

## Offspring diversity in *Hieracium* subgen. *Pilosella* (Asteraceae): new cytotypes from hybridization experiments and from open pollination

ANNA KRAHULCOVÁ AND FRANTIŠEK KRAHULEC

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**ABSTRACT:** The progeny derived from experimental crosses, in which the following species of *Hieracium* L. subgen. *Pilosella* (Hill.) S. F. Gray were used as parents, was analyzed with respect to chromosome numbers: diploid *H. lactucella* Wallr., tetraploid *H. pilosella* L., *H. caespitosum* Dumort. and *H. aurantiacum* L., pentaploid *H. glomeratum* Froel. and *H. piloselliflorum* Nägeli & Peter. In addition, some of selected F<sub>1</sub> hybrids as well as the hexaploid hybridogenous species *H. rubrum* Peter were open pollinated and their offspring was studied. In most crosses the sexuals served as seed parents, while the pollen donors were usually facultative apomicts. A considerable karyological diversity was recorded within the progeny of some of individual seed parents, hybridizing easily due to (at least facultative) sexuality. In addition, the haploid parthenogenesis and the participation of unreduced gametes (probably the fertilization of unreduced egg cell) contributed to offspring diversity. These reproduction modes, together with apomictic reproduction and fertilization of reduced egg cell, caused the rise of extremely diverse progeny of *H. rubrum*. The haploid parthenogenesis connected with aneuploidy was recorded as a new observation. Although the aneuploids are very rare in nature in Europe, the abundant and viable aneuploid progeny can be easily obtained from experimental crosses. Similarly, the viable triploids arising frequently from the crosses between diploid and tetraploid species, are in contrast to sparse occurrence of triploid cytotypes in the field. The reasons for these disproportions are discussed. Whereas the reduced competitive ability is expected in polyhaploids in the field, the natural polyploids originated spontaneously from the unreduced gametes may be more common. The possibilities to reveal the contribution of particular species comprising hybrid polyploids in the field are discussed.

**KEY WORDS:** Asteraceae, *Hieracium* subgen. *Pilosella*, aneuploids, cytotypes, haploid parthenogenesis, heteroploid crosses, hybridization, triploids, unreduced gametes

A. Krahulcová and F. Krahulec, Institute of Botany, Academy of Sciences of the Czech Republic, CZ–252 43 Průhonice, Czech Republic; e-mail: krahulcova@ibot.cas.cz

### INTRODUCTION

A noteworthy characteristic of *Hieracium* L. subgen. *Pilosella* (Hill.) S. F. Gray is its richness of forms, many of them coexisting within the same populations. The origin of this diversity has excited both taxonomists and geneticists, and led to hybridization experiments from as early as the mid 1800's (e.g. Mendel 1869; Peter 1884; Nägeli & Peter

1885). However, despite this early work the real reasons for the extensive variation, based on hybridization, polyploidy and apomixis, have remained unclear. Only later experimental studies on reproductive systems and embryogenesis, including the karyotyping of plants from natural populations, have led to an understanding of the main sources of variation in this group, i. e. hybridization, polyploidy and facultative apomixis (for their overview see in Krahulcová *et al.* 2000). It is important to note that, until now, the range of types obtained from garden crossing experiments has been more extensive than those found in natural populations. The reason for this is probably the different behaviour of plants in nature and in garden experiments. Some of the cytotypes and hybrids arising by artificial crosses may not arise in nature at all. For example, some types have very low vigour, and while they can be grown under the most suitable conditions in the garden, they probably would not survive in nature under competition with surrounding vegetation.

This paper reports on the first set of our garden crossing experiments, in which the ability of hybridizing plants to produce wholly new cytotypes among the progeny is recorded. It follows the experimental studies on *Hieracium* subgen. *Pilosella* performed mainly by M. Skalińska and Th. W. J. Gadella. Skalińska (1971a–c, 1973, 1976) detected two processes contributing to offspring diversity, namely haploid parthenogenesis and the fertilization of unreduced egg cells, by means of crossing experiments within the polyploid complex of *H. aurantiacum* L. The subject of Gadella's experimental work (Gadella 1982–1992) has been the polyploid complex of *H. pilosella* L. and its most related taxa. Aneuploids (very rare in nature in Europe) have been recorded among progeny of Gadella's crosses, giving evidence for both aneuploid pollen grains capable of fertilization and the viability of aneuploid hybrids under experimental conditions (Gadella 1987, 1991a). In other of Gadella's experiments (Gadella 1987, 1988, 1991a–b, 1992) polyploid "addition hybrids" have arisen among progeny, being indicative of both fertilization of unreduced egg cells and the production of viable unreduced pollen grains. For more detail on the experiments done by Skalińska and Gadella see Krahulcová *et al.* (2000). Recently, studies on sexual *versus* apomictic development of embryos, including the genetic background of apomixis, its mechanisms and variation within apomictic lines in *Hieracium* subgen. *Pilosella* were carried out (Koltunow *et al.* 1998, 2000; Bicknell *et al.* 2000).

## MATERIAL AND METHODS

### Plants

Plants used in the hybridization experiments were collected in the Krkonoše Mts (part of the Sudeten Mts) during the years 1995–1997 and cultivated in a lowland experimental garden in Průhonice. Chromosome numbers were examined from the root-tip meristem stained with lacto-propionic orcein (for detail of the method see in Krahulcová and Krahulec 1999). Voucher specimens of parental plants and their hybrids are deposited in the herbarium of the Institute of Botany, Průhonice (PRA). The mode of seed reproduction was studied according to the procedure described in Krahulcová and Krahulec (1999). The overview of species used for experiments with chromosome numbers and reproductive systems of particular parental plants is given in Table 1.

**Table 1.** List of species and individuals used in crossing experiments. The identity of locality labels means that all plants in question originated from the same locality. Several individuals of *Hieracium lactucella* and of *H. glomeratum* (marked by asterisk) belong likely to one clone of each species, as showed later their intraspecific identity of isozyme patterns (systems AAT-1, AAT-2, LAP, SHDH, PGM-1, PGM-2). The term apomictic reproduction refers to the facultative apomixis in tetraploids ( $2n = 36$ ) and hexaploids ( $2n = 54$ ), and probably to almost obligate apomixis in pentaploids ( $2n = 45$ ). The proportion of apomictic versus sexual reproduction in apomictic types was not studied in detail.

| Species                                  | Plant label | $2n$ | Reproduction | Locality label | Description of locality                     | Altitude | Coordinates              |
|--|-------------|------|--------------|----------------|---|----------|--------------------------|
| <i>H. rubrum</i> Peter                   | 11RU        | 54   | apomictic    | A              | meadow near Velká Úpa                       | 910 m    | 50°41'40"N<br>15°47'10"E |
|  | 13RU        | 54   | apomictic    | A              | "   | "        | "                        |
| <i>H. aurantiacum</i> L.                 | 30AU        | 36   | apomictic    | B              | meadow near Velká Úpa                       | 860 m    | 50°41'20"N<br>15°48'00"E |
|  | 32AU        | 36   | apomictic    | B              | "   | "        | "                        |
|  | 114AU       | 36   | apomictic    | C              | meadow near Pec p. Sněžkou                  | 920 m    | 50°41'20"N<br>15°43'10"E |
|  | 119AU       | 36   | apomictic    | D              | grassland along the road near Horní Mísečky | 1060 m   | 50°44'10"N<br>15°34'10"E |
| <i>H. lactucella</i> Wallr.              | 38LA        | 18   | sexual       | B              | meadow near Velká Úpa                       | 860 m    | 50°41'20"N<br>15°48'00"E |
|  | 40LA*       | 18   | sexual       | B              | "   | "        | "                        |
|  | 42LA*       | 18   | sexual       | B              | "   | "        | "                        |
|  | 43LA*       | 18   | sexual       | B              | "   | "        | "                        |
|  | 44LA*       | 18   | sexual       | B              | "   | "        | "                        |
| <i>H. pilosella</i> L.                   | 69PI        | 36   | sexual       | B              | "   | "        | "                        |
|  | 72PI        | 36   | sexual       | B              | "   | "        | "                        |
|  | 74PI        | 36   | sexual       | B              | "   | "        | "                        |
| <i>H. glomeratum</i> Froel.              | 10GL        | 45   | apomictic    | E              | meadow near Velká Úpa                       | 810 m    | 50°41'40"N<br>15°46'10"E |
|  | 25GL*       | 45   | apomictic    | B              | meadow near Velká Úpa                       | 865 m    | 50°41'20"N<br>15°48'00"E |
|  | 48GL*       | 45   | apomictic    | B              | "   | 880 m    | 50°41'30"N<br>15°47'50"E |
|  | 51GL*       | 45   | apomictic    | B              | "   | "        | "                        |
| <i>H. caespitosum</i> Dumort.            | 113CE       | 36   | apomictic    | C              | meadow near Pec p. Sněžkou                  | 910 m    | 50°41'20"N<br>15°43'10"E |
| <i>H. piloselliflorum</i> Nägeli & Peter | 76PF        | 45   | apomictic    | B              | meadow near Velká Úpa                       | 860 m    | 50°41'20"N<br>15°48'00"E |
|  | 82PF        | 45   | apomictic    | B              | "   | "        | "                        |

Additional species and cytotypes cultivated in the garden, which might serve as potential pollen donors for open pollinated seed parents

|                                     |            |  |        |
|-------------------------------------|------------|--|--------|
| <i>H. cymosum</i> L.                | 18         | <i>H. calodon</i> Tausch ex Peter        | 36     |
| <i>H. echioides</i> Lumn.           | 18, 27, 36 | <i>H. bifurcum</i> Bieb.                 | 36     |
| <i>H. apatellum</i> Nägeli & Peter  | 36         | <i>H. schultesii</i> F. W. Schultz       | 36, 45 |
| <i>H. iseranum</i> Uechtr.          | 36         | <i>H. stoloniflorum</i> Waldst. & Kit.   | 54     |
| <i>H. floribundum</i> Wimm. & Grab. | 36         | <i>H. pilosella</i> L.                   | 54     |
| <i>H. glomeratum</i> Froel.         | 36         | <i>H. piloselliflorum</i> Nägeli & Peter | 36, 54 |

The experiments, both open pollination and hand crossing, were carried out in the garden between May and June. All species and cytotypes of the *Pilosella* collection (with a basic chromosome number  $x = 9$ ), were cultivated together (Table 1). This allowed for the spontaneous pollination of open pollinated entomogamous plants. Plants used in hand crossing experiments, while present in the garden, were bagged to prevent open pollination.

It is important to point out the qualitative character of our data. The actual frequencies of particular new cytotypes among progeny (especially of polyhaploids and addition hybrids) remain unknown, because limited space for cultivation meant that only a proportion of the progeny from anyone cross was usually studied. Likewise the previous karyological examination of germinating seeds was limited, and only confirmed karyological variation among progeny, but did not allow to estimate the exact frequencies of particular cytotypes.

In the tables we refer directly to individual plants and not to average figures. The main reason for this is the high discontinuous variation among progeny of individual seed parents. However, this variation is of significant evolutionary importance, as has been shown e.g. by Arnold and Hodges (1995) or by Krahulcová *et al.* (1996).

### Crossing experiments

The sexual types were used as seed parents and the apomicts as pollen donors in most of the intentional crosses. In *Hieracium* subgen. *Pilosella* both the sexual and apomictic types can be successful pollen donors, producing viable pollen capable of fertilization (for review of literature see in Krahulcová *et al.* 2000). All the inflorescences of each parent were isolated in nylon bags before anthesis, i.e. before the outer ligular flowers of the capitulum had begun to open. In the stage of stigma receptivity (the bifurcate stigmas sticking out of the flowers), both parental capitula were rubbed together, to ensure the transport of pollen to stigmas. Pollination was usually repeated 2 to 3 times a week (on each occasion with a fresh new capitulum of the same pollen donor), until the capitulum of the seed parent began to fade. The nylon bags were left on pollinated capitula until the harvest of ripe seeds.

Emasculation was not carried out, because sexual plants (seed parents) were assumed to be self-incompatible (Gadella 1987). We have shown that self-incompatibility in sexual *H. pilosella* and *H. lac-tucella* Wallr. can break down under the influence of pollen of another species (Krahulcová *et al.* 1999). However, the clear detection of hybrid progeny intermediate to both parents was possible in most of the crosses, based both on morphology and ploidy level. Only the detection of tetraploid hybrids between two closely related species *H. pilosella* and *H. piloselliflorum*, similar to each other in morphology, was not clear in all cases (see below). When a facultatively apomictic species (e.g. *H. aurantiacum* or *H. rubrum* Peter) was used as a seed parent, the separation of hybrid progeny from those derived from apomixis was possible because of different morphology. In addition, the heteroploid crosses of such species (when used as seed parents) allowed to distinguish between the hybrid and matroclinal progeny by their ploidy level.

## RESULTS AND DISCUSSION

### Haploid parthenogenesis

Cytotypes which must have originated from the parthenogenetic development of reduced egg cells (Table 2) were recorded only twice among the mature progeny: once as a triploid descendant of a hexaploid *H. rubrum* (the open pollinated plant 11RU) and once as a diploid descendant of a tetraploid *H. aurantiacum* (the unsuccessful cross *H. aurantiacum*  $\times$  *H. pilosella*, 119AU  $\times$  72PI, Fig. 3A). In each case, the dwarf polyhaploid plants were

**Table 2.** Polyhaploids recorded among the progeny of *Hieracium rubrum*, *H. aurantiacum* and F<sub>1</sub> hybrid *H. pilosella* × *H. glomeratum* (for explanation see in text).

| Plant label | Seed parent<br>$2n$ | Number of polyhaploids | Progeny<br>$2n$ | Number of other individuals studied   |
|-------------|---------------------|------------------------|-----------------|---|
| 11RU        | 54                  | 1 (mature plant)       | 27              | 3 <i>rubrum</i> -like plants<br>4 hybrids (see Table 4)                               |
| 13RU        | 54                  | 3 (germinating seeds)  | 27              | 12 (see Table 4)  |
| 119AU       | 36                  | 1 (mature plant)       | 18              | 16 <i>aurantiacum</i> -like plants<br>3 hybrids <i>aurantiacum</i> × <i>pilosella</i> |
| 74PI×48GL-1 | 41                  | 1 (germinating seed)   | 20              | 17 hybrid seeds   |
|             |                     | 1 (germinating seed)   | 21              | ( $2n = 36-40$ )  |

distinct from the other morphotypes recorded within progeny. The both polyhaploids, as open pollinated, as well as emasculated, gave poor seed-set, but able to germinate. This is indicative for their apomictic seed reproduction. Moreover, the karyotyping of seeds germinated, which originated from an open pollinated trihaploid *H. rubrum*, showed that this F<sub>2</sub> progeny maintain the triploid chromosome number ( $2n = 27$ , Fig. 3B) of their trihaploid seed parent. In addition to both polyhaploids recorded among the mature progeny, the polyhaploids were also rarely recorded among germinating seeds used for karyological studies and therefore destroyed (Table 2). Those few originated either from open pollinated hexaploid *H. rubrum* (plant 13RU) or from open pollinated aneuploid F<sub>1</sub> hybrid *H. pilosella* × *H. glomeratum* Froel (cross 74PI × 48GL).

Haploid parthenogenesis has been recorded very rarely in *Hieracium* subgen. *Pilosella*, namely among progeny of experimental crosses in which the tetraploid or octoploid facultatively apomictic *H. aurantiacum* has been used as a seed parent (Skalińska 1971a, 1976). She recorded that both cytotypes gave rise to one polyhaploid plant each, diploid and tetraploid respectively. However, as the F<sub>1</sub> progeny were analyzed as mature plants, it is possible that polyhaploids might be more frequent among F<sub>1</sub> seedlings with defective development, which perished early (Skalińska 1971a). Recently, Bicknell (1997) succeeded in the cultivation of mature dihaploid descendant originated from triploid *H. aurantiacum*, i.e. from the seed parent having an odd ploidy level.

The polyhaploids obtained in our experiments were recorded either as mature plants or as germinating seeds (Table 2). The ability to produce polyhaploid progeny was confirmed in tetraploid *H. aurantiacum*. Moreover, trihaploids were found among progeny of hexaploid *H. rubrum*, i.e. of hybridogenous species having *H. aurantiacum* as one of parents. Its second putative parent is thought to be *H. pilosella* (Peter 1881; Nägeli & Peter 1885) or *H. flagellare* Willd. (= *H. caespitosum* × *H. pilosella*) – see e.g. Zahn (1930), Sell and West (1976). However, it appears that this mode of reproduction is not restricted to *H. aurantiacum* and its relatives. There are two records of polyhaploid germinating seeds (i.e. F<sub>2</sub> progeny), resulted from an open pollinated F<sub>1</sub> aneuploid hybrid between *H. pilosella* and *H. glomeratum* (Table 2). In this case however, the aneuploid F<sub>1</sub> hybrid gave rise to two polyhaploid descendants, which were aneuploid as well. Haploid parthenogenesis combined with aneuploidy is a new observation.

### The participation of unreduced gametes in hybridization

The polyploid hybrids which originated *via* participation of unreduced gametes (both female and male), have been called “addition hybrids” (e.g. Gadella 1987, 1988). Such hybrids were recorded in our experiments among progeny of open pollinated plants only (Table 3). Their seed parents were: hexaploid *H. rubrum* (plants 11RU and 13RU), tetraploid *H. aurantiacum* (plants 30AU and 32AU), two tetraploid F<sub>1</sub> hybrids *H. lactucella* × *H. glomeratum* (from the cross 47LA × 48GL) and the aneuploid F<sub>1</sub> hybrid *H. lactucella* × *H. glomeratum* (from the cross 40LA × 25GL). Only a proportion of these addition hybrids was cultivated and then examined as mature plants; the other hybrids of this type were recorded in the stage of germinating seeds (Table 3).

The addition hybrids in our experiments originated more probably from the fertilization of unreduced egg cells of open pollinated seed parents than through the unreduced pollen of the pollen donors. Although the putative fertilizing species (pollen donors) were unknown (Table 3), no participation of unreduced fertilizing pollen was recorded in additional intentional crosses, which included *H. pilosella*, *H. lactucella*, *H. aurantiacum*, *H. caespitosum* Dumort., *H. glomeratum* or *H. piloselliflorum* Nägeli & Peter as pollen donors. Nevertheless, the possibility of fertilization of a reduced egg cell of an open pollinated seed parent (either by reduced pollen of some highly polyploid pollen donor, or by unreduced pollen), cannot be excluded at all, as well as the doubling of chromosome number in proembryo. In some cases, a comparison between the hybrid in question and putative pollen donors cultivated in the garden with respect to chromosome numbers and morphological characters was a useful guide for paternity identification: e.g., the glabrous and glaucous leaves characteristic of diploid *H. lactucella* were ex-

**Table 3.** The hybrids derived probably from fertilization of unreduced embryo sac, as recorded among the progeny of open pollinated *H. rubrum*, *H. aurantiacum*, and F<sub>1</sub> hybrids *H. lactucella* × *H. glomeratum* (for explanation see in text). The unreduced pollen of putative tetraploid pollen parent is marked by asterisk; # = aneuploid pollen produced by pentaploid donor. Note: a possible origin of the octoploid hybrid ( $2n = 72$ ) given in bold may also be a fusion of two diploid gametes ( $n = 18$ ) followed by chromosome doubling in proembryo.

| Plant label | Seed parent<br>$2n$ | Fertilizing<br>pollen $n$  | Number of addition<br>hybrids | Progeny<br>$2n$ | Number of other<br>individuals studied |
|-------------|---------------------|----------------------------|-------------------------------|-----------------|--|
| 11RU        | 54                  | 18                         | 2 (mature plants)             | 72              | 5 (see Table 4)                        |
|             |                     | 9 ( <i>H. lactucella</i> ) | 1 (mature plant)              | 63              |  |
| 13RU        | 54                  | 18                         | 2 (germinating seeds)         | 72              | 13 (see Table 4)                       |
| 30AU        | 36                  | 18                         | 1 (mature plant)              | 54              | 0                                      |
| 32AU        | 36                  | 36*( $2n$ )                | <b>1 (mature plant)</b>       | 72              | 0                                      |
|             |                     | 18                         | 1 (mature plant)              | 54              |  |
| 47LA×48GL–2 | 36                  | 18                         | 4 (germinating seeds)         | 54              | 0                                      |
|             |                     | 27                         | 3 (germinating seeds)         | 63              |  |
| 47LA×48GL–6 | 36                  | 18                         | 1 (mature plant)              | 54              | 0                                      |
| 40LA×25GL–8 | 35                  | 18                         | 6 (germinating seeds)         | 53              | 4 ( $2n = 34$ – $35$ , 36)             |
|             |                     | 21–23#                     | 1 (germinating seed)          | 56–58           |  |

**Table 4.** The diverse cytotypes recorded among F<sub>1</sub> progeny of open pollinated hexaploid *H. rubrum* ( $2n = 54$ ). In addition hybrids, the fertilization of unreduced embryo sac is assumed (for explanation see in text). In those cases where the hexaploid level ( $2n = 54$ ) is maintained in progeny, the apomictic reproduction of the seed parent is considered as more probable than the possible hybridization between two hexaploid parents.

| Label of seed parent | Female gamete             | Putative pollen donor<br>$n$ | F <sub>1</sub> progeny<br>$2n$ | Number of individuals        | Origin                     |
|----------------------|---------------------------|------------------------------|--------------------------------|------------------------------|----------------------------|
| 11RU                 | 54 ( $2n$ , somatic cell) | –                            | 54                             | 3 <i>rubrum</i> -like plants | apomixis                   |
|                      | 27 ( $n$ )                | –                            | 27                             | 1 dwarf poor plant           | haploid<br>parthenogenesis |
|                      | 27 ( $n$ )                | 18                           | 45                             | 1 hybrid plant               | hybridization              |
|                      | 54 ( $2n$ )               | 9 ( <i>H. lactucella</i> )   | 63                             | 1 hybrid plant               | hybridization              |
|                      | 54 ( $2n$ )               | 18                           | 72                             | 2 hybrid plants              | hybridization              |
| 13RU                 | 54 ( $2n$ , somatic cell) | –                            | 54                             | 5 germinating seeds          | apomixis                   |
|                      | 27 ( $n$ )                | –                            | 27                             | 3 germinating seeds          | haploid<br>parthenogenesis |
|                      | 27 ( $n$ )                | 18                           | 45                             | 5 germinating seeds          | hybridization              |
|                      | 54 ( $2n$ )               | 18                           | 72                             | 2 germinating seeds          | hybridization              |

pressed in a heptaploid hybrid ( $2n = 63$ , Table 3 & 4), which facilitated the identification of *H. lactucella* as its pollen parent. Nevertheless, such identification of pollen parent based on distinct morphological characters was rare among the mature addition hybrids originated from open pollinated plants. A fertilization by pollen of some tetraploid pollen parent is assumed in most of these cases (Table 3 & 4); however, total of 12 tetraploid species could serve as potential pollen donors (Table 1).

The different genotypes of particular species (or cross combinations) may differ in their capability to produce addition hybrids. The data on F<sub>1</sub> hybrids *H. pilosella* × *H. glomeratum* used as seed parents may serve as an example (Table 3). A total of seven addition hybrids were found (as germinating seeds) among the progeny of the hybrid tetraploid plant 47LA × 48GL-2 (originated from the cross between diploid *H. lactucella* and pentaploid *H. glomeratum*), whereas no hybrids arising from reduced female gametes were recorded. Some genotypes may be especially prone to a diversity of reproductive modes and especially capable of hybridization, as the open pollinated *H. rubrum* (see below and Table 4, Fig. 1).

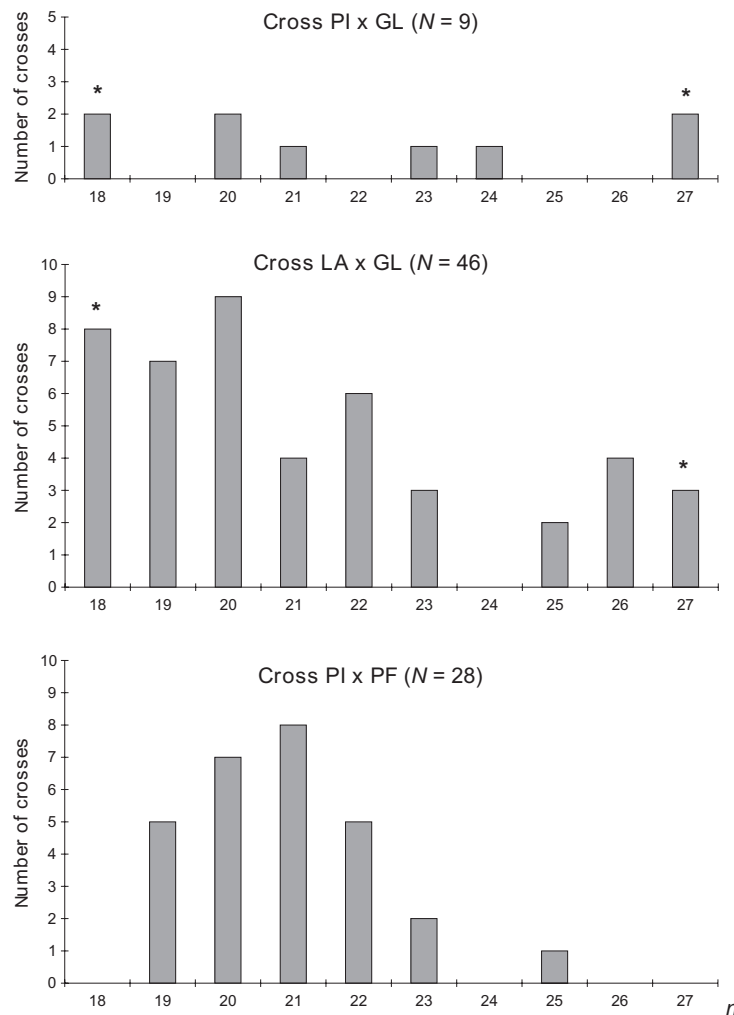
The production of unreduced egg cells requiring fertilization has also been recorded in diploid sexual *H. peleterianum*, tetraploid (sexual) and heptaploid (facultatively apomictic) *H. pilosella* (Gadella 1988), and in pentaploid, hexaploid and octoploid *H. aurantiacum* (all facultative apomicts – Skalińska 1973, 1976). In addition, several facultatively apomictic cytotypes belonging to both polyploid complexes of *H. aurantiacum* and *H. pilosella*, have been shown to occasionally form unreduced pollen capable of fertilization (Gadella 1988; Skalińska 1971a, 1973, 1976). In our experiments however, the fertilization by unreduced pollen was only once recorded reliably (Table 3). However, the doubling of chromosome number in proembryo was also possible in this case.

In general, both the processes of haploid parthenogenesis and the fertilization of unreduced egg cell may be considered as manifestations of (facultative) sexuality. Most of





*losella* × pentaploid *H. piloselliflorum*. The chromosome numbers of the fertilizing pollen grains were detected indirectly, based both on assumed regular meiosis in sexual seed parents (*H. lactucella*, *H. pilosella*) and on the known somatic chromosome numbers of individual F<sub>1</sub> hybrids (Table 5, Fig. 2). However, distinguishing between the true hybrids from the cross between two closely related species (the tetraploid *H. pilosella* and the pentaploid *H. piloselliflorum*) and the possible rare descendants of autogamous reproduction of *H. pilosella* (Krahulcová *et al.* 1999), was impossible especially in those tetraploid progeny almost identical in morphology with their tetraploid seed parent *H. pilosella*.



**Fig. 2.** Chromosome numbers ( $n$ ) in fertilizing pollen of pentaploid apomictic donor (*Hieracium glomeratum* Froel. – GL, *H. piloselliflorum* Nägeli & Peter – PF) crossed with sexual diploid (*H. lactucella* Wallr. – LA) or sexual tetraploid (*H. pilosella* L. – PI) seed parent (the whole capitula were crossed with each other). For data see in Table 5, for detailed explanation in text.  $N$  = total number of F<sub>1</sub> hybrids examined; \* = euploid gametes.

**Table 5.** Aneuploids recorded among the mature F<sub>1</sub> progeny of heteroploid crosses, where pentaploid *Hieracium glomeratum* Froel. or *H. piloselliflorum* Nägeli & Peter were used as pollen parents. For characteristics of particular parental species (according to plant labels) see Table 1. Total of five tetraploid plants originated from three crosses *H. pilosella* × *H. piloselliflorum* are not included into category of euploid hybrids (for explanation see in text).

| Label of seed parent | 2n | n  | Label of pollen parent | 2n | Male gametes comprising |                   | Number of aneuploid F <sub>1</sub> hybrids | 2n       | Number of euploid F <sub>1</sub> hybrids | 2n    |
|----------------------|----|----|------------------------|----|-------------------------|-------------------|--|----------|--|-------|
|                      |    |    |                        |    | aneuploid progeny n     | euploid progeny n |  |          |  |       |
| 74PI                 | 36 | 18 | 10GL                   | 45 | 20                      |                   | 1  | 38       | 0  |       |
| 72PI                 | 36 | 18 | 48GL                   | 45 |                         | 27                | 0  |          | 1  | 45    |
| 72PI                 | 36 | 18 | 51GL                   | 45 | 24                      | 18                | 1  | 42       | 1  | 36    |
|                      |    |    |                        |    |                         | 27                |  |          | 1*                                       | 45/46 |
| 74PI                 | 36 | 18 | 48GL                   | 45 | 20                      | 18                | 1  | 38       | 1  | 36    |
|                      |    |    |                        |    | 21                      |                   | 1  | 39       |  |       |
|                      |    |    |                        |    | 23                      |                   | 1  | 41       |  |       |
| 43LA                 | 18 | 9  | 10GL                   | 45 | 20                      |                   | 1  | 29       | 0  |       |
|                      |    |    |                        |    | 26                      |                   | 1  | 35       |  |       |
| 40LA                 | 18 | 9  | 10GL                   | 45 | 20                      |                   | 1  | 29       | 0  |       |
|                      |    |    |                        |    | 25                      |                   | 1  | 34       |  |       |
| 47LA                 | 18 | 9  | 51GL                   | 45 | 20                      | 18                | 1*   | 28/29/30 | 1  | 27    |
|                      |    |    |                        |    | 19                      |                   | 1  | 28       |  |       |
|                      |    |    |                        |    | 23                      |                   | 1  | 32       |  |       |
| 40LA                 | 18 | 9  | 25GL                   | 45 | 20                      | 18                | 3  | 29       | 1  | 27    |
|                      |    |    |                        |    | 21                      | 27                | 1  | 30       | 1*                                       | 35/36 |
|                      |    |    |                        |    | 23                      |                   | 2  | 32       |  |       |
|                      |    |    |                        |    | 26                      |                   | 2  | 35       |  |       |
| 47LA                 | 18 | 9  | 48GL                   | 45 | 19                      | 18                | 2  | 28       | 2  | 27    |
|                      |    |    |                        |    | 20                      | 27                | 1  | 29       | 2  | 36    |
|                      |    |    |                        |    | 21                      |                   | 1  | 30       |  |       |
|                      |    |    |                        |    | 22                      |                   | 1  | 31       |  |       |
|                      |    |    |                        |    | 26                      |                   | 1  | 35       |  |       |
| 43LA                 | 18 | 9  | 51GL                   | 45 | 19                      | 18                | 3  | 28       | 4  | 27    |
|                      |    |    |                        |    | 20                      |                   | 2  | 29       |  |       |
|                      |    |    |                        |    | 21                      |                   | 1  | 30       |  |       |
|                      |    |    |                        |    | 22                      |                   | 2  | 31       |  |       |
|                      |    |    |                        |    | 25                      |                   | 1  | 34       |  |       |
| 42LA                 | 18 | 9  | 25GL                   | 45 | 19                      |                   | 1  | 28       | 0  |       |
|                      |    |    |                        |    | 21                      |                   | 1  | 30       |  |       |
|                      |    |    |                        |    | 22                      |                   | 3  | 31       |  |       |
| 74PI                 | 36 | 18 | 76PF                   | 45 | 22                      | 18                | 1  | 40       |  |       |
| 69PI                 | 36 | 18 | 80PF                   | 45 | 19                      | 18                | 4  | 37       |  |       |
|                      |    |    |                        |    | 20                      |                   | 4  | 38       |  |       |
|                      |    |    |                        |    | 20?                     |                   | 1*   | 37/39    |  |       |
|                      |    |    |                        |    | 21                      |                   | 3  | 39       |  |       |
|                      |    |    |                        |    | 22                      |                   | 1  | 40       |  |       |
| 72PI                 | 36 | 18 | 82PF                   | 45 | 19                      | 18                | 1  | 37       |  |       |
|                      |    |    |                        |    | 20                      |                   | 2  | 38       |  |       |
|                      |    |    |                        |    | 21                      |                   | 5  | 39       |  |       |
|                      |    |    |                        |    | 22                      |                   | 3  | 40       |  |       |
|                      |    |    |                        |    | 23                      |                   | 2  | 41       |  |       |
|                      |    |    |                        |    | 25                      |                   | 1  | 43       |  |       |
| Total                |    |    |                        |    |                         |                   |  | 68       | 15                                       |       |
|                      |    |    |                        |    |                         |                   |  | (81.9%)  | (18.1%)                                  |       |

\* – somatic mosaic

**Table 6.** Aneuploids recorded among the F<sub>2</sub> progeny (\* – germinating seeds; + – mature plants) of selected open pollinated aneuploid F<sub>1</sub> hybrids.

| Seed parent<br>(F <sub>1</sub> hybrid) | 2n | F <sub>2</sub> progeny                              |          |                    |
|--|----|---|----------|--------------------|
|  |    | Number of<br>aneuploids                             | 2n       | Number of euploids |
| 74PI×10GL–2                            | 38 | 2*  | 37       | 8* (2n = 36)       |
|  |    | 1*  | 38       |                    |
| 74PI×48GL–1                            | 41 | 1+  | 29       | 1* (2n = 36)       |
|  |    | 1* 2+   | 37       |                    |
|  |    | 8* 2+   | 38       |                    |
|  |    | 3*  | 39       |                    |
|  |    | 4*  | 40       |                    |
|  |    | 2* descendants of haploid parthenogenesis (Table 2) |          |                    |
| 74PI×48GL–2                            | 38 | 3*  | 37       | 3* (2n = 36)       |
|  |    | 1*  | 39       |                    |
|  |    | 1*  | 41       |                    |
|  |    | 1*  | 42       |                    |
| 43LA×10GL–1                            | 35 | 1*  | 34       | 1* (2n = 36)       |
|  |    | 1*  | 35       |                    |
|  |    | 1*  | 39       |                    |
|  |    | 1*  | 44       |                    |
| 40LA×25GL–1                            | 32 | 1+  | 35       | 1+ (2n = 36)       |
| Total                                  |    | 37 (71%)  | 15 (29%) |                    |

Therefore, only the aneuploid progeny (i.e. the true hybrids without any doubt), and no tetraploid descendants obtained from this cross were included in the results of hybridization between *H. pilosella* and *H. piloselliflorum* (Table 5, Fig. 2).

Most of the progeny derived from selected open pollinated aneuploid F<sub>1</sub> hybrids was aneuploid as well, but with diverse chromosome numbers within half-siblings (Table 6). In addition, aneuploid individuals were rarely recorded among progeny of several heteroploid crosses, where the tetraploid apomictic *H. aurantiacum* or *H. caespitosum* served as pollen parents and the diploid sexual *H. lactucella* as a seed parent (Table 7).

Although aneuploids have only been recorded in a few isolated cases from nature in Europe (Finch ined. in Moore 1982; Krahulcová & Krahulec 1999), they have been easily obtained from experimental crosses. Such crosses usually involved an apomictic pollen donor with odd ploidy level, or the backcrossed pentaploid sexual seed parent (Gadella 1987, 1991a; our crosses – Table 5). In this respect, our experiments can be only compared with those crosses, where the tetraploid sexual *H. pilosella* has been pollinated by the pentaploid apomictic pollen donor of the same species. However, there are several differences between the design of experiments: all our crosses always involved two different parental species. Moreover, Gadella (1987, 1991) did not study the progeny of sexual diploids pollinated by apomictic pentaploids. Nevertheless, comparing the results of our and Gadella's crosses, the marked difference is the much higher fre-

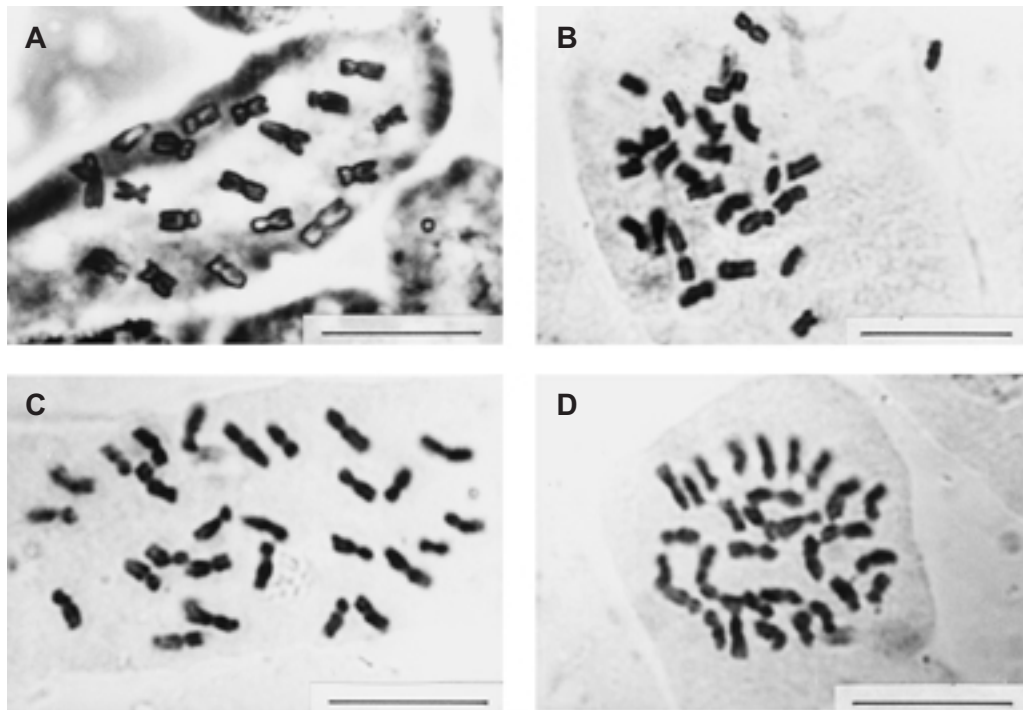
quency of aneuploids among F<sub>1</sub> hybrids in our crosses with pentaploids as pollen donors. Whereas Gadella (1991a: 472) obtained only 4% aneuploid progeny among 123 F<sub>1</sub> plants (the cross between tetraploid and pentaploid *H. pilosella*), the proportion of aneuploid F<sub>1</sub> hybrids in all our crosses together was more than 80% of the whole F<sub>1</sub> progeny (Table 5). The chromosome numbers recorded among F<sub>2</sub> aneuploid progeny, which originated from selected open pollinated F<sub>1</sub> aneuploid hybrids (Table 6), are diverse within individual half-siblings. This reflects the hybrid origin of such F<sub>2</sub> plants and consequently the sexual reproduction of their F<sub>1</sub> seed parents, as the progeny of apomicts should be homogeneous. The reproductive system of aneuploid hybrids is now under study.

The distribution of chromosome numbers among fertilizing pollen (Fig. 2) suggests that during meiosis in the pentaploid pollen donor, the chromosomes of four of its five sets form bivalents and are regularly distributed to opposite poles, while the remaining odd chromosome set is distributed more or less randomly. Thus, the chromosome numbers of fertilizing pollen produced by pentaploid parents range between the diploid and triploid level. However, the actual distribution of chromosome numbers of fertilizing pollen (crosses LA × GL and PI × PF in Fig. 2) suggests the preference of both diploid and aneuploid (with chromosome numbers more close to diploid than to triploid level) male gametes. The intragenomic pairing within four chromosome sets of pentaploid pollen donors (although detected indirectly), shows a tendency to their “diploidized” genetic behaviour and might demonstrate the hybrid (i.e. allopolyploid) nature. Indeed, both the pentaploids used in our crosses are considered as hybridogenous species: *H. glomeratum* = *H. caespitosum* × *H. cymosum*, *H. piloselliflorum* = *H. pilosella* × *H. floribundum* (Zahn 1930). The formation of 18 bivalents and 9 univalents in pollen mother cells giving rise to both euploid and aneuploid pollen grains has also been documented in some other pentaploid species, e.g. in *H. excellens* (Rosenberg 1917) and in pentaploid cytotype of *H. pilosella* (Gentcheff 1938).

The rare records of aneuploid progeny obtained from crosses between diploid sexual species and tetraploid facultative apomicts (Table 7), correspond to some extent to for-

**Table 7.** Aneuploids recorded among the mature F<sub>1</sub> progeny of crosses between the diploid sexual (*Hieracium lactucella* Wallr.) and the tetraploid facultatively apomictic species (*H. aurantiacum* L., *H. caespitosum* Dumort.).

| Label of seed parent | 2n | Label of pollen parent | 2n | Number of aneuploids among progeny | 2n | Number of triploid hybrids (2n = 27) |
|----------------------|----|------------------------|----|------------------------------------|----|--------------------------------------|
| 47LA                 | 18 | 114AU                  | 36 | 1                                  | 26 | 5                                    |
| 44LA                 | 18 | 114AU                  | 36 | 0                                  |    | 9                                    |
| 43LA                 | 18 | 114AU                  | 36 | 1                                  | 28 | 6                                    |
| 47LA                 | 18 | 113CE                  | 36 | 1                                  | 28 | 11                                   |
| 44LA                 | 18 | 113CE                  | 36 | 0                                  |    | 14                                   |
| 45LA                 | 18 | 113CE                  | 36 | 0                                  |    | 6                                    |
| 43LA                 | 18 | 113CE                  | 36 | 0                                  |    | 2                                    |
| 113CE                | 36 | 38LA                   | 18 | 0                                  |    | 1                                    |
| Total                |    |                        |    | 3 (5.3%)                           |    | 54 (94.7%)                           |



**Fig. 3.** Somatic metaphase in progeny of *Hieracium* subgen *Pilosella* originated from crossing experiments. **A:** a diploid *H. aurantiacum* L. (progeny of unsuccessfull cross 119AU  $\times$  72PI),  $2n = 18$  (phase contrast); **B:** a triploid descendant of open pollinated trihaploid *H. rubrum* Peter (derived of the plant 11RU),  $2n = 27$ ; **C:** a triploid  $F_1$  hybrid between diploid *H. lactucella* Wallr. and pentaploid *H. glomeratum* Froel. (cross 47LA  $\times$  51GL),  $2n = 27$ ; **D:** an aneuploid  $F_1$  hybrid between diploid *H. lactucella* and pentaploid *H. glomeratum* (cross 42LA  $\times$  25GL),  $2n = 31$ . Scale = 10  $\mu$ m.

mer studies on hybrids between *H. lactucella* and *H. aurantiacum* (Rosenberg 1917). In his experiments, hypotriploid progeny ( $2n = 24, 26$ ) have occasionally been recorded besides the prevailing triploid hybrids ( $2n = 27$ ). This has been attributed to incomplete pairing during microsporogenesis in tetraploid *H. aurantiacum*, which might reflect the hybrid origin of the particular individuals under study (Rosenberg 1917). Neither species *H. aurantiacum* and *H. caespitosum*, used as pollen donors in our crosses (Table 7), produced many aneuploid progeny. However, this ability itself gives no evidence for hybrid origin of these species. Specialized studies on meiosis and genome composition are necessary in this respect.

### Triploid hybrids

Triploids ( $2n = 3x = 27$ ) originated from heteroploid crosses between diploids ( $2n = 2x = 18$ ) and tetraploids ( $2n = 4x = 36$ ). The crosses between diploid sexual and tetraploid apomictic species resulted in mainly triploid, and rarely to aneuploid  $F_1$  hybrids (Table 7). Most

of triploid hybrids appeared to be sterile, and only a few of them produced seeds. Nevertheless, the fertile triploid hybrids were more frequent among progeny of the cross *H. lactucella* × *H. aurantiacum*, compared to those of the cross *H. lactucella* × *H. caespitosum*. With respect to morphology, the former cross resulted in more diverse morphotypes than the latter. The progeny of two open pollinated fertile triploid hybrids (*H. lactucella* × *H. caespitosum* and *H. lactucella* × *H. aurantiacum* respectively) were studied hitherto with respect to chromosome numbers. Only triploids were recorded, which suggests the apomictic seed reproduction of their triploid seed parents.

Nothing but triploids were recorded among F<sub>1</sub> hybrids obtained from the cross between two sexual species, the diploid *H. lactucella* and the tetraploid *H. pilosella*. The following three crosses were studied: 42LA × 74PI (9 triploid F<sub>1</sub> hybrids), 40LA × 69PI (10 triploid F<sub>1</sub> hybrids) and the reciprocal cross 72PI × 44LA (2 triploid F<sub>1</sub> hybrids). Undoubtedly, both crosses with the diploid *H. lactucella* as a seed parent were more successful than the reciprocal cross. Many germinating seeds were obtained if *H. lactucella* served as a seed parent, and so it was only possible to cultivate a proportion of F<sub>1</sub> seedlings. On the other hand, only two F<sub>1</sub> hybrids (both cultivated triploids) were obtained from the reciprocal cross *H. pilosella* × *H. lactucella* (three pairs of different parental plants were crossed). All triploid hybrids between *H. lactucella* and *H. pilosella* were found to be sterile or almost sterile. Several triploid hybrids originated also from the cross between diploid *H. lactucella* and pentaploid *H. glomeratum* (Table 5, Fig. 3C).

Gadella (1992) has resynthesised two triploid hybridogenous species, *H. floribundum* and *H. schultesii*. The former species is considered to be the hybrid between the diploid sexual *H. lactucella* and the tetraploid apomictic *H. caespitosum*, the latter as the hybrid between two sexuals – the tetraploid *H. pilosella* and the diploid *H. lactucella* (e.g. Gadella 1992). Whereas the triploid hybrids derived from the former cross have been apomictic, resembling the pollen parent in this respect, the triploids (besides rare addition hybrids) obtained from the latter cross have been sterile. Thus, the almost total sterility found in our triploid hybrids *H. lactucella* × *H. pilosella* (including the both plants from the reciprocal cross) corresponds to Gadella's results (Gadella 1992). Contrary, we recorded several fertile open pollinated plants among triploid F<sub>1</sub> progeny of those crosses, in which diploid *H. lactucella* was pollinated by tetraploid *H. aurantiacum* or *H. caespitosum* (Table 7).

### The occurrence of new cytotypes in nature

There are two principal questions: (1) Which of the reproductive processes discussed above occurs in nature? (2) What is their role in the formation of complicated population structures, undoubtedly influenced by hybridization as the most important factor?

However, the rare occurrence of aneuploids in Europe (from where only hypopentaploids have been reported) suggests that: (a) the heteroploid crosses involving an odd-ploid pollen donor have lower importance than other ones resulting in euploid progeny, or (b) that there is a strong selection against aneuploids in nature. Our experiments showed that more than 80% of progeny of heteroploid crosses with pentaploids as pollen

donors were aneuploid. In the Krkonoše Mts however, where the sympatric occurrence of several ploidy levels (including the pentaploids) in one locality is not rare, the aneuploid specimens were found twice out of about 200 of the plants examined karyologically (Krahulcová & Krahulec 1999 and unpublished data). On the other hand, under different environmental conditions in New Zealand, approximately 20% of clones sampled in the field were aneuploid (Chapman & Lambie 1999), although the present more extensive studies (Chapman, unpublished data) suggest lower frequency (about 5–10%).

Similarly, triploid hybrids can be easily produced from heteroploid crosses between diploid and tetraploid species (Table 7, text above). In spite of their high viability in experimental conditions, they occur sparsely as spontaneous hybrids in nature. Several references on hybridogenous triploid species (e.g., *H. piloselloides* and *H. floribundum*), are given in the literature (for their overview see in Krahulcová *et al.* 2000). However, no triploid individual has been recorded in our population studies on *Hieracium* subgen. *Pilosella* in the Krkonoše Mts, although three diploid species (*H. lactucella*, *H. caespitosum* subsp. *brevipilum* and *H. floribundum*) occur there sympatrically with several tetraploid species (Krahulcová & Krahulec 1999 and unpublished data). The possible sterility of triploid hybrids can be overcome by clonal growth. Therefore, the absence of triploids in our field studies might be attributed either to rare spontaneous hybridization between the putative parents, or to low survival of triploid hybrids in nature.

There is no evidence for the existence of polyhaploid plants in the field. The specimens of dihaploid *H. aurantiacum* and trihaploid *H. rubrum* obtained during our study are not growing well; both are rather poor and it is highly improbable that they could survive under field conditions. In fact, our dihaploid *H. aurantiacum* was of a similar general appearance to the dihaploid descendant of this species grown under micropropagation by Bicknell (1997: 169 – a photo). However, the rare survival of polyhaploids cannot be excluded, especially under conditions of low biotic stress, as e.g. in adventitious parts of the distribution area and/or in habitats with common disturbance. Till now, the natural polyhaploids are not reported at all (Petit *et al.* 1999).

It is probable that the importance of addition hybrids (i.e. the role of unreduced gametes) is high. The occurrence of several ploidy levels within populations studied by us in the Krkonoše Mts (Krahulcová & Krahulec 1999 and unpublished data) may be attributed (at least to some extent) to this process. In our population studies on several hybridogenous species (unpublished results) we recorded apomictic plants (within one species) having different genotypes at one ploidy level, evident from their different isozyme patterns. This suggests that the establishment of new hybrids is not rare. Similarly, the existence of different genotypes with the same ploidy level is common in facultatively apomictic populations in New Zealand (Chapman *et al.* 2000). It should be remarked that the ploidy levels higher than 6x, recorded in some of the addition hybrids in our experiments, have not been found in the Krkonoše Mts. Nevertheless, this pathway to the origin of spontaneous addition hybrids with high ploidy levels (up to certain limited level of ploidy), has thought to be an important mechanism of differentiation in polyploid complexes of *H. aurantiacum* (Skalińska 1976) and of *H. pilosella* (Gadella 1988).

There is a problem in determining the contribution of particular species comprising the hybrid polyploids. It is more probable that sexual types serve as seed parents and (facultatively) apomictic types as pollen donors than *vice versa*. The importance of unreduced gametes was shown in data presented here (Tables 3 & 4). It would be possible to do such study by means of species specific markers or by some of specialized cytological techniques (as GISH). On the other hand, the determination of species comprising spontaneous hybrids in the field is made more difficult by frequent hybridization connected with assumed introgression. If the role of introgression is high, then it would be difficult to reveal species specific markers, especially in molecular studies (isozymes and DNA). In such cases, the higher differentiation can be expected between particular isolated populations differing in basic species involved in hybridization and/or with different environmental conditions, than between species within one population with existing gene flow. Thus, the smoothing of borders between species is probable, due to hybridization and backcrossing.

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