, were scored as sexual. Those plants that did not form well-developed seeds in either open pollinated or hand-pollinated capitula were scored as seed-sterile. This method of determining the mode of reproduction is labelled as Method A in Electronic Appendix 1.

For some plants, Flow Cytometric Screening of Seeds was used for determining the embryo/endosperm ploidy level, used for detecting the reproductive origin of seed produced by the respective maternal plant (FCSS method, Matzk et al. 2000). Using this method (labelled as Method B in Electronic Appendix 1), seed doublets were analysed together with an internal standard in each sample (for details of the FCSS procedure modified for *Pilosella*, see for example Krahulcová et al. 2014, 2018). Alternatively, the FCSS analysis of pooled seed samples comprising 6–10 seeds (Krahulcová & Suda 2006, Krahulcová et al. 2011, 2018) allowed us to screen and quantify the embryo ploidy levels of samples. This modification of the FCSS method (Krahulcová & Suda 2006) mainly revealed and quantified that in facultatively apomictic plants the most frequent type of embryo had the maternal ploidy level (these embryos were used as an internal standard): in addition, the less frequent type of embryo had a ploidy level different from that of the maternal parent (e.g. Krahulcová et al. 2018). The high peak(s) for the embryo nuclei are clearly detectable in the histograms of pooled seed samples, usually along with small peak(s) for the endosperm nuclei. So, using this method (labelled as Method C in Electronic Appendix 1), the ratio of ploidy level of embryos to ploidy level of endosperm can reliably be determined for most seed in an analysed sample and the reproductive origin of the seed revealed.

In the absence of FCSS, the seed formed by either open pollinated of hand-pollinated capitula were sown on wet filter paper in Petri dishes. The seed was left to germinate and root-tip meristems stained with lacto-propionic orcein were used for chromosome counts (Krahulcová & Krahulec 1999). Comparing the chromosome number/karyotype of the maternal plant with that of the respective seed-progenies, we inferred either apomictic/polyhaploid progeny (maternal/polyhaploid karyotypes in the progeny), or sexual progeny (diverse karyotypes in the progeny, which must have originated from sexual mating).
This method of determining the mode of reproduction is labelled Method D in Electronic Appendix 1.

The clonal identity was inferred from the pattern of isozyme phenotypes consisting of four enzymes (AAT, LAP, 6-PGDH/PGM and EST). This was used to determine whether the tetraploid *Pilosella aurantiaca* from Háje was the same as the tetraploid clone common in central Europe (Krahulcová et al. 2012). For a description of the isozyme analysis see Krahulec et al. (2004). In previous studies, these methods provided sufficient resolution within individual cytotypes of numerous polyploid taxa of *Pilosella* (Krahulcová et al. 2009, 2012, 2014).

**Taxonomic concept and nomenclature**

In this paper we follow the taxonomic concept that is generally used for 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. The hybrid formulae for the respective species are given in Table 1. Names of particular taxa follow Kaplan et al. (2019). We do not give names to the (probably) newly detected hybrids because (i) we cannot eliminate the possibility that they have already been described; it is probable that such names exist hidden in dozens of names already published; (ii) we know that most of species of *Pilosella* commonly hybridize everywhere they meet, but most of the hybrids do not reproduce successfully via apomixis and thus will eventually disappear. For these reasons we do not propose names, especially for hybrids known from a single locality.

**Results and discussion of particular taxa**

**Localities and list of taxa recorded**

Praha-Háje, abandoned grassland between the fishpond at Kančík and former farm Miličov, 50°01’39”N, 14°32’28”E, alt. ~277 m a.s.l.; collection in 2008, 2009, 2010, 2012. First collection by Petr Petřík. – Basic species: *Pilosella aurantiaca* (L.) F. W. Schultz et Sch. Bip., *Pilosella bauhini* (Schult.) Arv.-Touv. subsp. *bauhini*, *Pilosella caespitosa* (Dumort.) P. D. Sell et C. West, *Pilosella officinarum* Vaill. – Intermediate species: *Pilosella rothiana* (Wallr.) F. W. Schultz et Sch. Bip., *Pilosella polymastix* (Peter) Holub. – Recent hybrids: *Pilosella ×brachiata* (DC.) F. W. Schultz et Sch. Bip., *Pilosella ×leptophyton* (Nägeli et Peter) S. Bräut. et Greuter, *P. ×brachiata< P. officinarum, P. aurantiaca × P. ×leptophyton*.

Praha-Hrnčíře, grassland along the road to Průhonice-Rozkoš, in the close vicinity of the cemetery, 350 m NNE of the church, 50°00’09.3”N, 14°31’06.5”E, alt. ~302 m a.s.l.; collected in 2013, 2014, 2017, 2018, 2019. First collected by F. Krahulec. – Basic species: *Pilosella aurantiaca, Pilosella bauhini* subsp. *bauhini*, *Pilosella bauhini* subsp. *magyarica* (Peter) S. Bräut., *Pilosella caespitosa, Pilosella officinarum, Pilosella
Table 1. – Taxa, ploidy/chromosome number and reproduction mode. The variable breeding system means a low penetration of apomixis (Hand et al. 2015) that is reflected in the structure of the progeny combining the products of the minority apomixis with products of haploid parthenogenesis and/or the sexual mating. Numbers of plants are given in parentheses.

| Name                        | Combination            | Locality   | DNA-ploidy | Number of chromosomes | Reproduction mode |
|-----------------------------|------------------------|------------|------------|-----------------------|-------------------|
| **Basic species:**          |                        |            |            |                       |                   |
| *P. aurantiaca*             | Háje 4x (5)            | Háje       | apomictic (2) |                       |                   |
|                            | Hrnčíře 4x (1)         | Hrnčíře    | apomictic (1) |                       |                   |
| *P. bauhini subsp. bauhini* | Háje 5x (5), 6x (2)    | Háje       | apomictic (3) |                       |                   |
|                            | Hrnčíře 5x (4), 2n = 54 (2), M | Hrnčíře    | apomictic (1) |                       |                   |
| *P. bauhini subsp. magyarica* | Hrnčíře 5x (4) 2n = 54 (1) | Hrnčíře    | apomictic (1) |                       |                   |
| *P. caespitosa*             | Háje 4x (7) 2n = 36 (1), M | Hrnčíře    | apomictic (2) |                       |                   |
|                            | Hrnčíře 4x (7) 2n = 36 (1), M | Hrnčíře    | apomictic (2) |                       |                   |
|                            | Hrnčíře 5x (2)         | Hrnčíře    | apomictic (1) |                       |                   |
| *P. officinarum*            | Háje 4x (4) sexual (4) | Háje       | sexual (4)  |                       |                   |
| *P. piloselloides*          | Hrnčíře 5x (3) 2n = 45 (1), M | Hrnčíře    | apomictic (4) |                       |                   |
| **Intermediate species:**    |                        |            |            |                       |                   |
| *P. densiflora*             | *P. bauhini – P. cymosa* | Hrnčíře    | 2n = 45 (1), M |                       | apomictic (1)     |
| *P. glomerata*              | *P. caespitosa – P. cymosa* | Hrnčíře    | 2n = 45 (1), M |                       | apomictic (1)     |
| *P. rothiana*               | *P. echioides ≥ P. officinarum* | Hrnčíře    | 2n = 45 (1), M |                       | apomictic (1)     |
| *P. bauhini-P. setigera*    | *P. bauhini – (P. cymosa – P. echioides)* | Hrnčíře    | 2n = 45 (1), M |                       | apomictic (2)     |
| *P. polymastix*             | *P. bauhini – P. caespitosa* | Háje       | 4x (1)     |                       | apomictic (1)     |
| *P. visianii*               | *P. officinarum ≤ P. piloselloides* | Hrnčíře    | 4x (2)     |                       | apomictic (1)     |
|                            | *P. officinarum ≤ P. piloselloides* | Repy       | 4x (1)     |                       | apomictic (1)     |
| **Recent hybrids:**          |                        |            |            |                       |                   |
| Hybrid complex at Háje      |                        |            |            |                       |                   |
| *P. xbrachiata*             | *P. bauhini ≤ P. officinarum* | Háje       | 4x (5)     |                       | apomictic (5)     |
| *P. xeleptophyton*          | *P. bauhini > P. officinarum* | Háje       | 4x (2)     |                       |                   |
|                            | *P. officinarum > P. brachiata* | Háje       | 4x (3) 2n = 36 (1) M | sexual (4) |                   |
|                            | *P. aurantiaca × P. leptophyton* | Háje       | 4x (1)     | sexual (1)           |                   |
|                            | *P. aurantiaca × P. leptophyton* | Háje       | 6x (2)     | sexual (1)           |                   |
| Hybrid complex at Hrnčíře:  |                        |            |            |                       |                   |
| *P. xbifurca*               | *P. officinarum × P. rothiana* | Hrnčíře    | 2n = 54 (1) | variable, parthenogenesis, low fertility (1) | seed-sterile (1) |
|                            | *P. aurantiaca × P. bifurca* | Hrnčíře    | 2n = 45 (1) |                       | seed-sterile (1) |
|                            | *P. aurantiaca × P. rothiana* | Hrnčíře    | 2n = 54 (1) |                       |                   |
| *P. xderubella*             | *P. aurantiaca × P. piloselloides* | Hrnčíře    | 2n = 45 (1) |                       | sexual (1)        |
| *P. xfuscoatra*             | *P. aurantiaca × P. caespitosa* | Hrnčíře    | 2n = 45 (1) |                       | apomictic (1), sexual (1) |
| *P. xrubra*                 | *P. aurantiaca > P. officinarum* | Hrnčíře    | 6x (1)     | variable, parthenogenesis potentially combined with sexuality (1) |                   |
| *P. xheterodoxa*            | *P. piloselloides × P. rothiana* | Hrnčíře    | 2n = 53/54 (1) |                       |                   |
| Hybrid complex at Repy:     |                        |            |            |                       |                   |
| *P. aurantiaca × P. visianii* | Repy 4x (1)           | Repy       | seed-sterile (1) |                       |                   |
piloselloides (Vill.) Soják. – Intermediate species: Pilosella densiflora (Tausch) Soják, Pilosella glomerata (Froel.) Fr., Pilosella rothiana, Pilosella bauhini – P. setigera, Pilosella visianii F. W. Schultz et Sch. Bip. – Recent hybrids: Pilosella × bifurca (M. Bieb.) F. W. Schultz et Sch. Bip., Pilosella aurantiaca × Pilosella × bifurca, Pilosella aurantiaca × Pilosella rothiana, Pilosella × derubella (Gottschl. et Schuhw.) S. Bräut. et Greuter, Pilosella × fuscoatra (Nägeli et Peter) Soják, Pilosella × heterodoxa (Tausch) Soják, P. × rubra Peter.

Praha-Řepy, lawn where the streets Makovského and Plzeňská cross, 50°03'47.0"N, 14°18'49.1"E, alt. ~327 m a.s.l.; collected 2015. First collected by Tomáš Mrázek. – Basic species: Pilosella aurantiaca (not collected). – Intermediate species: Pilosella visianii. – Recent hybrid: Pilosella aurantiaca × Pilosella visianii.

The ploidy level/chromosome number and mode of reproduction that were determined for taxa and cytotypes from particular localities, are presented in Table 1. For illustrating the relationships among putative parental species and their hybrids, separate diagrams are presented for all three populations: at the locality Háje (Fig. 1), Hrnčíře (Fig. 2) and Řepy (Fig. 3). Original data collected for individual plants are listed in Electronic Appendix 1.

Data confirming the previous findings from the Czech Republic

The tetraploid and apomictic P. aurantiaca that we recorded for the first time in Prague (Electronic Appendix 2), seems to be the most common biotype in other parts of this country (Chrtk 2019). The pentaploid and hexaploid cytotype of P. bauhini subsp. bauhini (both of which are apomictic) fall within the range of cytotypes that is already reported for the Prague area (Electronic Appendix 2). Pentaploids and hexaploids are the most common in P. bauhini in this country (Rotreklová 2004). In addition, we rarely recorded P. bauhini subsp. magyarica in the population at Hrnčíře, which is the first record of this subspecies in the Prague area. The only plant collected there was hexaploid. This cytotype of P. bauhini subsp. magyarica is common in the Balkan Peninsula (e.g. Krahalcová et al. 2016) and central Europe, the sexual tetraploid is the most common (Rotreklová 2004, Rotreklová et al. 2005, Marhold et al. 2007, Krahulcová & Krahulec 2020). In the Balkan Peninsula, Schuhwerk & Lippert (1998) report that both tetraploid and hexaploid cytotypes are present in Montenegro and North Macedonia.

Both the tetraploid and pentaploid cytotypes of P. caespitosa recorded here accord with previous findings for Prague (Electronic Appendix 2). These polyploid cytotypes of P. caespitosa are apomictic, both in other parts of this country (Chrtk 2019) and in Europe (Sell & West 1976). For P. officinarum only the tetraploid cytotype was recorded at two localities and the plants at Háje were sexual (Table 1). The sexual mode of reproduction was previously confirmed not only for tetraploids within the city of Prague, but also for the less common pentaploids and hexaploids found at other localities in Prague (Electronic Appendix 2). In addition, the heptaploid cytotype was rarely recorded in this area (Electronic Appendix 2). The tetraploid cytotype of P. officinarum we recorded (Table 1), is the most common cytotype in the western part of the Czech Republic (Mráz et al. 2008). The record of pentaploid and apomictic P. piloselloides agrees with earlier data from Prague (Electronic Appendix 2).
Pilosella densiflora occurs also at other localities in Prague, but the ploidy level of plants from this area is unknown (Křišťálová et al. 2010: 452). This taxon occurs in south-eastern Moravia, where it is tetraploid and sexual, but in other regions of this country it is pentaploid and apomictic (Chrtek 2004): this latter biotype corresponds to that of a plant we recorded in the population at Hrnčíře (Table 1).
The only plant of *P. polymastix* recorded in this study was tetraploid and apomictic, which conforms with that reported earlier for a population at Milín in central Bohemia (Krahulec et al. 2014). The record of the pentaploid and apomictic biotype of *P. glomerata* agrees with previous data from the city of Prague (Electronic Appendix 2). Similarly, the tetraploid and apomictic biotype of both *P. rothiana* and *P. visianii* agrees with published data from other localities in Prague (Electronic Appendix 2).

*Pilosella bauhini – P. setigera* (Electronic Appendix 3) is a biotype of hybrid origin and still undescribed, reported in the city of Prague by Křišťálová et al. (2010: 455, 457), but its karyological and reproductive attributes were not studied. We recorded a pentaploid ploidy level and apomictic mode of reproduction in both plants of this hybrid taxon (Table 1). All the plants reported by Křišťálová et al. (2010) from other localities in Prague, and those recorded recently by us, were morphologically very uniform; for that reason, we consider this hybrid as an already stabilized apomictic hybridogenous species. The influence of *P. cymosa* on *P. setigera* (*P. cymosa – P. echioides*) is reflected in the presence of stellate hairs on the upper surface of its leaves.

**Hybridization in the population at Háje (Fig. 1)**

Hybrids between *P. bauhini* and *P. officinarum*. These two are probably the most common species of *Pilosella* in Prague (Křišťálová et al. 2010). When they co-occur, they commonly hybridize (e.g. Krahulcová et al. 2009, 2014). The hybrids *P. xbrachiata* and *P. xleptophyton* recently recorded at Háje are evidently products of hybridization between co-occurring *P. bauhini* and *P. officinarum* (Table 1, Fig. 1). All the plants were tetraploid and those of *P. xbrachiata* apomictic (Table 1). These attributes are similar to the range in ploidy levels and modes of reproduction recorded for the hybrids of this parentage at other localities in Prague (Electronic Appendix 2). In addition to *P. xbrachiata* and *P. xleptophyton*, there were also plants in this population the morphology of which was closer to *P. officinarum* than *P. xbrachiata* (Fig. 1). The plants, presented here as *P. xbrachiata < P. officinarum*, were tetraploid and sexual. However, the influence of *P. bauhini* was indicated by the presence of a long ‘marker’ chromosome in the karyotype of one of these tetraploid and sexual hybrids (symbol M in Table 1 and Electronic Appendix 1). This chromosomal marker occasionally occurs in the karyotype of the pentaploid *P. bauhini* at other localities, but not in that of *P. officinarum* (Rotreklová 2004, Hand et al. 2015). Presence of hybrids closely related to *P. officinarum* is similar to the situation in the hybrid swarm *P. stoloniflora – P. officinarum* in Westphalia (Krahulcová et al. 2012).

A similar hybrid swarm occurred at another locality in Prague close to the train station Vysočany (Krahulcová et al. 2009, Urfus et al. 2014). This population was extremely complex in including many cytotypes of *P. bauhini* and different products of hybridization between *P. bauhini* and *P. officinarum*. Great variation was detected in the modes of reproduction associated with different chromosome numbers (see also the Electronic Appendix 2).

*Pilosella aurantiaca × P. xleptophyton* (Fig. 4, Electronic Appendix 4). Two different morphotypes and cytotypes of this recent hybrid were recorded. We found three plants with orange flowers that were similar in their morphology with those of the parents: two were hexaploid, most probably the 2n + n hybrid of the co-occurring tetraploid parents *P. aurantiaca* and *P. xleptophyton* (Table 1). One of these hexaploid hybrids was sexual and occasionally produced octoploid progeny of 2n + n origin (Electronic Appendix 1).
The second type of hybrid between *P. aurantiaca* and *P. ×leptophyton* recorded at this locality was tetraploid and sexual with a reduced seed-set; this plant probably originated from a homoploid n + n cross. Interestingly, the seed-progeny of this open-pollinated tetraploid hybrid included pentaploid, hexaploid and heptaploid embryos, which indicates sexual mating via both reduced and unreduced gametes (Electronic Appendix 1).
Hybridization in the population at Hrnčíře (Fig. 2)

Pilosella ×bifurca (Electronic Appendix 5). This recent hexaploid hybrid is intermediate in morphology between *P. rothiana* and *P. officinarum*; both are tetraploid at this locality, thus their hexaploid hybrid evidently originated from a conjugation of an unreduced and a reduced gamete. Cut capitula of this plant produced some seed autonomously without pollination (indication of parthenogenesis), however, even when this semi-sterile plant was open-pollinated, most of the embryos were trihaploid, indicating that haploid parthenogenesis is the main reproductive pathway (Electronic Appendix 1). Chrtek (2004) report triploid (2n = 27) and pentaploid cytotypes (2n = 45) in this hybridogenous taxon in the Czech Republic. It is reported that the pentaploid chromosome number of *P. ×bifurca* in Burgenland, Austria is (2n = 45) (Schuhwerk & Lippert, 1997).

*Pilosella aurantiaca* × Pilosella ×bifurca (Fig. 5). This recent hybrid with orange capitula is pentaploid and seed-sterile. Based on the ploidy level, this plant seems to be an n + n hybrid of the tetraploid *P. aurantiaca* and hexaploid *P. ×bifurca*. In agreement with this origin its morphology is more like that of *P. officinarum* than the following hybrid between *P. aurantiaca* and *P. rothiana* (compare the Fig. 5 and Fig. 6).

*Pilosella aurantiaca* × Pilosella rothiana (Fig. 6). This hexaploid plant also has orange capitula, but the leaves are narrower than those of the previous hybrid. The morphology of this plant is similar to that of the above hybrid combination.

*Pilosella xderubella* (Fig. 7). This is a hybrid between two apomictic parents, *P. aurantiaca* and *P. piloselloides*. Because this sexual plant is pentaploid, its putative origin may be from a reduced (2x) gamete of *P. aurantiaca* (4x) and reduced (3x) gamete of *P. piloselloides* (5x). There is no published data on the mode of reproduction of this hybrid. A different tetraploid cytotype (2n = 36) in Bavaria is cited as *P. xderubella* (Schuhwerk & Lippert 1997 under the name of Hieracium atramentarium). Pilosella xderubella is reported in Germany (Gottschlich & Schuhwerk 2000), Austria (Schuhwerk 2008, Brandstätter 2011) and eastern North America (Wilson et al. 2006).

*Pilosella ×fuscoatra* (Electronic Appendices 6, 7). This hybrid also has two apomictic parents, namely, *P. aurantiaca* and *P. caespitosa*. The two tetraploid clones at this locality differed in their mode of reproduction: one was apomictic and the other was sexual. The hybrid corresponding to *P. fuscoatra* is reported in the Krkonoše Mts, Czech Republic (Krahulcová et al. 2001): this plant was tetraploid and apomictic, but semi-sterile.

*Pilosella ×rubra* has been found in 2020; its ploidy level (6x) agrees with data from other regions where this recent hybrid occurs (Krahulcová et al. 2012).

*Pilosella ×heterodoxa* (Fig. 8). The plant we examined was hexaploid. Most of the achenes produced by open-pollinated flowers were damaged by insects and of the few achenes available for FCSS four were trihaploid, one tetraploid and one hexaploid (Electronic Appendix 1). So, the mode of reproduction of this hybrid seems to vary, indicating a combination of a parthenogenetic (trihaploid embryos and a hexaploid embryo of apomictic origin) and sexual mating (tetraploid embryo).

Hybridization in the population at Řepy (Fig. 3)

*Pilosella aurantiaca* × *Pilosella visianii* (Fig. 9). This hybrid between two tetraploid apomictic parents was detected only once at this locality. The plant was tetraploid and sterile, producing only empty achenes. The tetraploid and apomictic parent *P. visianii*
Fig. 5. – Pilosella aurantiaca × P. ×bifurca.
Fig. 6. – *Pilosella aurantiaca* × *P. × rothiana*.
Fig. 7. – *Pilosella xderubella.*
Fig. 8. – Pilosella ×heterodoxa.
occurs there in a lawn together with the second parent, \textit{P. aurantiaca}. It is highly likely it belongs to a tetraploid apomictic clone of \textit{P. aurantiaca} that is common in central Europe, both in primary habitats in mountains and secondary habitats in lowlands.
General discussion

Consequences of the spread of P. aurantiaca and P. rothiana

Pilosella aurantiaca is a native species in mountain grasslands. It is also cultivated as an ornamental plant and recently it spread from gardens into lawns, grasslands and ruderal habitats at low altitudes; this has happened in the Czech Republic and in many other regions in central Europe. The clonal identity of plants from Háje is the same (isozyme phenotype) as the tetraploid plants occurring in the Krkonoše Mts (Krahulec et al. 2004) and Šumava Mts (Krahulec et al. 2008). Plants of the same clone are common non-native plants in North America (Loomis & Fishman 2009) and New Zealand (the same isozyme phenotype, A. Krahulcová & F. Krahulec, unpublished). The first report of this species in lawns in Prague is for Praha-Ruzyně in the 1950s (Hejný 1971: 190). This species is now common at low altitudes (e.g. Doležal & Zámečník 2018, 2019). During the spread of P. aurantiaca it came into contact with many Pilosella taxa that are not present in the original distribution of P. aurantiaca in mountains.

Pilosella rothiana is a hybridogenous species, which originally occurred and still occurs in warm areas in the Czech Republic, usually in habitats in steppe areas, open forests etc. (Peckert 2002, Chrték 2004, Chrték in Kaplan et al. 2017). Secondary habitats of this species are various man-made places, such as train stations and road margins. Recently it has been repeatedly found in ruderal habitats in Prague and its surrounding (Chrték & Peckert 2002, Křišťálová et al. 2010), and also in regions distant from its native distribution area, such as, in eastern Bohemia (Doležal & Zámečník 2018), Krkonoše Mts (Křišťálová et al. 2010, Krahulcová et al. 2013) and Strakonice (leg. R. Paulič in Kaplan et al. 2017). The plants at Háje belong to the same tetraploid and apomictic isozyme phenotype as those studied earlier, namely those occurring along the highway north of Prague at Odolena Voda and the plant from the Krkonoše Mts (Křišťálová et al. 2010). It is not known if this genotype occurs at natural localities or whether it is a new one, selected for living in anthropic habitats. The clonal identity of the genotype that occurs in anthropic localities in Germany (Gottschlich et al. 2006, Siegel & Gottschlich 2015) is also unknown.

Hybridization of P. rothiana in anthropic habitats is until now not so common as that of P. aurantiaca (see next paragraph). Gottschlich et al. (2006) report the occurrence of Hieracium euchaeitiforme (P. euchaeitiformis = P. piloselloides × P. rothiana) at ruderal sites in Niedersachsen and Doležal & Zámečník (2018) report it in eastern Bohemia (as P. heterodoxa).

The hybridization of P. aurantiaca in anthropic habitats is recorded: the hybrids occur in secondary distribution areas in western Europe, mainly in Germany: P. stoloniflora (P. aurantiaca – P. officinarum) and P. rubra (P. aurantiaca > P. officinarum) in Westphalia, Germany (Gottschlich & Raabe 1991, Krahulcová et al. 2012), P. ×rubra in Hessen and Baden-Württemberg (Gottschlich & Emrich 2006, Gottschlich & Uhl 2017) and Bavaria (Schuhwerk 2010); P. ×derubella (P. aurantiaca – P. piloselloides) in Westphalia (Gottschlich & Schuhwerk 2000), Austria (Schuhwerk & Fischer 2003, Schuhwerk 2008), eastern North America (Wilson et al. 2006), P. stoloniflora in Lower Saxony (Gottschlich et al. 2006), Hessen (Gottschlich et al. 2003–2004), Latvia (Krasnopolska 2019), Canada (Wilson et al. 2006) and New Zealand (Webb et al. 1988), P. fuscoatra (P. aurantiaca – P. caespitosa) and P. stoloniflora in Belgium (Ronse & Gottschlich 2017), P. fuscoatra in
Germany (Meierott & Gottschlich 2015), USA (Wilson et al. 2006, Ronse & Gottschlich 2017), England (leg. Michael Wilcox, herb. PRA), Latvia (Krasnopolska 2019 – but this is a hybrid between *P. aurantiaca* and *P. onegensis*) and in Siberia (Tupicyna 1997). Hybrid of *P. aurantiaca* with *P. cymosa* subsp. *vaillantii* is reported in Latvia (Krasnopolska 2019). In the Czech Republic, hybrids of *P. aurantiaca* are reported occurring in areas other than mountains: *P. ×stoloniflora* and *P. ×rubra* (Petřík et al. 2003, Kotilínek & Chrtek in Lustyk & Doležal 2018, Doležal & Zámečník 2018, 2019), *P. ×norrliniiformis* (Pohle et Zahn) Soják (*P. aurantiaca* × *P. glomerata*; Lepší et al. 2013, Kotilínek & Chrtek in Lustyk & Doležal 2018, Chrtek 2019). The cultivation of *P. aurantiaca* with other *Pilosella* species in gardens provides an opportunity for hybridization. For example, *Pilosella fuscoatra* occurred as a spontaneous hybrid in the Botanical garden in Munich (Nägeli & Peter 1885) and again more recently in the Botanical garden in Klagenfurt, Austria (Schuhwerk & Fischer 2003).

Gottschlich & Emrich (2006) compare the number of hybrids of tall species of *Pilosella* (*P. aurantiaca*, *P. caespitosa*, *P. cymosa*, *P. piloselloides*) and report that the lowest number is for *P. aurantiaca*, because its contact with other species of *Pilosella* is limited. Our results clearly indicate that this could quickly change.

In the present paper we report (probably) undescribed hybrids of *P. aurantiaca* with three species of *Pilosella*: *P. rothiana* (two types of hybrid differing in ploidy level), *P. ×leptophyton* (two types of hybrid differing in ploidy level) and *P. visianii*. The hybrid between *P. aurantiaca* and *P. piloselloides* (*P. ×derubella*) is probably reported here for the first time in the Czech Republic, whereas the hybrid between *P. aurantiaca* and *P. caespitosa* (*P. ×fuscoatra*) is already reported at one locality in the Krkonoše Mts (Krahulcová et al. 2011) and in the Šumava Mts (Grulich 2015).

Because *P. rothiana* (*P. echioides > P. officinarum*) is still not as common as *P. aurantiaca*, their new hybrids are rare. Nevertheless, we found a hybrid between *P. rothiana* and *P. officinarum* (*P. ×bifurca*) and *P. piloselloides* (*P. ×heterodoxa*). For *P. ×heterodoxa* it is the second report for the Czech Republic, the first one being the adventitious occurrence of *P. rothiana* (Doležal & Zámečník 2018). Gottschlich et al. (2006) report the occurrence of *P. ×bifurca* and a hybrid between *P. piloselloides* and *P. rothiana* (under name *Hieracium euchaetiiforme* Zahn) in ruderal habitats in Lower Saxony.

**Problematic prediction of evolutionary success of recent hybrids**

It is extremely difficult to determine the evolutionary potential of the hybrids reported. The hybridogenous species of *Pilosella* usually reproduce apomictically (rarely stabilized sexually as mentioned above for *P. densiflora*) and at present are independent of their parents. On the other hand, most recent hybrids occurred as one individual or formed a small clone. Their mode of reproduction is very diverse and seems to be independent of the parental modes of reproduction (Table 2). Even the hybridization of two apomicts resulted in sexual plants.

Another phenomenon was recorded at Háje – if the hybrids are sexual, a hybrid swarm quickly develops and it is impossible to distinguish particular hybrids due to continuous variation. Similar results are reported by Urfus et al. (2014) who analysed the progeny of the same hybridizing parental species as reported here for Háje (*P. bauhini*, *P. officinarum*). It is evident that introgression is towards the sexual species, which was documented here.
by the presence of a long marker chromosome (M in Electronic Appendix 1), which is reported in one plant morphologically similar to *P. officinarum* (with lower density of stellate hairs on underside of leaves), which originated from the apomictic parent, *P. bauhini*. Such introgression towards sexual parent is reported in populations consisting of either *P. aurantiaca*, *P. officinarum* and their hybrids (Krahulcová et al. 2012) or *P. bauhini*, *P. officinarum* and their hybrids (Urfus et al. 2014).

It is difficult to predict results of potential hybridization from the composition of species (and cytotypes) co-occurring at a locality. The main reason is the unpredictability of the mode of reproduction, which governs the fate of the hybrids produced. In fact, the mode of reproduction of the hybrids seems to be independent of that of the hybridizing parents (Table 2).

This unpredictability can be demonstrated, for example, in two recent hybrids recorded at Hrnčíře, which corresponded to *P. × fuscoatra*. Although they are hybrids between two apomictic species, *P. aurantiaca* and *P. caespitosa*, one biotype was apomictic and the other sexual. A third biotype of *P. × fuscoatra* recorded in the Krkonoše Mts is semi-sterile and apomictic (Krahulcová et al. 2001). Currently the plants belonging to this third clone occur only in the close vicinity of the place where the hybrid was found in 1979 (F. Krahulec, personal observation). Another case of a hybrid between two facultative apomicts (*P. aurantiaca*, *P. piloselloides*) is *P. × derubella*, which is sexual. Some of the hybrids, especially those of 2n + n origin, are unstable because their progeny are very variable, as are those of the polyhaploids (*P. × bifurca*, *P. × heterodoxa*). *Pilosella × heterodoxa* is a hybrid between two apomictic parents (*P. piloselloides*, *P. rothiana*).

Species of *Pilosella* and their hybrids, which are often almost sterile, have another type of reproduction, clonal growth, by which they can multiply and increase the possibility of a rare reproduction event. In this respect they are similar to hybrid clones of sexual aquatic plants, which are known to form wide-spread and/or long-lasting clones in river systems, as for example, *Ranunculus* sect. *Batrachium* (Prančl et al. 2018) and *Potamogeton* (Zalewska-Gałosz et al. 2018).

**Changes over time in the genus Pilosella in Prague**

A paper published in 2010 (Křišťálová et al. 2010) based on data collected in 2003 along with samples collected in 2008 and 2009, included data for 49 localities at a very different spatial scale. In this paper we only considered two species-rich localities. The richest locality recorded in the first paper (Křišťálová et al. 2010) close to the highway at Jirny

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**Table 2.** – Reproduction mode of recent hybrids originated from combination of parents with particular reproduction modes. The numbers represent individual plants of the recent hybrids corresponding to diverse parental combinations (see Table 1). For the breeding system of individual parental taxa and of the respective recent hybrids see Table 1 and Electronic Appendix 1.

| Combination of parents | Breeding system of progeny |
|------------------------|---------------------------|
|                        | apomictic | sexual | variable | sterile |
| apomictic × apomictic   | 1         | 2      | 1        | 1       |
| apomictic × sexual      | 5         | 4      | 1        |         |
| apomictic × variable    |           |        |          | 1       |
was repeatedly visited; eight taxa were recorded there, all basic and hybridogenous species. The richest locality visited in the current study hosted 18 taxa. However, neither basic or hybridogenous species new for Prague were found. All new taxa reported in this paper are recent hybrids, which are unlikely to spread to other localities because of their mode of reproduction. The hybrids recorded shows the potential of basic and hybridogenous species to hybridize, if they grow together.

Species of Pilosella often colonize temporary habitats where they are confined to early successional stages. The richest locality, Hrnčíře, is different in this respect, it is a regularly managed grassland. Due to several years drought, there are many gaps in the sward of this grassland, which allowed the easy establishment of seedlings. This is probably one of the reasons why this locality is so rich. On the other hand, the locality at Háje is temporary and at present is almost covered by shrubs. Even temporary habitats are important as a source of seed for other localities in their vicinity. When we compare our data for the last twenty years with that collected more than one hundred years ago, we conclude that the present composition of Pilosella is poorer (Šprynar & Münzbergová 1998). The last data for intermediate species is that published by Zahn (1922–1930). At that time there were many grazed and locally disturbed habitats and for that reason many currently rare, absent species and hybridogenous species were more common then. The locality at Hrnčíře has only an area of 0.8 ha. Yet the species richness recorded there (18 species and recent hybrids) is exceptional.

See www.preslia.cz for Electronic Appendices 1–7

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Shrnutí

V této práci byly analyzovány tři lokality z jižní a západní periferie Prahy, kde jsme zaznamenali výskyt druhů rodu Pilosella a jejich recentní hybridizaci. Dokumentovány byly ploidie/počty chromozómů a reprodukční systémy většiny rostoucích taxonů. Ukázali jsme důsledky expanze P. aurantiaca a P. rothiana (stabilizovaný hybridogenní taxon z křížení P. echioides a P. officinarum) na antropicky ovlivněné lokality v okolí Prahy. Na lokalitě Háje byla populace tvořena hybridním rojem mezi P. bauhini a P. officinarum, dále jsme tam nalezli dva cytotypy (tetraploidní a hexaploidní) křížence mezi P. aurantiaca a P. ×leiptophyton. Kromě uvedených kříženců zde byl nalezen i hybridogenní druh P. polymastix (P. bauhini – P. caespitosa).

Loučka u hřbitova v Hrnčířích se ukázala jako jedna z nejbohatších u nás zkoumaných populací; na ploše 0,8 ha jsme naleží 17 taxonů rodu Pilosella. Byli našeněni kříženci P. aurantiaca s těmito druhy: P. caespitosa (P. ×fuscoatra), P. officinarum (P. ×ruba), P. piloselloides (P. ×derubella), P. rothiana a P. ×bifurca. K těmto hybridům přistoupili ještě další kříženci P. rothiana s P. piloselloides (P. ×heterodoxa) a P. officinarum (P. ×bifurca). Na třetí lokalitě v Praze-Řepích byl nalezen hybrid P. aurantiaca s P. visianii.

Pouze dva ze všech nalezených hybridů byly ustálenými (fakultativními) apomikty; ostatní byly sterilní, sexuální, či částečně sterilní s produkčí polyhaploidního potomstva. Přesto je velmi pravděpodobné, že tyto hybridizace mezi taxony, které se dříve běžně nesetkávaly, budou dále pokračovat a že někdy dojde k vytvoření stabilizovaného apomuktického typu. Většina z uvedených hybridů dosud nebyla popsána a jeden je zde uveden jako nový taxon pro ČR (P. ×derubella).
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