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# VARIATION IN *HIERACIUM* SUBGEN. *PILOSELLA* (*ASTERACEAE*): WHAT DO WE KNOW ABOUT ITS SOURCES ?

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**Abstract:** The present paper reviews mechanisms producing complicated patterns of variation within *Hieracium* subgen. *Pilosella*. The taxonomic complexity of this subgenus is due to highly variable basic species and intermediate (hybridogenous) species. The most important sources of variation are polyploidy, hybridization and (mostly) facultative apomixis of the aposporous type. The combination of hybridization, apomixis and clonal growth leads to the maintenance of various hybrids having originated from backcrossing and hybridization among more than two species, which is possible because of the fertile pollen of apomictic hybrids. Ever since Mendel's experiments, some of $F_1$ hybrids have been found to be highly variable, probably reflecting the high heterozygosity of some of the basic species. Variable progeny can also result from unreduced gametes, or the rare parthenogenetic development of reduced gametes. While these processes were detected in experiments, their role within field populations remains unknown. However, multiple origins of intermediate species, and introgression within basic species are highly likely to result in high levels of variation. While few population level studies have been undertaken in Europe, several such studies have been carried out on adventive populations in New Zealand, and these show a different pattern. Aneuploid plants, rare in Europe, are common in New Zealand, and there is frequently more than one ploidy level within a population.

## INTRODUCTION

The subgenus *Hieracium* subgen. *Pilosella* (HILL) GRAY is comprised of two types of species. There are the so-called "basic species" ("Hauptarten" in German) and the "intermediate species" (originally "Nebenarten", later "Zwischenarten"). The intermediate species are thought to be of hybrid origin. They often connect basic species almost continuously, as has already been stressed by NÄGELI & PETER (1885: 40); many of them are apomicts. Some of the ancient intermediate species differ slightly in morphology from recent hybrids, and have different areas of distribution (even in comparison with their putative parents). For these reasons some authors distinguish them as separate entities (compare a different evaluation of what was Zahn's "*H. aurantiacum* grex *croceum*" by SCHLYAKOV 1989: 353 and SELL & WEST 1976: 374). The basic species are also extremely polymorphic (with respect to morphology, and/or ploidy level and/or the reproductive system). The origin of this variation has had very little study, and future taxonomic evaluation should consider all these underlying factors.

The extensive number of forms with fixed morphological differences occurring in this subgenus result in problems with the taxonomic evaluation and classification of individual types. Such richness of forms has attracted interest from both plant taxonomists and geneticists. Indeed, research in this group has a long history, beginning in the second half of the last century with hybridization experiments (e.g. MENDEL 1869) and followed by the experimental and systematic work of NÄGELI and PETER (PETER 1884, NÄGELI & PETER 1885). These authors started the study of constancy of individual morphological characters under uniform environmental conditions, and of their heritability from parents to offspring. Later work, combining embryological, karyological and reproductive studies (e.g. OSTENFELD 1906, 1910, ROSENBERG 1906, 1907, 1917), explained "unexpected" results of former hybridization experiments as being due to facultative apomixis and polyploidy.

Continuing research on *Hieracium* subgen. *Pilosella* revealed that the extensive variation, together with abundance of intermediate types, was due to a combination of several different phenomena: (1) the presence of both sexual and facultatively apomictic types; (2) polyploid differentiation (even at the species level), leading to polyploid complexes; (3) frequent hybridization resulting in the origin of new taxa and (4) clonal growth, allowing quick spread and survival without seed production (e.g. GADELLA 1987, 1991b, BISHOP & DAVY 1994). In addition, high phenotypic plasticity contributing to the total variation (namely in *H. pilosella* L.) has been stressed by some authors (TURESSON 1972, GADELLA 1987, 1991b, BISHOP & DAVY 1994). In spite of this complexity, much knowledge has accumulated for some of these species, especially *H. pilosella* and *H. aurantiacum* L., which are attractive for experimental studies (e.g., the series of papers by Gadella and Skalińska, cited bellow). It is important to note that the behaviour of plants may differ in nature as compared with cultivated, garden-grown experimental plants. Some of the cytotypes and hybrids arising by artificial hybridization may not arise in nature at all, or, if they do, they may not survive.

Many studies have been carried out on particular factors influencing the variation within *Hieracium* subgen. *Pilosella*. We feel that further progress in the understanding of its complicated structure should reflect the information obtained from a number of specialized, mostly experimental studies. For this purpose we reviewed the literature concerning the variation in this taxonomic group. Some of these abundant literature sources are extremely extensive and not always clearly organized. The purpose of this paper is therefore (1) to review published papers which both describe and explain variation within *Hieracium* subgen. *Pilosella*; (2) to identify those sources of variation which, as well as being detected in experiments, have also been found in the field.

Finally, another important aspect of the study of variation in *Hieracium* subgen. *Pilosella* should be emphasized here. In contrast to Europe, several species of this group, especially *H. pilosella*, have become problematic weeds in New Zealand (WEBB et al. 1988). While no description of species population structure in this subgenus has been published from Europe, several New Zealand studies have investigated different aspects of population variation within *H. pilosella*. Most of this research has been undertaken in order to understand the ecology and evolution of this species, and to implement effective biological control (e.g. MAKEPEACE 1981, JENKINS & JONG 1997, CHAPMAN et al. 2000). The molecular/genetic mechanisms controlling apomixis in this taxonomic group are also under intensive study (JEFFERSON & BICKNELL 1996, KOLTUNOW et al. 1998).

## A SURVEY OF VARIATION IN *HIERACIUM* SUBGEN. *PILOSELLA*

### Chromosome variation

#### Polyploidy

Chromosome numbers have been examined in almost 70 species; in approximately half of them (31 species) more than one ploidy level (based on x=9) was found (SCHUHWERK 1996, SCHUHWERK & LIPPERT 1997, 1998, KRAHULCOVÁ & KRAHULEC 1999). While heptaploids (7x) are reported as the highest ploidy level occurring in natural populations of the subgenus (see below), an octoploid (8x) plant in hybrid swarm between *H. pilosella* and *H. bauhini* BESSER has recently been recorded from a disturbed site in Prague, Czechia (KRAHULCOVÁ & KRAHULEC, unpubl.). The best examples of polyploid complexes are *H. pilosella* and *H. aurantiacum*, whose reproductive systems and cytogeography have undergone intensive study (e.g. GADELLA 1972, 1984, 1991a,b, SKALIŃSKA 1967, 1970, 1971b, 1973). The co-occurrence of several species with a range of ploidy levels may be common, although few studies have been undertaken to quantify this. Eight species displaying a total of four ploidy levels have been described from a locality in Saxony, Germany (BRÄUTIGAM & BRÄUTIGAM 1996). JENKINS & JONG (1997) reported three species with a total of two ploidy levels from a single site in New Zealand. Individuals of the same species, but differing in ploidy level may also grow together at one locality. CHAPMAN & LAMBIE (1999) recorded up to four ploidy levels in *H. pilosella* from a single site in New Zealand.

Differences exist between the two best examined polyploid complexes, *H. pilosella* and *H. aurantiacum*. *H. pilosella* consists of five ploidy levels in Europe, 2x, 4x, 5x, 6x and 7x (GADELLA 1984, 1991a), although tetraploids are most common (GADELLA 1984, 1991a). While triploid *H. pilosella* does not occur as a "pure" species in the field in Europe, this ploidy level is relatively common among interspecific hybrids, both spontaneous and artificial (GADELLA 1991b). The situation is different in New Zealand, however, where pentaploids are most common (MAKEPEACE 1981, JENKINS & JONG 1997), comprising about 60% of the individuals within any population studied to date (CHAPMAN & LAMBIE 1999). Tetraploids, hexaploids, and aneuploids have also been recorded (CHAPMAN & LAMBIE 1999). The fact that earlier New Zealand studies only recorded pentaploids may reflect sampling densities, or it may illustrate evolution within this species in New Zealand. Additional immigration is unlikely, as pasture grass seed (of which *H. pilosella* was a contaminant) is now produced within New Zealand.

In contrast to *H. pilosella*, diploid *H. aurantiacum* has never been found in nature in Europe, although most studies have been limited to Poland. However, in hybridization experiments, diploids were very occasionally recorded as parthenogenetic descendants of tetraploids (SKALIŃSKA 1971a). Furthermore, a diploid plant presumably of a similar origin, has been recorded among cultivated progeny of open pollinated triploid *H. aurantiacum*, collected in the field in New Zealand (BICKNELL 1997). Triploid *H. aurantiacum* is rare in nature in Europe (as distinct from *H. pilosella*), the more common ploidy levels being 4x, 5x, 6x and 7x (e.g. SKALIŃSKA 1967, 1970, SKALIŃSKA et al. 1968). As in *H. pilosella* (GADELLA 1982, 1987), ploidy levels higher than those found in nature can occur among the progeny of experimental hybridization, where counts as high as 12x have been reported in *H. aurantiacum* (SKALIŃSKA 1976).

### Aneuploidy and chromosomal rearrangements

Aneuploidy is extremely rare in European *Hieracium* L., including the subgenus *Pilosella* (e.g. BRÄUTIGAM & BRÄUTIGAM 1996); the only report referring to hyperpentaploid *H. stoloniflorum* WALDST. et KIT. (2n=46) comes from Europe, but without any details concerning the origin of plants or the locality (FINCH, ined. in MOORE 1982). In addition, two cases of aneuploid variation at the pentaploid level have recently been recorded in the Czech Republic: the first in *H. piloselliflorum* NÄGELI et PETER (2n=44) from the Sudeten Mts. (KRAHULCOVÁ & KRAHULEC 1999), the second in hybrid swarm between *H. pilosella* and *H. bauhini* (2n=48) from Prague (KRAHULCOVÁ & KRAHULEC, unpubl.).

In contrast to the European situation, aneuploidy is relatively common in field populations of *H. pilosella* in New Zealand (or at least, in clones collected from the field and grown in the greenhouse). Approximately 20% of the clones sampled by CHAPMAN & LAMBIE (1999) were aneuploid, with somatic chromosome numbers of 39, 40, 41, 42 and 44. These numbers suggest that both tetraploids and pentaploids are losing or gaining individual chromosomes, which suggests that hybridization between tetraploids (usually obligatory sexuals in Europe – see p. 324) and pentaploids is occurring in the field in New Zealand. An aneuploid *H. aurantiacum* with chromosome number between triploid and tetraploid (2n=31) was also recently recorded in New Zealand (KOLTUNOW et al. 1998).

In hybridization experiments with *H. pilosella*, aneuploids have sometimes been found in progeny of crosses where the pollen parent had an odd chromosome number (usually the pentaploid) (GADELLA 1991a). This author refers to the absence of aneuploid clones in areas of sympatry of tetra- and pentaploid cytotypes of *H. pilosella* in nature in the Netherlands. He suggests that this is probably due to aneuploids being less well-adapted than euploid hybrids. Aneuploids were also rare in hybridization experiments with facultatively apomictic *H. aurantiacum*. For example, a single aneuploid plant occurred among the $F_1$ progeny of a pentaploid seed parent crossed with a hexaploid *H. aurantiacum* (SKALIŃSKA 1971a). Rare aneuploids have also been recorded among the progeny of the fertile triploid $F_1$ hybrids between diploid *H. lactucella* WALLR. (seed parent) and tetraploid *H. aurantiacum* (CHRISTOFF 1942).

Detailed karyotype descriptions have not been made, due perhaps, to small chromosome size (ca. 2–5 µm) and polyploidy. However, a single long chromosome, different from the other chromosomes of the genome, was found in a pentaploid *H. caespitosum* DUMORT., an introduced taxon to New Zealand (JENKINS & JONG 1997). A similar "marker" chromosome was revealed in the karyotypes of *H. caespitosum* and of three closely related apomictic species, collected in the Sudeten Mts., the Czech Republic: *H. floribundum* WIMM. et GRAB., *H. iseranum* (UECHTR.) ZAHN (all 2n=36), and *H. glomeratum* FROEL. (both 2n=36 and 2n=45) (KRAHULCOVÁ & KRAHULEC 1999). Such "anomalies" might result from chromosomal rearrangements or the multiplication of repetitive DNA sequences; their impact on the genotype structure in natural populations is not possible to estimate without specialized studies.

## Reproductive systems and their relation to ploidy level

The mode of seed production has been studied in detail, especially within two taxonomic groups of *Hieracium* subgen. *Pilosella*: the section *Pilosella* (= sect. *Pilosellina* FR., including the polyploid complex of *H. pilosella* and diploid species *H. peleterianum* MÉRAT and *H. hoppeanum* SCHULT.), and the polyploid complex of *H. aurantiacum*. The method used to determine the breeding system, e.g., in *Hieracium* and *Taraxacum* F.H. WIGG., is simple and based on a comparison of seed-set (1) in open pollinated (or cross pollinated) capitula, (2) in isolated capitula without emasculation and (3) in emasculated and isolated capitula (e.g. GADELLA 1984, 1987, RICHARDS 1997); the emasculation of the whole capitulum is easy, owing to the arrangement of anthers and stigmas within the florets comprising the inflorescence. The following modes of reproduction can be deduced from the different combinations of presence/absence of well-developed achenes in capitula after the three types of pollination treatment mentioned above: amphimictic (sexual) reproduction with or without self-incompatibility, apomictic reproduction (which may be facultative) or sterility.

Three modes of reproduction and propagation are effective in *Hieracium* subgen. *Pilosella*: seed production which may be sexual (amphimictic) or apomictic, and vegetative propagation by means of stolons (clonal growth). A combination of both amphimictic and apomictic seed production in a single capitulum is relatively common (facultative apomixis, amphi-apomixis); this potential for sexual reproduction allows for hybridization between amphi-apomictic seed parents. Detailed embryological studies on this subgenus (ROSENBERG 1906, 1907) revealed apomixis of the aposporic type, characterized by the development of the embryo sac from a somatic cell of the nucellus. This was the first information about apospory in the angiosperms (POGAN & WCISŁO 1989, ASKER & JERLING 1992). Indeed, apospory is one of the significant characters distinguishing *Hieracium* subgen. *Pilosella* from the subgen. *Hieracium*, which is characterized by apomixis of the diplosporic type (ASKER & JERLING 1992, RICHARDS 1997). The results of hybridization experiments between amphimictic and apomictic accessions of *H. pilosella* with different ploidy levels (GADELLA 1987, 1991a, 1992) are in concordance with the proposed genetic regulation of apospory in the *Ranunculus auricomus* group (NOGLER 1984). According to this model, apospory is conferred by a dominant allele at a single locus, but this allele cannot be transmitted by haploid (probably functionless) gametes. BICKNELL & BORST (1996) also found evidence for dominant inheritance of apomixis in *H. pilosella*.

As far as is known, diploids of the following species of *Hieracium* subgen. *Pilosella* are amphimictic and self-incompatible: *H. lactucella* (OSTENFELD 1910, SKALIŃSKA 1967, GADELLA 1984, KRAHULCOVÁ & KRAHULEC 1999), *H. peleterianum*, *H. hoppeanum*, *H. castellanum* BOISS. et REUT. (GADELLA 1984), and also the diploid cytotypes of *H. pilosella* (GADELLA 1984), *H. onegense* (NORRL.) NORRL. (SKALIŃSKA & KUBIEŃ 1972 – under the name of *H. pratense* subsp. *silvicolum* ZAHN) and *H. echioides* LUMN. (KASHIN & CHERNISHOVA 1997).

Few studies have been made on the mode of reproduction of naturally-occurring triploids. These triploids usually occur as spontaneous hybrids and may be sterile (e.g. the triploid *H. piloselloides* Vill. subsp. *piloselloides* – GADELLA 1984), or stabilized by apomixis (the triploid *H. floribundum* – SKALIŃSKA 1967, 1968). BICKNELL (1997) reports on the production of a diploid apomictic progeny from an open-pollinated triploid apomictic *H. aurantiacum*. This progeny probably originated from the parthenogenetic development of a diploid egg cell (BICKNELL 1997, see also p. 321 and 332). Similarly, two apomictic polyhaploid plants, one dihaploid descendant of a tetraploid *H. aurantiacum*, and one trihaploid descendant of a hexaploid *H. rubrum* PETER, were recorded among the progeny of experimental crosses (KRAHULCOVÁ & KRAHULEC, unpubl.). It remains unclear, however, if such plants, markedly smaller than other seedlings in the progeny, could survive in the field. No mature diploid apomictic plants representing any species or hybrid of *Hieracium* subgen. *Pilosella* have been recorded in nature.

The relationship between ploidy and breeding system is more complicated in cytotypes with higher ploidy levels than in diploids and triploids, and appears to vary among particular polyploid complexes. In the *H. pilosella* complex, tetraploids, widespread in Europe, are mostly sexual and self-incompatible, except for rare spontaneous tetraploid hybrids with neighbouring apomicts, usually pentaploids (GADELLA 1984, 1987). In New Zealand, however, facultative apomicts, including tetraploids are the norm, and only two sexual cytotypes have been recorded (HOULISTON, unpubl.). Work is currently underway to determine levels of sexual reproduction in the field in New Zealand.

Self-incompatibility, which is known to operate in sexual species of *Hieracium* subgen. *Pilosella* (see above), can be broken under the influence of pollen from another species. This phenomenon (the mentor effect) has been recorded in diploid *H. lactucella,* and in tetraploid *H. pilosella* (KRAHULCOVÁ et al. 1999). In each of these cases autogamy was confirmed in each of the sexual species used as seed parents in crosses.

GADELLA (1984) stated that in Europe pentaploid *H. pilosella* is almost exclusively apomictic, although TURESSON & TURESSON (1960) reported a possibility of sexual reproduction. POGAN & WCISŁO (1995), in their detailed embryological studies, also recorded rare sexual reproduction in pentaploid *H. pilosella* (see p. 330). Recently, one accession of a fully sexual pentaploid *H. pilosella* has been found in Czechia (KRAHULCOVÁ & KRAHULEC, unpubl.). Hexaploid *H. pilosella* is both sexual (usually the plants growing at higher altitudes in mountains) and apomictic (predominantly the plants from lowlands), whereas heptaploids, rare in nature, are apomictic (GADELLA 1984).

In contrast to *H. pilosella,* all cytotypes known from nature which comprise the polyploid complex of *H. aurantiacum,* are facultative apomicts (SKALIŃSKA 1968, 1971a,b,c, 1973). However, the frequency of sexual reproduction is higher in cytotypes with an even number of genomes. Tetraploids and pentaploids belonging to species other than *H. pilosella* and *H. aurantiacum* are apomictic or amphi-apomictic, e.g. the tetraploid *H. caespitosum* (SKALIŃSKA 1967, SKALIŃSKA & KUBIEŃ 1972 – both under the name of *H. pratense* TAUSCH, GADELLA 1984) or the pentaploid *H. cymosum* L., *H. echioides* (KASHIN & CHERNISHOVA 1997) and *H. bauhini* (GADELLA 1984 – under the name of *H. praealtum* subsp. *bauhini* (BESSER) PETUNN.). Recently, a group of hybridogenous species from the Krkonoše Mts. (the Sudeten Mts.) was studied (including tetraploids, pentaploids and hexaploids), most of them being related to *H. lactucella, H. pilosella* and *H. caespitosum.* The species examined were predominantly apomictic, except for sexual tetraploid cytotypes of *H. apatelium* NÄGELI et PETER and *H. schultesii* F.W. SCHULTZ (KRAHULCOVÁ & KRAHULEC 1999).

## Hybridization

SCHULTZ (1848, 1856, 1858) was probably the first to carry out hybridization experiments on *Hieracium* subgen. *Pilosella.* Further experimentation was made by MENDEL (1869), continued by PETER (1884), NÄGELI & PETER (1885) and OSTENFELD (1906, 1910). Many putative hybrids have also been identified from the field, combining morphological characters of their putative parents. Most sympatric species of *Hieracium* subgen. *Pilosella* are able to hybridize spontaneously (GADELLA 1987). Hybridization involves both the sexuals and apomicts, and the different ploidy levels can be combined as parental types (e.g. SKALIŃSKA 1967, GADELLA 1987). The apomicts take part in hybridization usually as pollen donors (e.g. SKALIŃSKA 1971a, GADELLA 1982, 1987), but the hybridization of amphi-apomictic types as seed parents is also possible, e.g. in *H. aurantiacum* (SKALIŃSKA 1968, 1971a,b,c). As the pollen of apomicts is able to fertilize sexuals, the genes for apomixis can be transferred, so that both apomictic and sexual offspring may be produced by sexual plants (GADELLA 1982). This phenomenon was observed in natural populations where the sexual tetraploid and the apomictic pentaploid *H. pilosella* grow intermingled or in close proximity (GADELLA 1982), and was later confirmed in hybridization experiments (GADELLA 1987). Experimental hybridization between two facultatively apomictic species resulted in both sexual and apomictic offspring (CHAPMAN & BICKNELL 2000).

If any hybrids establish and are fertile, they can backcross with one of the parents or with another species, creating so-called "triple hybrids", or, rarely quadruple hybrids (GADELLA 1987). Apomictic hybrids can continue hybridization both with sexuals and with other apomicts, which can lead to a series of intermediate types and taxonomic complexity. Examples of species which readily hybridize spontaneously include *H. aurantiacum*, *H. caespitosum*, *H. cymosum*, *H. echioides*, *H. lactucella*, etc. (GADELLA 1987). There is a group of taxa in *Hieracium* subgen. *Pilosella* which are facultative or almost obligate apomicts and which probably originated as hybrids; many of them display odd multiples of chromosome sets (SKALIŃSKA 1967, 1971a, SKALIŃSKA & KUBIEŃ 1972, GADELLA 1987, 1992). Some examples of such stabilized species with known chromosome numbers, and which probably originated as spontaneous hybrids, are given in Tab. 1.

## Sporogenesis

Meiosis during micro- and megasporogenesis seems to be quite regular in sexual species, namely in diploid *H. lactucella* (ROSENBERG 1907, 1917, GENTCHEFF 1938, CHRISTOFF 1942) and in tetraploid sexual *H. pilosella* (POGAN & WCISŁO 1995). Regular meiosis has also been reported to occur in pollen mother cells of tetraploid facultatively apomictic *H. aurantiacum* (CHRISTOFF 1942). Actually, the genetic behaviour of such tetraploid cytotypes appears to be "diploidized", which may reflect their wide alloploid nature.

In apomicts, however, regular meiosis during pollen development need not be the rule. E.g., tetraploid plants of *H. aurantiacum* examined by ROSENBERG (1917), were characterized by incomplete pairing leading to aneuploids with functional pollen. This phenomenon was attributed to the hybrid origin of the plants under study (ROSENBERG 1917). In apomicts with an odd number of chromosome sets, microsporogenesis seems to be less disturbed than megasporogenesis. While meiosis was irregular during both male and female sporogenesis in the pentaploid apomictic *H. pilosella* (POGAN & WCISŁO 1995), pollen stainability reached 40–60%. Chromosome pairing during megasporogenesis in this cytotype of *H. pilosella* was even more irregular. The arising megaspores were mostly chromosomally unbalanced and suppressed by aposporous embryo sacs. Even the sporadic well-developed megaspores rarely produce fully-organized sexual embryo sacs, so that even persisting reduced egg cells will rarely survive long enough to be fertilized (see p. 330–331). Generally, the pollen of pentaploid species (and perhaps also of other cytotypes with odd numbers of chromosome sets) arises from almost regular meiosis in pollen mother cells, where the univalents (originated from odd chromosome sets) are probably randomly distributed to daughter nuclei. This process, leading to both euploid and aneuploid pollen grains, has been recorded in, e.g., *H. excellens* BŁOCKI (member of the *H. tauschii* ZAHN group – ROSENBERG 1917). The other documented cases of "regular" meiosis in pollen mother cells of pentaploids are probably due to a similar process, involving the arrangement of 18 bivalents and 9 univalents; subsequently, the univalents might be randomly distributed, or (some of them) eliminated (ROSENBERG 1917,

Table 1. Examples of natural hybridogenous species of *Hieracium* subgen. *Pilosella* and of their putative parents (according to SELL & WEST 1975). Only those hybridogenous species with known ploidy levels (given in parentheses) are included. * Country where the plants for karyological data of hybrids came from.

| Parental species | | Hybrid | Country * | Reference to ploidy in hybrids |
|---|---|---|---|---|
| *H. pilosella* L.<br>(2x, 4x, 5x, 6x, 7x) | *H. peleterianum* MÉRAT<br>(2x) | *H. longisquamum* PETER<br>(3x) | The Netherlands | GADELLA 1984 |
| *H. pilosella* L.<br>(2x, 4x, 5x, 6x, 7x) | *H. lactucella* WALLR. (2x) | *H. schultesii* F.W. SCHULTZ<br>(3x, 4x, 5x) | France, Corse (3x) | CONTANDRIOPOULOS 1957 |
| | | | Germany, Bavaria (3x) | SCHUHWERK & LIPPERT 1997 |
| | | | Czech Republic (4x, 5x) | KRAHULCOVÁ & KRAHULEC 1999 |
| *H. pilosella* L.<br>(2x, 4x, 5x, 6x, 7x) | *H. praealtum* VILL. ex GOCHNAT<br>(4x, 5x, 6x) | *H. brachiatum* LAM. et DC.<br>(4x, 5x, 6x) | Germany, Saxony (4x, 6x) | BRÄUTIGAM & BRÄUTIGAM 1996 |
| | | | Germany, Bavaria (4x, 5x) | SCHUHWERK & LIPPERT 1997 |
| *H. pilosella* L.<br>(2x, 4x, 5x, 6x, 7x) | *H. aurantiacum* L.<br>(3x, 4x, 5x, 6x, 7x) | *H. stoloniflorum* WALDST. et KIT.<br>(3x, 4x, 5x, 6x) | Germany, Bavaria (3x, 6x) | SCHUHWERK & LIPPERT 1997 |
| | | | Germany, Saxony (4x) | BRÄUTIGAM & BRÄUTIGAM 1996 |
| | | | Great Britain, Scotland (5x) | JENKINS & JONG 1997 |
| | | | New Zealand (6x) | JENKINS & JONG 1997 |
| | | | Czech Republic (6x) | KRAHULCOVÁ & KRAHULEC 1999 |
| *H. aurantiacum* L.<br>(3x, 4x, 5x, 6x, 7x) | *H. flagellare* WILLD.<br>(4x, 5x, 6x) | *H. rubrum* PETER (6x) | Germany, Bavaria | SCHUHWERK & LIPPERT 1997 |
| | | | Czech Republic | KRAHULCOVÁ & KRAHULEC 1999 |
| *H. aurantiacum* L.<br>(3x, 4x, 5x, 6x, 7x) | *H. lactucella* WALLR. (2x) | *H. fuscum* VILL. (4x, 5x) | Poland (4x) | SKALIŃSKA 1967 |
| | | | Germany, Bavaria (4x, 5x) | SCHUHWERK & LIPPERT 1997 |
| *H. caespitosum* DUMORT.<br>(2x, 3x, 4x, 5x) | *H. lactucella* WALLR. (2x) | *H. floribundum* WIMM. et GRAB.<br>(3x, 4x) | Poland (3x) | SKALIŃSKA 1967 |
| | | | Russia, Far East (4x) | PROBATOVA et al. 1989 |
| | | | Germany, Bavaria (4x) | SCHUHWERK & LIPPERT 1997 |
| | | | Czech Republic (4x) | KRAHULCOVÁ & KRAHULEC 1999 |

CHRISTOFF & POPOFF 1933, GENTCHEFF 1938). In this way, the pollen of pentaploids (as well as of other cytotypes with odd numbers of chromosome sets) may consist of both euploid and aneuploid grains. The supposed ability of pollen of pentaploids to fertilize was subsequently verified by successful hybridization experiments using the pentaploid *H. pilosella* as the pollen parent (GADELLA 1987, 1991a, 1992).

### Participation of unreduced gametes in hybridization proved by experiments

The ability to form unreduced gametes capable of fertilization has been demonstrated in experiments involving mostly representatives from *Hieracium* sect. *Pilosella* and *H. aurantiacum*. In these experiments, more attention has been given to the functioning of unreduced macrospores than to microspores. Within the section *Pilosella*, unreduced egg cells were sometimes produced by sexual seed parents (diploid *H. peleterianum* and tetraploid *H. pilosella*), giving rise to so-called "addition hybrids" (i.e. B-III hybrids in the sense of RUTISHAUSER 1967) resulting in offspring with increased ploidy level (GADELLA 1988). The unreduced pollen which is occasionally produced by apomicts (e.g., in pentaploid and hexaploid cytotypes of *H. pilosella*) can also lead to the formation of B-III hybrids. The total frequency of B-III hybrids (originating from either unreduced macro- or microspores) is, however, very low. In the section *Pilosella*, GADELLA (1988) found only 1.26% of hybrid offspring to be addition hybrids. Despite the low percentage of addition hybrids in experiments, he supposed their frequency to be sufficient for the production of a considerable number of such hybrids in natural populations of moderate size. In fact, addition hybrids within the polyploid complex of *H. pilosella* are very rare in nature, which may be explained by pollen competition, by the formation of precocious embryos in apomicts, or by the low adaptive value of addition hybrids with higher ploidy levels in natural populations (GADELLA 1988).

Facultatively apomictic cytotypes of *H. aurantiacum* (pentaploid, hexaploid and octoploid) were rarely able to form addition hybrids by means of fertilization of unreduced egg cells (SKALIŃSKA 1973, 1976). This mechanism was also rarely found to operate with the facultatively apomictic heptaploid *H. pilosella* as a seed parent (GADELLA 1988). However, various cytotypes of *H. aurantiacum* can occasionally form unreduced pollen grains (SKALIŃSKA 1971a), which may contribute to the formation of addition hybrids as well.

### The role of unreduced gametes in natural populations

The results of hybridization experiments, together with knowledge of natural populations with respect to chromosome numbers, allows for some speculation on microevolution processes due to hybridization involving unreduced gametes (GADELLA 1988, SKALIŃSKA 1967, 1971b, 1973). GADELLA (1988) reported an isolated example of a successful hybrid of increased ploidy (heptaploid *H. pilosella*) in the Netherlands, which probably originated from a reduced macrospore of a tetraploid and from unreduced pentaploid pollen. In this case, a large isolated heptaploid population was found to occur within the vicinity of tetraploid and pentaploid populations of *H. pilosella*, no other cytotypes being found in their neighbourhood. SKALIŃSKA (1973) assumed an analogous origin for a hexaploid hybrid between the pentaploid *H. aurantiacum* (supposed donor of an unreduced egg cell) and the diploid *H. lactucella* (donor of the reduced pollen) in a natural population in Poland. She also based her hypothesis on results of former hybridization experiments, and on particular cytotypes occurring in nature. In contrast to hybridization experiments, no natural cytotypes of *H. aurantiacum* have been found with a ploidy level higher than 7x, which may reflect poor survival of such addition hybrids under natural conditions (SKALIŃSKA 1973). The role of chromosome addition in hybrids *via* unreduced parental gametes seems therefore to be limited in nature, at least in the case of new cytotypes of *H. pilosella* and *H. aurantiacum* with very high ploidy levels.

### Resynthesis of interspecific hybrids known from nature

Many intermediate species have already been described by PETER (1884) and NÄGELI & PETER (1885) on the basis of progeny from artificial hybridization, e.g. *H. arvicola* NÄGELI et PETER, *H. calomastix* PETER, *H. polymastix* PETER, *H. melinomelas* PETER, *H. fuscoatrum* NÄGELI et PETER, *H. longisquamum* NÄGELI et PETER. GADELLA (1991b, 1992) succeeded in creating a series of interspecific hybrids, which were probably analogous to some of the assumed hybridogenous species found in natural populations. Sympatry and shared morphological characteristics between hybrids and putative parents have long been used as criteria in the classification of intermediate types in nature (e.g. PETER 1884). The important contribution of Gadella's hybridization experiments comes from both karyological examinations and breeding system studies of hybrid progeny. Three species from *Hieracium* sect. *Pilosella (H. hoppeanum, H. peleterianum* and *H. pilosella)* and one species each from the other sections *(H. lactucella* and *H. caespitosum)* were included in these hybridization experiments. It was shown that:

(1) A cross between two sexual parents (interspecific or intraspecific) gave rise to both sexual and sterile offspring, while apomictic offspring could not be obtained in this way (GADELLA 1987, 1991a, 1992). These results support the model for dominant inheritance of apospory postulated for the *Ranunculus auricomus* group (NOGLER 1984). In our opinion, spontaneous mutation of the genes responsible for apospory/sexuality are possible, although some other mechanism might regulate this genetic system. However, nothing is known about the frequency of origin of new apomictic types in nature; their origin is hard to explain by hybridization between two sexual types.

(2) Triploid hybrids were either sterile (e.g., triploid *H. schultesii* = diploid sexual *H. lactucella* × tetraploid sexual *H. pilosella*) or apomictic (e.g., triploid *H. floribundum* = diploid sexual *H. lactucella* × tetraploid apomictic *H. caespitosum* – GADELLA 1992).

(3) The most diverse reproductive systems were displayed by the tetraploid interspecific hybrids: they were sexual (e.g., tetraploid *H. schultesii* = hexaploid sexual *H. pilosella* × diploid sexual *H. lactucella* – GADELLA 1992), sterile (some of the hybrids between diploid sexual *H. hoppeanum* and sexual hexaploid *H. pilosella* – GADELLA 1991b) or apomictic (e.g., *H. duplex* PETER = tetraploid sexual *H. pilosella* × tetraploid apomictic *H. caespitosum* – GADELLA 1992).

It should be emphasized that the hybridogenous species occurring in nature may have a more complicated evolutionary history than the simple interspecific hybrids obtained in the previously mentioned experiments. Some naturally-occurring hybridogenous species are diverse in both ploidy level and reproductive system (KRAHULCOVÁ & KRAHULEC 1999). In addition, some ploidy levels cannot be explained by simple hybridization between the putative parental species, and therefore the participation of unreduced gametes and/or backcrossing should be considered (KRAHULCOVÁ & KRAHULEC 1999).

## The manifestation of different ratios of parental genomes in hybrids

Unequal representation of parental chromosome sets in hybrids usually results from hybridization between parents with different ploidy levels, and may be common in *Hieracium* subgen. *Pilosella*, as has been shown experimentally (GADELLA 1982, 1988, 1991a, SKALIŃSKA

1973, 1976). When sporogenesis is regular and both parents form one type of gamete each, the hybrid offspring are uniform with respect to chromosome number, but the proportion of parental genomes in hybrids will be unequal. This may explain why some hybridogenous species of *Hieracium* subgen. *Pilosella* (intermediate species, "Zwischenarten") are morphologically more similar to one of their putative parents, regardless of dominance and recessivity of particular characters. *Hieracium floribundum* may serve as an example. It was classified as an intermediate species between *H. caespitosum* and *H. lactucella* (ZAHN 1930), with the prevalence of morphological characters of the first species. Further investigations by SKALIŃSKA (1967) supported this, and her chromosome counts of *H. floribundum* and of its natural putative parents from Poland could be explained by an immediate hybridization product between tetraploid *H. caespitosum* and diploid *H. lactucella*. The prevalence of morphological characters of *H. caespitosum* in this triploid hybrid was explained by its genome composition (two sets of *caespitosum* + one set of *lactucella*). However, such simple hybridization cannot explain the origin of the naturally-occurring tetraploid *H. floribundum* (KRAHULCOVÁ & KRAHULEC 1999).

In those cases where parental types involved in hybridization are able to form several types of gametes, the situation may be more complicated. Here, a range of offspring can be produced from the same parental crosses. Offspring genotypes will depend on the different numbers of parental chromosomes comprising the hybrid individual. As has been shown in experiments (GADELLA 1982, 1988, 1991a, SKALIŃSKA 1973, 1976), two factors are usually responsible for chromosomal diversity in hybrid offspring in *Hieracium* subgen. *Pilosella*: (a) the ability of parental species to form both reduced and unreduced gametes capable of fertilization, and (b) the production of several types of functional male gametes (euploid or aneuploid) by pollen parents with an odd number of genomes. An example of a range of hybrid progeny being produced from crosses between the same parents is given by GADELLA (1992). He resynthetized the hybridogenous species *H. schultesii* by crossing tetraploid *H. pilosella* and diploid *H. lactucella*. Mostly triploid, but also a few pentaploid sterile hybrids of *H. schultesii* were produced. The morphological characters of the pentaploids more closely resembled the tetraploid parent *H. pilosella* than did the triploids. This can be explained by different ratios of parental genomes in these hybrids (GADELLA 1992), that is two *pilosella* genomes : one *lactucella* genome in triploids and four *pilosella* genomes : one *lactucella* genome in pentaploids (the unreduced egg cell of the tetraploid parent was assumed to take part in hybridization).

No direct evidence of the hybrid genome composition (e.g., by genome *in situ* hybridization (GISH)) has been obtained for hybrids of *Hieracium* subgen. *Pilosella*. This approach is recommended for future research to provide much needed information on the genome structure of hybrid species in this taxonomic group. The use of GISH may, however, be limited in hybrids between closely-related taxa, because of the lack of clear differentiation between parental genomes.

## Facultative apomixis

This is the combination of sexual and apomictic seed production within one plant. The term amphi-apomixis has been applied to this condition by some authors (e.g. TURESSON & TURESSON 1960, 1963). It was first noticed, and subsequently studied in detail, in the *H. aurantiacum* polyploid complex (SKALIŃSKA 1967, 1969, 1971a,b,c, 1973, 1976). In addition, facultative apomixis has been proven experimentally in several cytotypes of

*H. pilosella* (TURESSON 1972) and in tetraploid *H. caespitosum* (SKALIŃSKA & KUBIEŃ 1972). Experimental hybridization with *H. aurantiacum* showed that in amphi-apomictic cytotypes offspring could either keep the genome structure of the seed parent or have a different genotype, including a change in ploidy level. This diversity of offspring is due to the ability of the seed parent to form both reduced and unreduced egg cells, which may develop *via* parthenogenesis or may be fertilized. Therefore, four options for genome composition are possible in offspring (Fig. 1). Among progeny arising from hybridization experiments within *H. aurantiacum*, even twin embryos have been found, originating through meiosis and apomixis, respectively; they were characterized by diverse ploidy levels (SKALIŃSKA 1971c).

### Fertilization of meiotic embryo sac *versus* apospory

Embryogenesis has been studied in detail in several amphi-apomictic species, such as *H. aurantiacum* (SKALIŃSKA 1971a,b,c), tetraploid *H. caespitosum* (SKALIŃSKA & KUBIEŃ 1972), selected cytotypes of *H. pilosella* (TURESSON 1972, POGAN & WCISŁO 1995), triploid *H. piloselloides* and aneuploid *H. aurantiacum* (KOLTUNOW et al. 1998). Initially, the megaspore mother cell undergoes meiosis and a sexual embryo sac (ES) is formed. Meiosis may be more or less disturbed, depending on chromosomal conditions (balanced or unbalanced). An apomictic ES starts its development from nucellar tissue, but diverse timing of differentiation of aposporous initial cells has been observed. For example, in facultatively apomictic *H. pilosella*, the aposporous initials appear at the stage of fully-developed sexual ESs, and then they suppress and destroy them (TURESSON 1972). This process, running almost synchronously in all florets, is usually complete before the capitula open (TURESSON 1972). Aposporous initials have never been observed in triploid *H. piloselloides* before the meiotic tetrads have degenerated (KOLTUNOW et al. 1998). In contrast, aposporous initials arising in an asynchronous way, i.e. independently from the developmental stage of sexual ESs, have been detected in pentaploid *H. pilosella* (POGAN & WCISŁO 1995). It remains unclear if the differentiation of aposporous initial cells is the direct cause of degradation of the sexual ES (KOLTUNOW et al. 1998), as has been assumed by TURESSON (1972).

Occasionally, persisting sexual ESs capable of fertilization can occur in amphi-apomictic cytotypes with an even number of chromosome sets, which are therefore able to form viable megaspores *via* regular meiosis (SKALIŃSKA 1971a,b,c, 1973, TURESSON 1972). In cytotypes with odd chromosome numbers, e.g., pentaploids, the meiotic ESs rarely survive because of chromosomal imbalance (SKALIŃSKA 1971a,b,c, TURESSON 1972). The exception to this rule was found in pentaploid *H. pilosella*, where fully organized meiotic ESs were sometimes observed in older capitula just before their opening (POGAN & WCISŁO 1995). This suggests that the odd-ploid cytotypes need not be obligatory apomicts and that they might be able (in very rare cases) to act as seed parents in a sexual cross. The pollen of pentaploids is, however, usually capable of fertilization, so that such plants can take part in hybridization as pollen parents as well (e.g. GADELLA 1987). Similarly, aneuploids (e.g., aneuploid *H. aurantiacum*) may produce viable pollen, and can sometimes even serve as seed parents in a sexual cross (KOLTUNOW et al. 1998).

TURESSON (1972) suggested that in amphi-apomictic *H. pilosella*, the ratio of sexual to apomictic embryos may be determined by environmental conditions, changing the timing of development between sexual (meiotic) and aposporous (unreduced) ESs within an ovule. If the plants flower quickly (e.g., under conditions of high temperature, low humidity and intense insolation in early summer), sexual egg cells may be fertilized. In this case, the apomictic

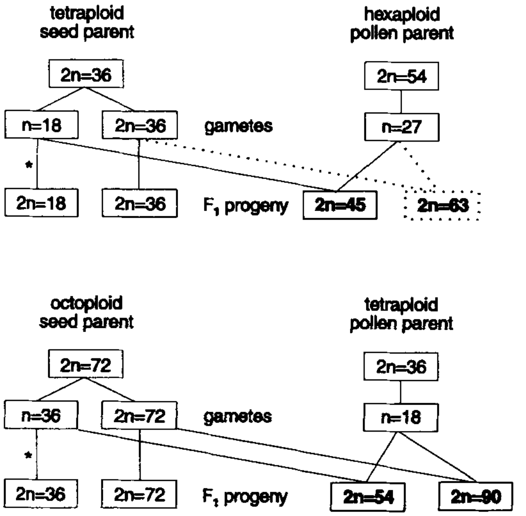

Fig. 1. Diversification in progeny originated from experimental hybridization within the polyploid complex of *H. aurantiacum* (according to SKALIŃSKA 1971a,b, 1973, 1976, adapted). While the tetraploid *H. aurantiacum* occurs commonly in nature, the octoploid has never been found in the field (it originated as a product of experimental hybridization only). Actually, the tetraploid seed parent gave rise to three cytotypes in progeny only, the fourth one is hypothetical (dotted line). The hybrids are given in bold typeface, the haploid parthenogenesis is marked by an asterisk (for detailed explanation see p. 330 and 331–332).

ESs do not succeed in suppressing the sexual ones because of lack of time (TURESSON 1972). KNOX (1967) noted changes in the incidence of apomictic to sexual ESs in *Dichanthium aristatum* (POIR.) C.E. HUBB. (*Poaceae*) associated with photoperiods prevailing during the development of inflorescences.

The regulation of sexuality and the establishment of sexually-derived progeny of amphi-apomictic types is difficult to study directly in the field. Field studies using *H. aurantiacum* as a pollen parent and *H. pilosella* as a seed parent are underway in New Zealand (HOULISTON et al., unpubl. data). The use of molecular markers for apomixis (transposons carrying dominant marker sequences and ensuring positive or negative selection of offspring cultivated on special media) might provide new possibilities for the study of this phenomenon (JEFFERSON & BICKNELL 1996).

### Fertilization of unreduced embryo sacs and haploid parthenogenesis

The former phenomenon has been discussed on p. 327, but with major emphasis on sexual (amphimictic) types able to form unreduced female gametes capable of fertilization. In amphi-apomictic types fertilization of unreduced female gametes may also be considered a manifestation of their facultative sexuality. This phenomenon has been recorded in e.g. pentaploid, hexaploid and octoploid *H. aurantiacum* (SKALIŃSKA 1971a,b, 1973, 1976 – Fig. 1), in heptaploid *H. pilosella* (GADELLA 1988) and in tetraploid *H. caespitosum* (CHAPMAN & BICKNELL 2000). Many meiotic ESs produced by pentaploids, however, degenerate early due to aneuploidy (SKALIŃSKA 1973, 1976). SKALIŃSKA (1971a,b) has also shown in hybridization experiments with *H. aurantiacum* that even the apomictic (unreduced) ESs of such cytotypes are rarely fertilized. In some aposporous embryo sacs the first mitotic division of the egg cell may be delayed relative to normal development of the endosperm (SKALIŃSKA 1969, 1971b). If this delay lasts until anthesis, the undivided egg cell may be fertilized (SKALIŃSKA 1969, 1971b). Progeny arising from the fertilization of unreduced ESs (so-called addition hybrids), have higher ploidy than their seed parents, due to chromosome addition from the male gametes to unreduced ESs. Furthermore, choice of pollen donor may influence the degree of fertilization of unreduced egg cells, as has been found in several apomicts representing other genera than *Hieracium* (ASKER & JERLING 1992).

Haploid parthenogenesis has been found to operate in very rare instances in tetraploid and octoploid facultative apomicts of *H. aurantiacum* (SKALIŃSKA 1971a, 1976). A polyhaploid (diploid and tetraploid respectively) was recorded among the offspring of both cytotypes used as seed parents. These had to have arisen *via* parthenogenetic development of a reduced egg cell (Fig. 1). In addition, several other seedlings in the progeny with defective development perished early, and it was assumed that they arose in a similar way (SKALIŃSKA 1971a). An experimentally-chromosome-doubled decaploid *H. hoppeanum* also gave rise to polyhaploid progeny (ASKER & JERLING 1992). BICKNELL (1997) recorded a diploid apomictic plant under micropropagation, the descendant of a triploid apomictic *H. aurantiacum* collected in New Zealand. This diploid, markedly smaller than other progeny of the triploid seed-parent, probably originated as a dihaploid from a reduced egg cell developing parthenogenetically (BICKNELL 1997). The possibility of survival of such polyhaploid plants in the field under competition of surrounding vegetation remains unclear (see also p. 323).

## Variation at the level of the gene

### Variable $F_1$ hybrid progeny due to heterozygosity of parents

A conspicuous morphological heterogeneity of $F_1$ progeny has been obtained from hybridization experiments between *H. lactucella* as seed parent and *H. aurantiacum* as pollen parent (MENDEL 1869, OSTENFELD 1910, ROSENBERG 1917, CHRISTOFF 1942). This type of hybridization has led to the origin of newly-stabilized types, and may well take place in nature as well (OSTENFELD 1910). The variation among $F_1$ hybrids was almost continuous with respect to e.g. flower colour, number and size of capitula, shape and hairiness of leaves and type of clonal growth. Parental characteristics occurred together in various combinations. Some of the $F_1$ hybrids were fertile apomicts, giving rise to uniform mother-like $F_2$ progeny. Other $F_1$ hybrids produced offspring with chromosome numbers different from them. These $F_1$ plants must have been sexual or amphi-apomictic (ROSENBERG 1917, CHRISTOFF 1942). A karyological examination confirmed that most of the $F_1$ plants were true triploid hybrids ($2n=27$) between diploid and tetraploid parental species (ROSENBERG 1917, CHRISTOFF 1942). During meiosis in pollen mother cells, 9 bivalents and 9 univalents were formed. The rest of the $F_1$ hybrids also had 9 bivalents and a variable number of univalents during meiosis, indicating the variable number of chromosomes of the *H. aurantiacum* genome (ROSENBERG 1917). Thus, the morphological variation among the $F_1$ progeny might to some extent, be attributed to irregular meiosis in the pollen parent, occasionally producing aneuploid gametes (p. 325). More often, however, it may be due to the presumed heterozygous state of the apomictic *H. aurantiacum* used in the experiments (ROSENBERG 1917). CHRISTOFF (1942) suggests that mutations in the genome of this taxon probably resulted in increased heterozygosity.

### Variation detected by ISSR (Inter sequence simple repeats)

A study of genetic variation within and among populations of *H. pilosella* by means of ISSR (ZIETKIEWICZ et al. 1994) has recently been undertaken in New Zealand. DNA fingerprinting demonstrated high levels of clonal diversity; variation within populations was almost as great as that across populations (CHAPMAN et al. 2000). This pattern of variation was most simply explained by sexual reproduction.

## Clonal growth and the influence of environmental factors on morphology and reproduction

The ability for vegetative spread by stolons is necessary for the maintenance and reproduction of sterile and semisterile amphimictic hybrids, which otherwise could not survive. However, it is also an important factor influencing the competitive ability of all other fertile types, both amphimictic and apomictic (TURESSON & TURESSON 1960, GADELLA 1987, 1991a, BISHOP & DAVY 1994). In addition, clonal growth together with apomixis facilitates the maintenance and spread of local genotypes, contributing to total genetic diversity.

Phenotypic plasticity, applicable to both sexual and apomictic types, was mentioned in the section *Pilosella*, especially in the polyploid complex of *H. pilosella* (e.g. TURESSON 1972, GADELLA 1987, 1991b, BISHOP & DAVY 1994). The most plastic morphological characters are rosette size and the number and length of stolons, which determine the type of clonal growth. Some local genetic variants, able to reproduce by vegetative means, are considered as ecologically different forms (ecotypes) of *H. pilosella* (TURESSON 1972, BISHOP & DAVY 1994). These plastic traits and their stability after transplantation were studied in transplantation experiments, a method already tested in the early experimental studies on *Hieracium* subgen. *Pilosella* by NÄGELI & PETER (1885).

More experimental work is needed in the area investigating the manifestation of sexuality versus apomixis in plants under different environmental conditions. The views published in the literature admit the different extent to which environmental factors may influence the manifestation of sexuality in facultative apomicts (ASKER & JERLING 1992). Most probably the mode of reproduction is regulated genetically, but it is also influenced by seasonal changes of temperature and light regimes (ASKER & JERLING 1992). Preliminary experimental studies on this topic are currently underway by HOULISTON et al. (unpubl.). The hypothesis concerning higher expression of sexual reproduction in amphi-apomictic *H. pilosella* caused by extreme environmental conditions during the flowering time (TURESSON 1972) has not been confirmed experimentally. The details concerning this hypothesis and new methods which might be useful in such studies have already been discussed briefly above (pp. 330–331).

## DISCUSSION AND CONCLUSIONS

The results of hybridization experiments performed by MENDEL (1869) on *Hieracium* subgen. *Pilosella* led to his doubting the universal validity of his Laws. As far as we know, these experiments involved both the diploid sexual species (*H. lactucella*) and polyploid species, sexual or facultatively apomictic (*H. pilosella*, *H. echioides*, *H. caespitosum*, *H. aurantiacum*, *H. praealtum* VILL. ex GOCHNAT). However Mendel knew nothing about polyploidy and apomixis. We now know that the occurrence of progeny strongly resembling seed parents can be explained by apomixis. Other possible explanations of "unexpected" phenomenon in Mendel's crosses, i.e. the heterogeneity of $F_1$ progeny include: (1) a combination of sexual and apomictic reproduction of seed parent (amphi-apomixis), (2) the different viable combinations of reduced and unreduced parental gametes, leading to different ratios of parental genomes in hybrids, (3) aneuploidy of $F_1$ progeny or (4) a heterozygous parental composition. The latter refers specifically to *H. aurantiacum* as a pollen parent in hybridization experiments repeated later (OSTENFELD 1910, CHRISTOFF 1942). All these phenomena have been discussed.

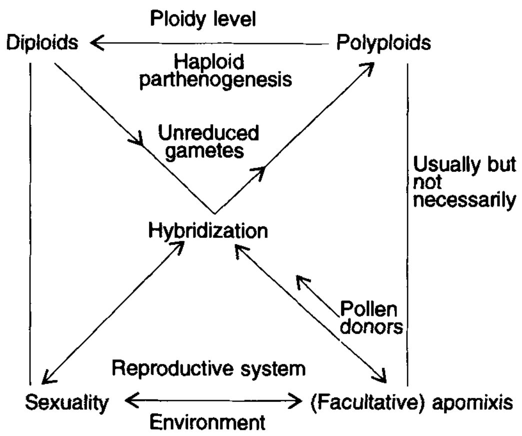

Fig. 2. Background of variation in *Hieracium* subgen. *Pilosella* (represented schematically).

Many field populations are heterogeneous, that is, individuals of different ploidy and/or reproductive systems occur together at the same locality. Several hypotheses concerning microevolutionary processes in such populations have been proposed, based mostly on data obtained from experiments (Skalińska 1967, 1976, Turesson 1972, Gadella 1987):

(1) The polyploid differentiation in *H. pilosella* and *H. aurantiacum* through chromosome addition in intraspecific hybrids has already been mentioned (pp. 327–328). New cytotypes with higher ploidy levels exceeding the parental types may be produced in this way in a step-wise fashion, but probably up to a limited ploidy level, lower than those reached in experiments (Skalińska 1976). *Arnica alpina* (L.) Olin et Ladau and *Hierochloë odorata* (L.) P. Beauv. are examples of other karyologically polymorphic apomictic species in which a similar method of differentiation in natural populations can be assumed (Skalińska 1976).

(2) Gene transfer from apomictic pollen donors is probably responsible for genome enrichment in sexuals or facultative apomicts (Gadella 1987). In experimental hybridization between tetraploid sexual and pentaploid apomictic *H. pilosella*, the transfer of both sexuality and apospory to tetraploid and pentaploid offspring occurred. In nature however, no sexual pentaploids (assumed to be homozygous recessives) were found (except for a single record mentioned on p. 324), but amphi-apomictic tetraploids did occur in the neighbourhood of pentaploid apomicts (Gadella 1987). This natural hybridization resulted in reduced germination rates of achenes of these amphi-apomictic hybrid tetraploids. On the basis of these experiments, and from observations in nature, a unidirectional gene-flow from apomicts to sexuals is assumed to occur in natural populations (Gadella 1987). We do not know anything, however, about the viability and competitive ability of other possible products of hybridization between apomictic and sexual types in nature.

(3) Hybridization (involving both sexual and apomictic types) seems to be the most important factor in speciation within *Hieracium* subgen. *Pilosella* (e.g. Ostenfeld 1910, Skalińska 1967, Turesson 1972). The products of spontaneous hybridization usually undergo backcrossing followed by both segregation of particular characters, and gene mutations (Skalińska 1967). It is assumed that mutation is important in the speciation process. For example, mutation might be responsible for new forms of almost totally apomictic pentaploids in *H. pilosella*, which are of uniform phenotype within one locality, but morphologically different between localities (Turesson 1972). A recurrent origin of these apomictic lines seems to be a more probable explanation in this case. As evidenced in our current hybridization experiments (Krahulcová & Krahulec, unpubl.), plants of putative hybrid origin are similar in morphology to those originating from artificial hybridization. However, their chromosome number and/or reproductive system is often different, suggesting a more complicated history.

Introgressive hybridization between different species of *Hieracium* subgen. *Pilosella* has not been studied. However, indirect evidence suggests that its presence is highly probable. Intermediate species (even those with apomictic reproduction) have fertile pollen. Recurrent backcrossing produces plants more and more similar to one of the parents (basic species). In this way certain genes can enrich the gene pool of basic species; they may be manifest as characters of "alien" origin, blurring the species borders. Some early work has already stressed the absence of sharp borders for particular basic species (e.g., NÄGELI & PETER 1885). ZAHN (1930) used the symbol "»" to indicate the small influence of the other species on the basic one, or described it in words. His remarks were based on intimate knowledge of individual species. The following examples illustrate this phenomenon: "*H. pratense* 1. *sudetorum* (*pratense* » *aurantiacum*)"; "*H. obscurum* 2. *ciliosum* (*H. florentinum* » *floribundum*)" or "*H. pilosella* 93. *pernigrescens* (*flagellare* ähnlich [similar to *flagellare*])" (ZAHN 1930). The exact role of introgressive hybridization should be studied in greater detail; in our opinion it is a process strongly influencing the observed patterns of variation.

Experimentation has played a major role in our understanding of the complicated patterns of variation within *Hieracium* subgen. *Pilosella*. Polyploidy, hybridization and facultative apomixis are most important (Fig. 2.). Hybrids probably arise repeatedly not only from simple interspecific crossing, but also from backcrossing and/or crossing among more than two species. The various hybrids produced may survive and reproduce due to a combination of hybridization and apomixis. Moreover, the fertile pollen of apomictic hybrids enables crossing between sexual and apomictic types. Another possibility for the production of variable progeny is the formation of unreduced gametes, or a rare parthenogenetic development of reduced female gametes. High variation among some of $F_1$ interspecific hybrids has repeatedly been detected in experiments. It has been explained by high levels of heterozygosity in some of the basic species. For the present, the role of such processes leading to diversity in progeny remains unknown in field populations. Polyploids with odd chromosome numbers produce gametes with aneuploid numbers.

Hybridization, leading to both new ploidy levels and aneuploids means that careful attention needs to be given to chromosome counts. Hybrids, common under field conditions, may comprise a proportion of polyhaploids and/or addition hybrids with high chromosome numbers. While many of these do not survive as adult plants, they may be detected during analysis of germinating seeds. We propose to investigate this difference using both germinating seeds and established plants.

A comparison of cytogeographic studies among European and New Zealand populations has exposed several differences. The most obvious difference is the common occurrence of aneuploid plants in New Zealand as compared with Europe. Further comparisons will have to wait until more detailed population-level studies have been undertaken in Europe.

The taxonomic complexity of *Hieracium* subgen. *Pilosella* is explained by both basic species and by intermediate species, many of which have been proven to be of hybrid origin. Both are extremely variable. The basic species are likely influenced by introgressive hybridization. The variation of intermediate species is more eclectic: they may arise by backcrossing, by repeated hybridization events and/or by fusion of reduced and unreduced gametes. Individual morphotypes are preserved by apomixis and/or clonal growth.

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## REFERENCES

ASKER S. E. & JERLING L. (1992): *Apomixis in plants*. CRC Press, Boca Raton.

BICKNELL R. A. (1997): Isolation of diploid, apomictic plant of *Hieracium aurantiacum*. *Sexual Pl. Reprod.* 10: 168–172.

BICKNELL R. A. & BORST N. K. (1996): Isolation of reduced genotypes of *Hieracium pilosella* using anther culture. *Pl. Cell Tissue Organ Cult.* 45: 37–41.

BISHOP G. F. & DAVY A. J. (1994): Biological flora of the British Isles. *Hieracium pilosella* L. (*Pilosella officinarum* F. SCHULTZ & SCHULTZ-BIP.). *J. Ecol.* 82: 195–210.

BRÄUTIGAM S. & BRÄUTIGAM E. (1996): Determination of the ploidy level in the genus *Hieracium* subgenus *Pilosella* (HILL) S.F. GRAY by flow cytometric DNA analysis. *Folia Geobot. Phytotax.* 31: 315–321.

CHAPMAN H. M. & BICKNELL R. (2000): Recovery of a sexual and an apomictic hybrid from crosses between the facultative apomicts *Hieracium caespitosum* and *H. praealtum*. *New Zealand J. Ecol.* 24: 81–86.

CHAPMAN H. M. & LAMBIE S. (1999): [Reports]. In: STACE C.A. (ed.), IOPB chromosome data 15, *Int. Organ. Pl. Biosyst. Newslett.* 31: 12.

CHAPMAN H. M., PARH D. & ORAGUZIE N. (2000): Genetic structure and colonising success of a clonal weedy species, *Pilosella officinarum* (*Asteraceae*). *Heredity* 84: 401–409.

CHRISTOFF M. (1942): Die genetische Grundlage der apomiktischen Fortpflanzung bei *Hieracium aurantiacum* L. *Z. Indukt. Abstammungs-Vererbungsl.* 80: 103–125.

CHRISTOFF M. & POPOFF A. (1933): Zytologische Studien ber Gattung *Hieracium*. *Planta* 20: 440–447.

CONTANDRIOPOULOS J. (1957): Nouvelle contribution a l'ètude caryologique des endémiques de la Corse. *Bull. Soc. Bot. France* 104: 533–538.

GADELLA T.W.J. (1972): Biosystematic studies in *Hieracium pilosella* L. and some related species of the subgenus *Pilosella*. *Bot. Not.* 125: 361–369.

GADELLA T.W.J. (1982): Cytology and reproduction of *Hieracium pilosella* L. and some related diploid species. *Acta Bot. Neerl.* 31: 140–141.

GADELLA T.W.J. (1984): Cytology and the mode of reproduction of some taxa of *Hieracium* subgenus *Pilosella*. *Proc. Kon. Ned. Acad. Wetensch. C* 87: 387–399.

GADELLA T.W.J. (1987): Sexual tetraploid and apomictic pentaploid populations of *Hieracium pilosella* (*Compositae*). *Pl. Syst. Evol.* 157: 219–246.

GADELLA T.W.J. (1988): Some notes on the origin of polyploidy in *Hieracium pilosella* aggr. *Acta Bot. Neerl.* 37: 515–522.

GADELLA T.W.J. (1991a): Variation, hybridization and reproductive biology of *Hieracium pilosella* L. *Proc. Kon. Ned. Acad. Wetensch.* 94: 455–488.

GADELLA T.W.J. (1991b): Reproduction, variation and interspecific hybridization in three species of *Hieracium* section *Pilosellina* (*Compositae*). *Polish Bot. Stud.* 2: 85–103.

GADELLA T.W.J. (1992): Notes of some triple and inter-sectional hybrids in *Hieracium* L. subgenus *Pilosella* (HILL) S.F. GRAY. *Proc. Kon. Ned. Akad. Wetensch.* 95: 51–63.

GENTCHEFF G. (1938): Zytologische und embryologische Studien ber einige *Hieracium*-Arten. *Planta* 27: 165–195.

JEFFERSON R.A. & BICKNELL R. (1996): The potential impacts of apomixis: a molecular genetics approach. In: SOBRAL B.W.S. (ed.), *The impact of plant molecular genetics*, Birkhäuser, Boston, pp. 87–101.

JENKINS T.A. & JONG K. (1997): Significance of polyploid variation in New Zealand *Pilosella* and *Hieracium* (*Asteraceae*). *Bot. J. Scotland* 49: 75–87.

KASHIN A.S. & CHERNISHOVA M.P. (1997): Chastota apomiksisa v populyatsiyakh nekotorykh vidov *Taraxacum* i *Hieracium* (*Asteraceae*) (The frequency of apomixis in populations of some *Taraxacum* and *Hieracium* species (*Asteraceae*)). *Bot. Zhurn.* 82: 14–24.

KNOX R.B. (1967): Apomixis: seasonal and population differences in a grass. *Science* 157: 325–326.

KOLTUNOW A.M., JOHNSON S.D. & BICKNELL R.A. (1998): Sexual and apomictic development in *Hieracium*. *Sexual Pl. Reprod.* 11: 213–230.

KRAHULCOVÁ A., CHRTEK J. & KRAHULEC F. (1999): Autogamy in *Hieracium* subgen. *Pilosella*. *Folia Geobot.* 34: 373–376.

KRAHULCOVÁ A. & KRAHULEC F. (1999): Chromosome numbers and reproductive systems in selected representatives of *Hieracium* subgen. *Pilosella* in the Krkonoše Mts (the Sudeten Mts). *Preslia* 71: 217–234.

MAKEPEACE W. (1981): Polymorphism and the chromosomal number of *H. pilosella* L. in New Zealand. *New Zealand J. Bot.* 19: 255–257.

MENDEL G. (1869): Ueber einige aus künstliche Befruchtung gewonnenen *Hieracium*-Bastarde. *Verh. Naturf. Vereins Brünn* 8: 26–31.

MOORE D.M. (1982): *Flora europaea. Check-list and chromosome index.* Cambridge University Press, Cambridge.

NÄGELI C. VON & PETER A. (1885): *Die Hieracien Mittel-Europas. Monographische Bearbeitung der Piloselloiden mit besonderer Berücksichtigung der mitteleuropäischen Sippen.* München.

NOGLER G.A. (1984): Gametophytic apomixis. In: JOHRI B. M. (ed.), *Embryology of angiosperms,* Springer, Berlin & Heidelberg, pp. 475–518.

OSTENFELD C.H. (1906): Castration and hybridization experiments with some species of *Hieracia. Bot. Tidskr.* 27: 225–248.

OSTENFELD C.H. (1910): Further studies on the apogamy and hybridization of the *Hieracia. Z. Indukt. Abstammungs-Vererbungsl.* 3: 241–285.

PETER A. (1884): Über spontane und künstliche Gartenbastarde der Gattung *Hieracium* sect. *Piloselloidea. Bot. Jahrb.* 5: 203–286.

POGAN E. & WCISŁO H. (1989): Cytological investigations on *Hieracium pilosella* L. from Poland. I. Karyological studies. *Acta Biol. Cracov., Ser. Bot.* 31: 19–28.

POGAN E. & WCISŁO H. (1995): Embryological analysis of *Hieracium pilosella* L. from Poland. *Acta Biol. Cracov., Ser. Bot.* 37: 53-61.

PROBATOVA N.S., SOKOLOVSKAYA A.P. & RUDYKA E.G. (1989): Chisla khromosom nekotorykh vidov sosudistykh rastenii o-va Kunashir, Kuril'skie ostrova (Chromosome numbers in some species of vascular plants from Kunashir Island (The Kuril islands)). *Bot. Zhurn.* 74: 1675–1678.

RICHARDS A. J. (1997): *Plant breeding systems.* Ed. 2. Chapman & Hall, London, Weinheim, etc.

ROSENBERG O. (1906): Über die Embryobildung in der Gattung *Hieracium. Ber. Deutsch. Bot. Ges.* 24: 157–161.

ROSENBERG O. (1907): Cytological studies on the apogamy in *Hieracium. Bot. Tidskr.* 28: 143–170.

ROSENBERG O. (1917): Die Reduktionsteilung und ihre Degeneration in *Hieracium. Svensk Bot. Tidskr.* 11: 145–206.

RUTISHAUSER A. (1967): Fortpflanzungsmodus und Meiose apomiktischer Blütenpflanzen. In: ALFERT M. et al. (eds.), *Protoplasmatologia* VI/F/3, Springer, Vienna & New York, pp. 1–245.

SCHUHWERK F. (1996): Published chromosome counts in *Hieracium.* http://www.botanik.biologie.uni-muenchen.de/botsamml/projects/chrzlit.html.

SCHUHWERK F. & LIPPERT W. (1997): Chromosomenzahlen von *Hieracium* (*Compositae, Lactuceae*) Teil 1. *Sendtnera* 4: 181–206.

SCHUHWERK F. & LIPPERT W. (1998): Chromosomenzahlen von *Hieracium* (*Compositae, Lactuceae*) Teil 2. *Sendtnera* 5: 269–286.

SCHULTZ F. (1848): Postscriptum et observations sur d'autres hybrides et sur la manière de comprendre le genre et l' espèce. *Arch. Fl. France Allemagne* 1: 150–153.

SCHULTZ F. (1856): Plantes hybrides. *Arch. Fl. France Allemagne* 2: 254–256.

SCHULTZ F. (1858): Herbarium normale. Herbier des plantes nouvelles, peu connues et rares d'Europe, principalement de France et d'Allemagne. *Arch. Fl. France Allemagne* 2: 258–288.

SCHLYAKOV R. N. (1989): Rod 33. Yastrabinochka – *Pilosella* HILL. In: TSVELEV N.N. (ed.), *Flora evropeiskoi chasti SSSR 8*, Nauka, Leningrad, pp. 300–377.

SELL P.D. & WEST C. (1975): 558P. *Pilosella* HILL. In: STACE C.A. (ed.), *Hybridization and the flora of the British Isles*, Academic Press, London, New York etc., pp. 434–439.

SELL P.D. & WEST C. (1976): *Hieracium.* In: TUTIN T.G. et al. (eds.), *Flora europaea 4,* Cambridge University Press, Cambridge, London etc., pp. 358–410.

SKALIŃSKA M. (1967): Cytological analysis of some *Hieracium* species, subgenus *Pilosella,* from mountains of southern Poland. *Acta Biol. Cracov., Ser. Bot.* 10: 127–141.

SKALIŃSKA M. (1968): Studies in twin plants of *Hieracium. Acta Biol. Cracov., Ser. Bot.* 11: 179–186.

SKALIŃSKA M. (1969): Apomixis in *Hieracium aurantiacum* L. *Genet. Polon.* 10: 91–93.

SKALIŃSKA M. (1970): Further cytological studies in natural populations of *Hieracium aurantiacum* L. *Acta Biol. Cracov., Ser. Bot.* 13: 111–117.

SKALIŃSKA M. (1971a): Experimental and embryological studies in *Hieracium aurantiacum* L. *Acta Biol. Cracov., Ser. Bot.* 14: 139–152.

SKALIŃSKA M. (1971b): Further studies in apomixis of *Hieracium aurantiacum* L. *Genet. Polon.* 12: 209–212.

SKALIŃSKA M. (1971c): Further studies in twins of *Hieracium aurantiacum* L. *Acta Biol. Cracov., Ser. Bot.* 14: 43–53.

SKALIŃSKA M. (1973): Further studies in facultative apomixis of *Hieracium aurantiacum* L. *Acta Biol. Cracov., Ser. Bot.* 16: 121–133..

SKALIŃSKA M. (1976): Cytological diversity in the progeny of octoploid facultative apomicts of *Hieracium aurantiacum. Acta Biol. Cracov., Ser. Bot.* 19: 39–46.

SKALIŃSKA M. & KUBIEŃ E. (1972): Cytological and embryological studies in *Hieracium pratense* TAUSCH. *Acta Biol. Cracov., Ser. Bot.* 15: 39–50.

SKALIŃSKA M., POGAN E., JANKUN A. et al. (1968): Further studies in chromosome numbers of Polish angiosperms. Seventh contribution. *Acta Biol. Cracov., Ser. Bot.* 11: 199–224.

TURESSON B. (1972): Experimental studies in *Hieracium pilosella* L. II. Taxonomy and differentiation. *Bot. Not.* 125: 223–240.

TURESSON G. & TURESSON B. (1960): Experimental studies in *Hieracium pilosella* L. I. Reproduction, chromosome number and distribution. *Hereditas* 46: 717–736.

TURESSON G. & TURESSON B. (1963): Observations on chromosome number and reproduction in some *Piloselloids. Bot. Not.* 116: 157–160.

WEBB C.J., SYKES W.R. & GARNOCK-JONES P.J. (1988): *Flora of New Zealand 4. Naturalized pteridophytes, gymnosperms, dicotyledons.* Botany Division, D.S.I.R., Christchurch.

ZAHN K.H. (1930): *Hieracium.* In: ASCHERSON P. & GRAEBNER P. (eds.), *Synopsis der Mitteleuropäischen Flora XII/1,* Borntraeger, Leipzig, pp. 1–492.

ZIETKIEWICZ E., RAFALSKI A. & LABUDA D. (1994): Genome fingerprinting by simple sequence repeats (SSR) – Anchored polymerase chain reaction amplification. *Genomics* 20: 176–183.

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