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MATING INTERACTIONS BETWEEN COEXISTING DIPLOID, TRIPLOID AND TETRAPLOID CYTOTYPES OF *HIERACIUM ECHIOIDES* (*ASTERACEAE*)

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Abstract: Experimental crosses between diploids, triploids and tetraploids of *Hieracium echioides* were made to examine mating interactions. Specifically, cytotype diversity in progeny from experimental crosses, intercytotype pollen competition as a reproductive barrier between diploids and tetraploids, and differences in seed set between intra- and intercytotype crosses were studied. Only diploids were found in progeny from 2x × 2x crosses. The other types of crosses yielded more than one cytotype in progeny, but one cytotype predominated in each cross type: diploids (92%) in 2x × 3x crosses, tetraploids (88%) in 3x × 2x crosses, triploids (96%) in 2x × 4x crosses, triploids (90%) in 4x × 2x crosses, tetraploids (60%) in 3x × 3x crosses, pentaploids (56%) in 3x ×4x crosses, triploids (80%) in 4x × 3x crosses and tetraploids (88%) in 4x × 4x crosses. No aneuploids have been detected among karyologically analyzed plants. Unreduced egg cell production was detected in triploids and tetraploids, but formation of unreduced pollen was recorded only in two cases in triploids. Triploid plants produced x, 2x and 3x gametes: in male gametes x (92%) gametes predominated whereas in female gametes 3x (88%) gametes predominated.

Cytotype diversity in progeny from crosses where diploids and tetraploids were pollinated by mixture of pollen from diploid and tetraploid plants suggested intercytotype pollen competition to serve as a prezygotic reproductive barrier. No statistically significant difference in seed set obtained from intra- and intercytotype crosses between diploids and tetraploids was observed, suggesting the absence of postzygotic reproductive barriers among cytotypes.

Keywords: *Hieracium* subgen. *Pilosella*, Pollen competition, Reproductive barriers, Unreduced gametes

INTRODUCTION

Polyploidy is a widespread phenomenon among angiosperm plant species, with estimates of the proportion of polyploid angiosperm taxa ranging from 30% to 70% (STEBBINS 1950, AVERETT 1980, MASTERSON 1994). Two main types of polyploidy are recognized: (i) autopolyploidy, which results from duplication of a single genome (e.g. within a population of a given species), and (ii) allopolyploidy, which results from the combination of two or more differentiated genomes (typically as the product of interspecific hybridization; DE WET 1980, RAMSEY & SCHEMSKE 1998). In this study, the term polyploidy will be used in the sense of autopolyploidy.

Polyploidization is considered as an important evolutionary force. However, processes and mechanisms responsible for the origin of polyploidy are still not entirely understood. The same holds for the factors influencing the establishment and coexistence of polyploid cytotypes in diploid populations (THOMPSON & LUMARET 1992, RAMSEY & SCHEMSKE 1998, PETIT et al. 1999).

The most common mechanism of polyploid origin is believed to be through production of unreduced gametes (also referred to as 2n gametes), i.e., gametes with a somatic chromosome number (HARLAN & DE WET 1975, THOMPSON & LUMARET 1992, BRETAGNOLLE & THOMPSON 1995, RAMSEY & SCHEMSKE 1998). There are two main models explaining the pathways of polyploid formation in diploid populations (i) frequency-dependent minority cytotype exclusion (LEVIN 1975, VAN DIJK & BAKX-SCHOTMAN 1997, HUSBAND 2000) and (ii) the triploid bridge hypothesis (RAMSEY & SCHEMSKE 1998, BURTON & HUSBAND 2001, HUSBAND 2004).

Experimental studies suggest that triploid progeny derived from reciprocal crosses between diploids and tetraploids have low fitness (low seed set and germination rates) or are even lethal (e.g. LUMARET & BARRIENTOS 1990, JAY et al. 1991, BRETAGNOLLE & THOMPSON 1996, BURTON & HUSBAND 2000, HARDY et al. 2001). The postzygotic reproductive isolation caused by triploid lethality was described as a triploid block (MARKS 1966, THOMPSON & LUMARET 1992, PETIT et al. 1999). According to the model of frequency-dependent minority cytotype exclusion, the rarer polyploid (tetraploid) cytotype, which receives the majority of the pollen from diploids, produces only a few tetraploid and viable triploid seeds and consequently it will be eliminated from a population (LEVIN 1975, VAN DIJK & BAKX-SCHOTMAN 1997, HUSBAND 2000). To prevent minority cytotype exclusion, prezygotic reproductive barriers are supposed to be an important requirement for establishment and coexistence of polyploid cytotypes in diploid populations (THOMPSON & LUMARET 1992).

On the contrary, according to the triploid bridge hypothesis, triploids are expected to be an important element in the polyploidization process. As the fusion of two unreduced gametes during the initial stage of polyploid formation seems to be an unlikely event, it is more likely that tetraploids arise in two steps, via a triploid intermediary, through a process called triploid bridge (RAMSEY & SCHEMSKE 1998, BURTON & HUSBAND 2001, HUSBAND 2004). Because the triploid block is usually not complete, triploids are first formed via the union of reduced and unreduced gametes. Subsequently, backcrosses of triploids to diploids or crosses between triploids can generate tetraploids. Further crosses between tetraploids and triploids may increase the number of unreduced gametes within a population and so enhance the probability of tetraploid establishment in a diploid population (YAHARA 1990, RAMSEY & SCHEMSKE 1998, HUSBAND 2004).

Sympatric occurrence of conspecific diploids and polyploids is a suitable situation for the study of evolutionary processes and dynamics in polyploids. The perennial species *Hieracium echioides* LUMN. appears to be such a case. It belongs to *Hieracium* subgen. *Pilosella* (HILL) GRAY, which is well-known for its huge variation in morphology, ploidy level (seven ploidy levels have been found in natural populations) and breeding system (both sexuality and agamospermy of aposporous type) (KRAHULCOVÁ et al. 2000).

Hieracium echioides is distributed in Central and eastern Europe, and in steppe regions of Asia. Westwards it reaches Germany, the Czech Republic and eastern Austria (BRÄUTIGAM 1992). Four cytotypes have been found in nature in Central Europe, diploids ($2n = 2x = 18$), triploids (2n = 3x = 27), tetraploids (2n = 4x = 36) and pentaploids (2n = 5x = 45) (SCHUHWERK & LIPPERT 1997, PECKERT 2001, ROTREKLOVÁ et al. 2002, 2005). Co-occurrence of several cytotypes has been detected at some localities in the Czech Republic and in north-eastern Austria. In contrast, only diploids have been recently found in north-eastern Germany and Poland. Both diploids and tetraploids were reported from Slovakia and Hungary (MÁJOVSKÝ et al. 1970, 2000, ROTREKLOVÁ et al. 2002, 2005, PECKERT et al. 2005). However, tetraploid plants from southern Slovakia and northern Hungary studied by PECKERT et al. (2005) slighly but consistently differed morphologically from those from other parts of the distribution range. Tetraploid counts published by MÁJOVSKÝ et al. (1970, 2000) refer most probably to the same type, whose taxonomic position is not satisfactorily resolved; exclusion of these populations from *H. echioides* in future might be expected. Pentaploids have been recently found at the locality of Havranické vřesoviště heathland in south-western Moravia (Czech Republic), together with the other cytotypes (ROTREKLOVÁ et al. 2005). Polyploid cytotypes have nearly always been recorded in sympatry with diploids; an exclusively polyploid population has not been found in *H. echioides*, except for tetraploids from southern Slovakia and northern Hungary (PECKERT et al. 2005).

In Central Europe, all cytotypes proved to be sexual and self-incompatible (PECKERT 2001, ROTREKLOVÁ et al. 2002, 2005). Self-incompatibility in *Hieracium* subgen. *Pilosella* may be disturbed by the presence of foreign pollen grains on the stigma (so called “mentor effect”) (KRAHULCOVÁ et al. 1999), however, the impact of this phenomenon on reproductive strategy in *H. echioides* was not tested. Agamospermy has only been detected in morphologically slightly different tetraploids from southern Slovakia and northern Hungary (see above). However, KASHIN & CHERNISHOVA (1997) detected cytoembryologically the occurrence of embryo sacs with apomictic characters in pentaploid and even in diploid plants of *H. echioides* from the Saratov region in Russia. Except of the aforementioned populations from southern Slovakia and northern Hungary, *H. echioides* seems to be morphologically rather invariable in Central Europe; no differences between cytotypes were observed (PECKERT, unpubl.).

In this study we focused on interactions among sympatric cytotypes of *H. echioides*. We addressed the following questions: (i) what is the cytotype diversity in progeny from experimental crosses between 2x, 3x and 4x plants?, (ii) is there intercytotype pollen competition as a reproductive barrier in crosses between diploids and tetraploids?, and (iii) are there any differences in seed set between intra- and inter-cytotype crosses?

MATERIAL AND METHODS

Plant material

All examined plants came from the heathland Havranické vřesoviště (Czech Republic, distr. Znojmo, 0.5–1 km W to SW of the village of Havraníky, 300 m a.s.l., 48°48´ N, 15°59´ E). Sympatric occurrence of three cytotypes (diploids, triploids and tetraploids) has been previously confirmed there (ROTREKLOVÁ et al. 2002). About 60 plants from this locality were collected (in 2001 and 2002) and cultivated in pots under field conditions in the Experimental Garden of the Institute of Botany, Průhonice. Either ploidy level was detected using flow cytometry analysis or chromosome number was counted. Among the 63 cultivated plants, 17 plants were found to be diploid, 28 triploid, 14 tetraploid and 4 pentaploid (these pentaploids are referred to in ROTREKLOVÁ et al. 2005).

Voucher specimens are deposited in herbarium PRC (Charles University, Praha).

Flow cytometry

Ploidy level of both collected plants and offspring from crosses was estimated by a Partec PA II flow cytometer (Partec GmbH., Germany). A two-step procedure of sample preparation was used (OTTO 1990). A piece of fresh leaf of *H. echioides* was chopped up together with the internal standard (*Zea mays* L. cv. CE-777, 2C = 5.43 pg; LYSÁK & DOLEŽEL 1998) with a sharp razor blade in a plastic Petri dish with 1 ml ice-cold Otto I buffer (0.1 M citric acid, 0.5% Tween 20). The suspension was filtered through a 42 µm nylon mesh and centrifuged at 150 g for 5 min. The supernatant was removed and the nuclei were resuspended in 100 µl of fresh Otto I buffer. After incubation for 10 min at room temperature, the staining solution containing 1 ml Otto II buffer (0.4 M $Na_2HPO_4 . 12 H_2O$), fluorochrome DAPI (4 µg/ml) and ß-mercaptoethanol (2 µg/ml) was added.

Mixed samples were used to analyze the ploidy level of offspring from experimental crosses. The samples where more than one ploidy level was detected were reanalyzed to obtain accurate data. For each sample, usually 5000 nuclei were analyzed. The coefficient of variance (CV) of obtained peaks varied from 1.41 to 4.69. The cytometer was adjusted so that the fluorescence of G1 nuclei of diploid plants was localized on channel 200 (Fig. 1).

Karyology

Selected triploid parental plants and selected offspring from experimental crosses were karyologically analyzed to detect aneuploidy. Root tips, which were collected in the morning, were pre-treated with a saturated solution of p-dichlorbenzene for 3 hours at room temperature, fixed in cold acetic acid-ethanol (1 : 3) overnight, and stored in 70% ethanol at 4 °C. Root tips were macerated in a mixture of ethanol and hydrochloric acid (1 : 1) for 2 min at room temperature, and cut meristems were squashed in a drop of lacto-propionic orcein (DYER 1963). The karyological analysis was aimed at progeny resulting from crosses involving triploids. The following numbers of plants were analyzed: 11 parental triploid plants, three progeny plants from crosses 2x × 3x, eight from 3x × 2x, seven from 3x × 3x, three from 3x × 4x, six from 4x × 3x and four from 4x × 4x crosses.

Crossing experiments

Two kinds of crosses were included in the crossing design: (i) inter- and intra-cytotype crosses between 2x, 3x and 4x plants from the Havranické vřesoviště heathland, i.e., 2x × 2x, 2x × 3x, 2x × 4x, 3x × 2x, 3x × 3x, 3x × 4x, 4x × 2x , 4x × 3x and 4x × 4x, (ii) to detect pollen competition and preferences, 2x and 4x mother plants (plants from the Havranické vřesoviště heathland) were pollinated by a mixture of pollen from both diploid and tetraploid plants.

Crossing experiments were conducted in 2002–2004. Randomly selected plants were transferred to an unheated glasshouse and inflorescences of each plant were isolated in nylon

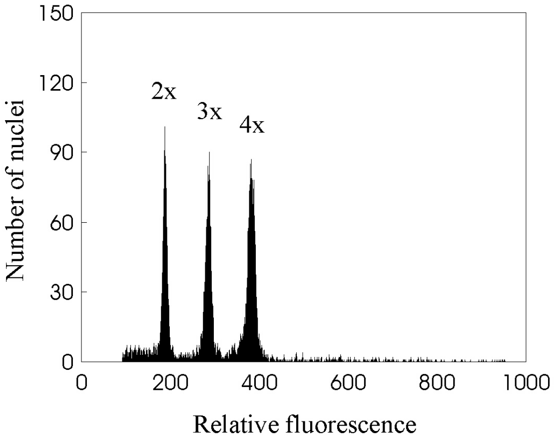

Fig. 1. Flow cytometric profile of mixed sample of diploid, triploid and tetraploid plants of *Hieracium echioides*.

bags before anthesis to prevent pollination. Because *H. echioides* was proved to be self-incompatible (PECKERT 2001, ROTREKLOVÁ et al. 2002) it was not necessary to emasculate flowers (heads) prior to pollination. In total, 16 diploid, 18 triploid and 16 tetraploid plants were used in crosses. Three capitula per maternal plant were pollinated by rubbing the whole parental capitula together. The pollination was repeated two to three times on different days, on each occasion using a new freshly opened head of the pollen plant. Pollinated heads were marked and ripe achenes were harvested approximately two weeks after pollination.

Analysis of crossing experiments

The number of full achenes was counted in each harvested capitula and was expressed as a proportion of the total number of flowers in a capitulum (seed set). The full achenes were sown next spring in the soil in the glasshouse. Only achenes obtained from crosses in summer 2004 were sown immediately in that autumn. Plants older than one month were analyzed for ploidy level using flow cytometry.

The data obtained were statistically analyzed using NCSS 2001-PASS 2000 statistical package. Variation in ploidy level of offspring from reciprocal crosses was compared using χ^2 tests. Variation in seed set between intracytotype (2x × 2x and 4x × 4x) and intercytotype (2x × 4x and 4x × 2x) crosses was compared using two-sample *t*-test.

RESULTS

Distribution of cytotypes among progeny from crosses between diploids, triploids and tetraploids

All conducted crosses produced progeny, whose ploidy levels are summarized in Table 1 (the first nine rows). Distribution of ploidy levels among progeny differed significantly depending on reciprocal crosses in all cases (χ^2 tests: $P < 0.001$). Diploid, triploid and

Table 1. Distribution of progeny cytotypes in crosses between different cytotypes of *Hieracium echioides.* The maternal parent is given as the first in the cross type. n – number of crosses per cross type. Frequencies of progeny cytotypes within a cross type are given in round parentheses. The number of karyologically analyzed plants in progeny is given in square brackets following the total number of plants. The two last rows show the cytotype composition of progeny in crosses where diploid and tetraploid plants were pollinated by a mixture of pollen from diploid and tetraploid plants.

		Offspring ploidy				
Cross type	n	2x	3x	4x	5x	6x
2x × 2x	5	347 (1.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
2x × 3x	4	23 (0.92) [3]	2 (0.08)	0 (0.00)	0 (0.00)	0 (0.00)
2x × 4x	3	5 (0.04)	117 (0.96)	0 (0.00)	0 (0.00)	0 (0.00)
3x × 2x	4	6 (0.09) [2]	2 (0.03)	56 (0.88) [6]	0 (0.00)	0 (0.00)
3x × 3x	9	2 (0.07)	0 (0.00)	16 (0.60) [5]	7 (0.26) [2]	2 (0.07)
3x × 4x	3	0 (0.00)	20 (0.34) [1]	6 (0.10)	33 (0.56) [2]	0 (0.00)
4x × 2x	3	0 (0.00)	97 (0.90)	2 (0.02)	9 (0.08)	0 (0.00)
4x × 3x	3	0 (0.00)	33 (0.80) [5]	6 (0.15) [1]	0 (0.00)	2 (0.05)
4x × 4x	5	0 (0.00)	0 (0.00)	221 (0.88) [2]	0 (0.00)	29 (0.12) [2]
2x × 2x/4x	2	3 (0.06)	51 (0.94)	0 (0.00)	0 (0.00)	0 (0.00)
4x × 2x/4x	3	0 (0.00)	26 (0.39)	31 (0.47)	4 (0.06)	5 (0.08)

tetraploid progeny predominated. In addition, a small number of pentaploids and hexaploids was also detected. Only diploids were produced in 2x × 2x crosses. The reciprocal crosses between diploids and tetraploids produced mainly triploid progeny (96% in 2x × 4x and 90% in 4x × 2x crosses). The 2x × 3x crosses resulted mainly in diploid progeny (92%). On the contrary, the 3x × 2x crosses resulted mainly in tetraploid progeny (88%). The 3x × 3x crosses resulted predominantly in tetraploid progeny (60%). The 3x × 4x crosses produced mainly pentaploids (56%), but an important proportion formed also triploids (34%). The 4x × 3x crosses produced mainly triploids (80%). Finally, the 4x × 4x crosses resulted mostly in tetraploid progeny (88%).

Triploid plants produced x, 2x and 3x gametes; x gametes (92%) predominated in male gametes whereas 3x gametes (88%) predominated in female gametes (percentage calculated on the basis of results of reciprocal crosses between diploids and triploids).

Formation of unreduced female gametes was observed in tetraploids too. Progeny, which was formed by the union of reduced and unreduced gametes, occurred in the crosses where tetraploids were pollinated by pollen from diploids. In progeny originating from crosses between tetraploid plants, hexaploids that arose from the union of reduced and unreduced gametes formed 12%. On the contrary, no unreduced gametes were recorded in the crosses between diploids and tetraploids where tetraploids served as the pollen donor.

No aneuploids were detected among 42 karyologically analyzed plants.

Pollen competition experiments

Results are presented in Table 1 (two last rows). Crosses with 2x maternal plants pollinated by pollen from both 2x and 4x plants yielded predominantly triploids (94%). In contrast, 4x maternal plants pollinated by pollen from both 2x and 4x plants yielded triploids (39%) and tetraploids (47%). Pentaploids and hexaploids (e.g. progeny originated via the union of unreduced pollen from tetraploids) comprised the remaining 14%.

Seed set in crossing experiments

The seed set obtained in particular cross types is shown in Table 2. The highest seed set was observed in 2x × 2x crosses (0.84 ± 0.05), the lowest in 3x × 3x crosses (0.07 ± 0.02). Seed set from intracytotype crosses (2x × 2x, 4x × 4x) and seed set from intercytotype crosses (2x × 4x, 4x × 2x) did not differ significantly (*t*-test; $t = 1.5747$, $P = 0.1377$).

DISCUSSION

Polyploidy in *Hieracium echioides*

This study brings the first detailed information about polyploidy in *H. echioides*. The experimental reciprocal crosses between diploids, triploids and tetraploids proved cytotypes to be able to hybridize with each other. Some conclusions about interactions among cytotypes and population dynamics can be made from the cytotype diversity in progeny from different cross types.

Only diploids were detected in progeny from 2x × 2x crosses in *H. echioides*. Similarly, only diploids were found in progeny originating from crosses between diploid plants of *H. echioides* from different diploid populations from Central Europe (PECKERT, unpubl.). The presumption that diploids either do not produce unreduced gametes or do so only with very low frequency was used as a basic assumption to reveal the origin of progeny from crosses using diploids as one of the parental plants.

Formation of unreduced egg cells was discovered in triploids and tetraploids. In triploids, production of triploid and in two cases also diploid egg cells was detected. Similarly in tetraploids, production of tetraploid and in two cases also triploid egg cells was detected. On the contrary, formation of unreduced pollen was recorded only in two cases (two different plants) in triploids (diploid pollen was detected based on 2x × 3x crosses). All pentaploid and hexaploid plants recorded among progeny from experimental crosses must have originated via participation of unreduced gametes. Whereas pentaploids have been found in nature, hexaploids have been obtained in experimental crosses only. The ability to form unreduced egg cells was demonstrated in hybridization experiments in different species of *Hieracium* subgen. *Pilosella*, including diploid species (GADELLA 1988, SKALIŃSKA 1973, 1976, KRAHULCOVÁ & KRAHULEC 2000, KRAHULCOVÁ et al. 2004). The formation of unreduced pollen seems to be less common than formation of unreduced egg cells in *Hieracium* subgen. *Pilosella* but was recorded in facultatively apomictic plants of *Hieracium* subgen. *Pilosella* (GADELLA 1988, SKALIŃSKA 1971, 1973, 1976). Occurrence of unreduced gametes has been reported in many groups of plants, including diploids (reviewed in HARLAN & DE WET 1975, RAMSEY & SCHEMSKE 1998). Our results need not fully reflect natural circumstances. However, we are aware that the production of unreduced gametes can be affected by environmental factors (reviewed in RAMSEY & SCHEMSKE 1998). Our experimental hybridizations were made in glasshouse, hence in conditions wholly different from natural ones. In addition, variation in unreduced gamete production at the individual plant level is

Table 2. Mean ± s.e. number of full achenes and seed set (proportion of full achenes in a capitulum) for crosses between different cytotypes of *Hieracium echioides*. The maternal parent is given as the first in the cross type. n – number of crosses per cross type.

Cross type	n	No. of full achenes	Seed set
2x × 2x	5	47.23 ± 4.50	0.84 ± 0.05
2x × 3x	4	14.25 ± 1.98	0.32 ± 0.09
3x × 2x	4	14.38 ± 3.11	0.36 ± 0.07
3x × 3x	9	2.65 ± 0.76	0.07 ± 0.02
3x × 4x	3	23.67 ± 4.67	0.45 ± 0.09
4x × 3x	3	9.25 ± 2.75	0.22 ± 0.06
4x × 4x	5	26.93 ± 4.92	0.54 ± 0.08
2x × 4x	3	29.89 ± 3.07	0.58 ± 0.11
4x × 2x	3	25.94 ± 4.30	0.49 ± 0.09
2x × 2x/4x	2	39.34 ± 0.67	0.78 ± 0.01
4x × 2x/4x	3	21.78 ± 4.81	0.47 ± 0.10

known as well; certain individuals have the ability to produce unreduced gametes, whilst others do not (BRETAGNOLLE & THOMPSON 1995). Differences in production of unreduced gametes at an individual level have been observed, e.g. in *Hieracium rubrum* PETER (KRAHULCOVÁ et al. 2004).

Interestingly, five diploid plants (progeny of different individuals) were obtained from 2x × 4x crosses. There are two possible explanations of this phenomenon: (i) self-fertilization, or (ii) production of haploid pollen by tetraploids. Although *H. echioides* was proved to be self-incompatible (PECKERT 2001), presence of pollen grains of another individual on the stigma may induce compatibility with its own pollen (so called “mentor effect”) (e.g. RICHARDS 1997). The occurrence of this phenomenon has been detected in *Hieracium* subgen. *Pilosella* (KRAHULCOVÁ et al. 1999). Production of haploid gametes in tetraploids was recorded in hybridization experiments in *Chamerion angustifolium* (L.) HOLUB (BURTON & HUSBAND 2001). However, this was not detected in 4x × 4x crosses in *H. echioides*.

Some progeny obtained in experimental crosses and the majority of triploid parents of *H. echioides* were analyzed karyologically to detect aneuploidy. In nature, aneuploidy is extremely rare in *Hieracium* subgen. *Pilosella* in Europe (BRÄUTIGAM & BRÄUTIGAM 1996, KRAHULCOVÁ et al. 2000). Aneuploids, however, were recorded in hybridization experiments, particularly in progeny from crosses where plants with an odd chromosome number served as the pollen donor (GADELLA 1987, 1991, KRAHULCOVÁ & KRAHULEC 2000). Therefore, the karyological analyses were aimed at progeny originating from crosses with participation of triploid parents. However, no aneuploids have been detected in such plants of *H. echioides*. Nevertheless, it does not mean that aneuploids are lacking in *H. echioides*. Aneuploids may be rare. Furthermore, aneuploids may have died before analysis (relatively significant mortality in seedlings was noticed in the first month of their life in *H. echioides*). Occurrence of double-peak on FCM-histogram in a few cases (unfortunately these plants died before karyological re-analyzation) suggests that a detailed karyological analysis would probably find aneuploids in *H. echioides*.

Triploids in experimental crosses and wild populations

The results of experimental hybridizations between diploids, triploids and tetraploids suggest that triploids are able to participate in matings with other cytotypes of *H. echioides*. Triploids are produced in these matings as well. Particularly in crosses between diploids and tetraploids, almost only triploids are formed. In addition, analysis of progeny obtained from crosses where diploid and tetraploid plants were pollinated by a mixture of pollen from diploid and tetraploid plants suggests that intercytotype pollen competition affects the formation of progeny. In crosses where diploids served as the maternal plant, diploid pollen from tetraploids was much more successful in fertilization than haploid pollen from diploids. As a consequence of this phenomenon, predominantly triploids were produced in such crosses with a mixed pollen source. In crosses where tetraploids served as the maternal plant, tetraploid pollen donors were also more successful than diploid ones, but the difference in success of pollen was not so strong as in diploid maternal plants. Greater success of pollen from tetraploids (i.e., diploid pollen) when in mixtures with pollen from diploids (haploid pollen) has also been observed in *Chamerion angustifolium* (HUSBAND et al. 2002). This phenomenon is accounted by increased pollen-tube growth rates or increased sheltering of deleterious mutations in diploid pollen (reviewed in HUSBAND et al. 2002). Unilateral pollen priority results in greater isolation of tetraploids than diploids. This fact may have an important impact on population dynamics of polyploid taxa. However, the effect of pollen competition on the formation and establishment of polyploidy has been overlooked in theoretical models (e.g. FOWLER & LEVIN 1984, FELBER 1991, RODRÍGUEZ 1996).

Triploids were not only produced in experimental crosses but are also abundantly represented in natural populations of coexisting cytotypes of *H. echioides* (PECKERT & CHRTEK, unpubl.). They are supposed to be an important element in polyploidy in *H. echioides*, despite their lower fecundity compared with diploids and tetraploids. As female gametes of triploids participating in crosses proved to be mostly triploid, triploids may have contributed to the formation of polyploids and support the stable coexistence of diploids and tetraploids in *H. echioides*, according to the triploid bridge hypothesis. Specifically, the 3x × 2x crosses produced predominatly tetraploid progeny (88%). Similarly, the reciprocal crosses between triploids and tetraploids produced exclusively polyploid progeny, but progeny with odd chromosome numbers, i.e., triploids and pentaploids, predominated.

Furthermore, the comparison of seed set production between 2x × 2x, 4x × 4x and 2x × 4x, 4x × 2x crosses showed that the seed set in intracytotype crosses did not statistically differ from that in intercytotype crosses. This fact suggests the absence of postzygotic reproductive isolating barriers such as the triploid block in *H. echioides*. In *Hieracium* subgen. *Pilosella*, most species are able to hybridize when they occur sympatrically (GADELLA 1987). Hybridization is also possible between different ploidy levels of the same species. Experimental studies in *Hieracium pilosella* L. showed that interspecific hybridizations resulting in progeny with odd chromosome numbers may also occur. Such plants possess lower fertility or may be sterile or apomictic (GADELLA 1987, 1991).

Reproductive barriers

Our results from the examination of polyploidy in *H. echioides* are not entirely consistent with results from other studies on polyploid taxa. Specifically, a certain impact of triploid block was observed in most examined cases of sympatric occurrence of diploids and polyploids (THOMPSON & LUMARET 1992). The discrepancy may be due to weak (or even no) reproductive barriers in *Hieracium* subgen. *Pilosella* even at the interspecific level. Another explanation might rely on a different evolutionary history of mixed populations of two or more ploidy levels in *H. echioides* from that in most examined polyploid taxa. It is supposed that most of the hitherto examined populations of sympatric occurrence of diploid and polyploid cytotypes, if not all, resulted from secondary contact between cytotypes (PETIT et al. 1999). The distribution of cytotypes is mostly parapatric and only a few intercytotype hybrids are normally recorded (e.g. SOLTIS 1984, VAN DIJK & BAKX-SCHOTMAN 1997, HUSBAND & SCHEMSKE 1998, SEGRAVES et al. 1999, HARDY et al. 2000). The near absence of exclusively tetraploid populations in *H. echioides* suggests a primary origin of tetraploids in diploid populations. To confirm this hypothesis, it would be neccessary to ascertain the degree of genetic differentiation between diploids and tetraploids of *H. echioides*.

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