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Some notes on the origin of polyploidy in *Hieracium pilosella* aggr.

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SUMMARY

A cytological analysis of 1192 artificially produced hybrids in the collective apo-amphimictic species complex, *Hieracium pilosella/H. peleterianum*, showed that 16 addition hybrids were formed; 15 of these were produced by the fusion of one reduced and one unreduced gamete and one by the fusion of two unreduced gametes. Zygotic chromosome doubling could be unambiguously ruled out. Polyspermaty and somatic doubling in meristematic tissue of the creeping stolons did not occur. The percentage (1·26%) of unreduced gametes is low but sufficient for the production of considerable numbers of addition hybrids, even in moderately large populations. The fact that addition hybrids are rare under natural conditions suggests that the adaptive value of these hybrids is inferior.

Key-words: Hieracium pilosella aggr., polyploidy, unreduced gametes.

INTRODUCTION

It is generally recognized that a thorough knowledge of the origins of polyploidy is essential for understanding the evolution of plant species. The process of chromosome doubling is responsible for the production of many plant species. Stebbins (1971) estimated that 30–35% of all angiosperms are polyploids, whereas Grant (1981) came to the conclusion that 47% of all angiosperms are of polyploid origin. Goldblatt (1980) and Lewis (1980) estimated that 58% of monocotyledones and 43% of dicotyledones are polyploids. For ferns an even higher percentage is given (up to 95%).

Harlan and DeWet (1975) and DeWet (1980) discussed the mode(s) of origin of polyploidy. Usually polyploidization is described as 'hybridization, followed by chromosome doubling'. The classic explanation is that a sterile diploid hybrid becomes an allotetraploid as a result of chromosome doubling. However, the mechanisms by which chromosome sets are added are less well understood. Chromosome doubling may imply a somatic event, or polyploids may arise through the fusion of unreduced gametes. The somatic event may occur by zygotic chromosome doubling or by meristematic chromosome doubling. The latter phenomenon may also give rise to a polyploid chimaera. Zygotic chromosome doubling, first proposed by Winge (1917), seems to be very rare and is almost impossible to distinguish unambiguously from the fusion of gametes, one or both of which are unreduced.

It was proved in a convincing way that *Primula kewensis* Watson derived from spontaneous somatic chromosome doubling (Newton & Pellew 1929).

Certain meiotic disturbances may occasionally occur and, as a result, some of the gametes are unreduced. Such gametes can unite with normal reduced gametes (or with other unreduced gametes) to give rise to individuals with an increased ploidy level. Harlan & DeWet (1975) concluded that almost all polyploids arise from unreduced gametes. Other mechanisms such as polyspermaty (Hagerup 1947) or somatic events do occur but seem to be of minor importance.

Since many hybridization studies do not involve large-scale cytological screening of F1 hybrids in a quantitative way, exact data on the incidence of unreduced gametes among angiosperms are scarce. Therefore, the author decided to summarize the results of hybridization studies on some taxa of the *Hieracium pilosella* aggr., which is an apo-amphimictic species complex. A total of 1192 F1 hybrids was studied cytologically, together with their parents. In addition, the frequency of somatic doubling was studied by comparing cloned rooted plantlets derived from stolons of various cytotypes with the original stock.

MATERIALS AND METHODS

The parental plants were collected in their natural habitats. As a rule three to five plants from each population (or clone) were dug up and transferred to the experimental garden. The plants, belonging to the diploid species *Hieracium peleterianum* Mérat ($2n = 18$) and to the cytologically variable species *Hieracium pilosella* L. ($2n = 36, 45, 54, 63$), originated from various European populations. The diploids were collected in The Netherlands (Isle of Terschelling), Denmark (Jylland) and Italy (Aosta). Plants belonging to *H. pilosella* with the chromosome number $2n = 36$ were mainly collected in The Netherlands, Luxembourg, France and Spain, with $2n = 45$ (pentaploids) in The Netherlands and Ireland, hexaploids ($2n = 54$) in the Swiss and Austrian Alps and heptaploids ($2n = 63$) on the Dutch Isle of Vlieland.

The plants were grown in pots. In order to prevent contamination of the cultures new vegetatively formed rosettes were usually re-potted for use in the following season. The chromosome numbers were subsequently checked. The screening for unreduced egg cells and pollen grains was carried out by counting the chromosome number of the parents and of the resulting hybrids. The mode of reproduction of the parents was also studied using the method described by Gadella (1987).

RESULTS

Chromosome numbers of cloned individuals

Stolons (325) were cut from rosettes and the chromosome number of each of the cloned plants was compared with that of the original biotype. All 325 cloned plants had the same chromosome number as the plant from which they were derived. This means that meristematic chromosome doubling could not be demonstrated in this sample.

The occurrence of unreduced gametes

A total of 268 capitula was pollinated. They produced 30 187 achenes, 11 028 of which turned out to be viable. Of the F1 plants, 1192 were investigated cytologically.

Three groups of F1 plants could be distinguished.

(a) Plants that have the same chromosome number as the mother plant and show exactly the same morphological character combination as the mother plant (strictly maternal). This is found irrespective of whether both parents are homoploid or heteroploid.

(b) Plants that have intermediate morphological characters and intermediate chromosome number if the parents were characterized by different chromosome numbers (heteroploid).

(c) Plants that have intermediate morphological characters and with chromosome numbers not exactly intermediate between the parents. One or both parents produced unreduced gametes, which were subsequently involved in the production of the F1 plants. Such hybrids are addition hybrids (B-III hybrids sensu Rutishauser 1967) or U-hybrids (sensu Asker 1977).

Fifteen plants turned out to belong to the third category. Table 1 lists these addition hybrids and shows the way in which both the parents and addition hybrids reproduce.

The female plants were all sexual with the exception of two apomictic heptaploid ($2n=63$) plants of *H. pilosella*. Two crosses were interspecific and in these cases both parents were sexual. In all other crosses the male parent was apomictic (i.e. the plant producing these pollen grains reproduced agamospermously), which proves that apomictic pollen donors are able to fertilize sexual plants and particularly other apomictic plants. In four types of crosses the female parent produced some unreduced gametes, in four other types some pollen grains were unreduced. Apomictic progeny cannot be obtained from a cross between two sexually reproducing parents. Heteroploid crosses usually yield euploid progeny. Heteroploid crosses involving sexual tetraploids as female parents and apomictic pentaploids as male parents, however, yielded both aneuploid (7·2%) and euploid progeny (92·8%). The euploid F1 plants resulting from these crosses turned out to be tetraploids or pentaploids. The aneuploids had the chromosome numbers $2n=38$ (3·8%), $2n=39$ (26·9%), $2n=40$ (30·8%), $2n=41$ (11·6%), $2n=42$ (23·1%) and $2n=44$ (3·8%). Most of the crossing experiments were carried out between tetraploids (♀) and pentaploids (♂). The crosses summarized in Table 1 yielded only some addition hybrids, most of the resulting progeny had a chromosome number intermediate between the parents. From the cross 36♀ × 18♂, 46 sterile triploid ($2n=27$) F1 plants were produced. The cross 18♀ × 54♂ yielded 16 sterile tetraploid plants. From the cross 36♀ × 54♂, 29 apomictic pentaploid F1 plants were obtained. The offspring of the cross 36♀ × 63♂ turned out to be pentaploid and apomictic (77 plants). Pollination of heptaploid ($2n=63$) capitula always resulted in purely maternal heptaploid offspring (25 plants).

Table 2 gives a survey of the number of pollinated capitula, the number of (viable) achenes and the number of F1 plants investigated cytologically. Only diploid, tetraploid and heptaploid plants produced some addition hybrids. The diploids and tetraploids reproduce sexually, the heptaploids usually agamospermously. Most of the progeny of the pentaploid and hexaploid plants turned out to be completely maternal in their external appearance. Moreover, they had the same chromosome number as the pollinated plant. This indicates that these plants reproduce agamospermously. Many achenes in both apomictic and sexual capitula are empty and do not contain a viable embryo. Even under conditions of open pollination, in the presence of many insects and many sexual pollen donors, not a single capitulum was formed with 100% fully developed viable achenes.

Table 2 shows, however, that nine pentaploid plants formed reduced egg cells that were subsequently fertilized. Such plants were never found in natural populations but resulted from the cross $2n=36$♀ (sexual) × $2n=54$♂ (sexual), or the reciprocal cross. Hexaploid plants from natural populations are either sexual or apomictic. The production of unreduced egg cells could not be demonstrated in these pentaploid hybrids. From Table 2 it can be seen that five unreduced egg cells (=0·56%) were fertilized, whereas 886

Table 1. A list of the addition hybrids produced by fertilization of unreduced gametes in intraspecific and interspecific artificial crosses of the genus *Hieracium*, subgenus *Pilosella*. Parents producing unreduced gametes are indicated by an asterisk. Only addition hybrids are listed; the other hybrids are described in the text

						F1	
2*n* P♀	Species P♀	Mode of reproduction P♀	2*n* P♂	Species P♂	Mode of reproduction P♂	2*n*	Mode of reproduction and number of addition hybrids
18*	*H. peleterianum*	sexual	54	*H. pilosella*	sexual	45	sexual (1)
36*	*H. pilosella*	sexual	18	*H. peleterianum*	sexual	45	sexual (6)
36	*H. pilosella*	sexual	45*	*H. pilosella*	apomictic	63	apomictic (3)
36*	*H. pilosella*	sexual	45*	*H. pilosella*	apomictic	81	apomictic (1)
36*	*H. pilosella*	sexual	54	*H. pilosella*	apomictic	63	apomictic (1)
36	*H. pilosella*	sexual	63*	*H. pilosella*	apomictic	81	apomictic (1)
63*	*H. pilosella*	apomictic	54	*H. pilosella*	apomictic	90	apomictic (2)

Table 2. A list of the number of plants pollinated with pollen from the same or other cytotypes of the same or other species of the genus *Hieracium*. The number of capitula pollinated, the numbers of viable achenes and the number of cytologically studied F1 plants are indicated as well as the number and percentage of addition hybrids

	P♀			F1				
Chromosome number	Pollinated capitula	Pollinated florets	Viable achenia	Cytologically studied	Reduced fertilized egg cells	Unreduced fertilized egg cells	Percentage addition hybrids formed	Unreduced not fertilized egg cells
$2n=18$	45	7314	2545	200	199	1	0·5	0
$2n=36$	137	13 464	4981	610	608	2	0·32	0
$2n=45$	36	3523	1515	160	9	0	0	151
$2n=54$	38	4729	1896	169	70	0	0	99
$2n=63$	12	1157	91	53	0	2	3·77	51
All cytotypes	268	30 187	11 028	1192	886	5	0·42	301

Table 3. The number and percentage of addition hybrids resulting from fertilization by unreduced pollen grains, produced by diploid, tetraploid, pentaploid, hexaploid and heptaploid pollinating plants

P♂		F1		
Pollen donor chromosome number	Number of capitula used for pollination	F1 plants tested cytologically	Addition hybrid	
			Number	%
18	42	188	6	3·19
36	60	268	0	0
45	76	339	4	1·17
54	47	205	0	0
63	43	192	1	0·52
All cytotypes	268	1192	11	0·92

(=99·44%) were both reduced and subsequently fertilized. Only five capitula (=1·8%) produced at least one unreduced egg cell that was capable of being fertilized, whereas in 263 capitula (=98·2%) only reduced egg cells or unreduced parthenogenetic embryo sacs were formed.

The proportion of unreduced male gametes to female gametes is very difficult to determine. Each floret produces only one egg cell but the number of pollen grains is far in excess of 2500 per floret. Unreduced pollen grains have a larger diameter. The author did not, however, measure the diameter of the pollen grains but confined himself to cytological studies of F1 plants in order to infer the percentage of capitula producing at least some viable unreduced pollen grains. Measuring the diameter of the pollen grains does not permit conclusions to be drawn regarding their viability. Some viable unreduced pollen grains were formed in at least five capitula (=1·8%), whereas in 263 capitula (=98·2%) either reduced pollen grains were produced or pollen grains not involved in fertilization. Usually, the apomictic forms of *Hieracium pilosella* produce not only a varying number of abnormal pollen grains but also a fairly large number of normal ones, generally with euploid, but sometimes with aneuploid chromosome numbers. From Table 1 it can be concluded that six pollen grains of *H. peleterianum* Mérat, two of pentaploid *H. pilosella* L. and one of heptaploid *H. pilosella* L. were involved in the formation of addition hybrids. All other pollinations of pentaploid, hexaploid and heptaploid plants resulted in the formation of strictly maternal offspring with the same chromosome number as the pollinated plant. This made it convincingly clear that fertilization of apomictic plants is exceptional. The offspring of apomictic plants usually originated from unreduced unfertilized egg cells.

Combining the results of unreduced pollen grains and egg cells (involved in fertilization) one finds that a total number of 15 individuals (=1·26%) out of 1192 cytologically investigated F1 plants was formed by the fusion of one unreduced with a reduced gamete or by the fusion of two unreduced gametes.

Table 3 refers to the percentage of addition hybrids formed by fusion of female gametes with unreduced male ones, arranged per cytotype. In general the percentages are low, both for egg cells and pollen grains: from 0 to 3·77% (Tables 2 and 3).

DISCUSSION

In this study polyspermaty and somatic doubling from meristematic tissue could not be demonstrated. It is usually very difficult to distinguish between zygotic chromosome doubling and the fusion of unreduced gametes. The present study, however, has made it convincingly clear that zygotic chromosome doubling is not the cause of polyploidy. If it was, the cross $2n=18♀ \times 2n=54♂$ would have resulted in a hybrid with the chromosome number $2n=72$, whereas the number actually found was $2n=45$. This means that the female parent produced at least some unreduced egg cells. The same is true of the cross $2n=36♀ \times 2n=54♂$, which produced a hybrid with $2n=63$ instead of $2n=90$. The latter number would have resulted if zygotic chromosome doubling had been involved. The results of all other crosses can be explained in the same way, i.e. all higher polyploids originated from the fusion of gametes at least one of which was unreduced.

Data on the frequency of the occurrence of unreduced gametes in angiosperms are scarce (Franke, 1975; Harlan & DeWet, 1975). Franke lists 31 families. Harlan & DeWet, on the other hand, mention 26 families in which the chromosome increase in the F1 was caused by one or both gametes and 18 families in which the chromosome increase occurred in the F2. The frequency of the occurrence of functional unreduced gametes was studied in various plants groups, especially in commercially important plants.

Crosses involving unreduced gametes also occur in nature. 't Hart (1987) described the hybrid *Sedum* × *lorenzoi* ($2n=124$, 146), which is an allotriploid. He assumed that it resulted from a backcross of the hybrid between *S. ochroleucum* ($2n=68$) and *S. rupestre* subsp. *rupestre* ($2n=112$) with its parents. Some plants of the interspecific hybrid between *S. forsterianum* × *S. rupestre* also produced some unreduced male gametes. The high percentage of polyploidy in the genus *Sedum* may be explained by the frequency of functional unreduced gametes. In *Rumex acetosella* aggr., Den Nijs (1974) reported the occurrence of unreduced gametes in some French populations and found that they give rise to polyploids. Quantitative data, however, are lacking in these studies. In tuber-bearing species of *Solanum*, Den Nijs & Peloquin (1977) screened the number of clones producing unreduced gametes. Unreduced pollen grains were detected by their larger size, whereas screening for unreduced egg cells was based on the results of $2n \times 4n$ crosses. Eight out of ten diploid species tested turned out to contain clones which formed some unreduced egg cells. The percentage of clones producing unreduced pollen grains varied from 0 to 16%. The authors pointed out that there is a very high correlation between the occurrence of unreduced gametes and polyploidy. This conclusion can be confirmed by the present author. Price (1957), Kandasami (1961) and Roach (1968) studied unreduced gametes in interspecific crosses of *Saccharum* and described the derivation of the hybrids: $n+n$, $2n+n$, $n+2n$, $2n+2n$. About 5% of the hybrids turned out to be diploids and 50% triploids. No tetraploids were formed which shows that the fusion of two unreduced gametes is a rare phenomenon. This could be confirmed in *Hieracium pilosella* s.l.: one addition hybrid (6·25% of all addition hybrids) was produced by the fusion of two unreduced gametes. In the genera *Bothriochloa* and *Dichantium* (Harlan & DeWet 1975), in which diploidy, polyploidy and apomixis occur, 39% of the interspecific and intergeneric hybrids originated from the fusion of at least one unreduced gamete. Two out of 296 hybrid plants originated from the fusion of two unreduced gametes. In *Zea mays*, Alexander & Beckett (1963) found the frequencies of unreduced egg cells to range from 0 to 348 per 10 000 zygotes, or from 0 to 3·5%, in in-bred lines. Apparently the production of unreduced gametes is under genetic control. Rutishauser (1967) summarized the results of investigations on the frequency of fertilization of unreduced gametes in pseudogamous apomicts, e.g. in *Potentilla, Poa, Rubus*, and in *Calamagrostis*, an autonomous agamospermous group.

In general, fertilization of unreduced gametes is rare. Apparently the proportion of pseudogamous to sexual reproduction is under genetical control and dependent on ecological factors. Some strains of the genera mentioned before reproduced exclusively in a pseudogamous way, other strains formed at least some B-III hybrids, i.e. hybrids resulting from the fertilization of unreduced egg cells. In *Hieracium pilosella* s.l., reproduction is either sexual or by autonomous agamospermy. In this case fertilization of unreduced gametes is rare as deduced from the percentage of plants with a higher level of polyploidy among the offspring. Of all the cytologically studied experimentally produced hybrids, 1·26% appears to be addition hybrids.

The frequency of unreduced gametes is low but it does not differ significantly from the percentages found in sexual species (e.g. in the genera *Zea* and *Solanum*) and other agamospermous taxa (e.g. *Potentilla, Poa*).

In spite of the low frequency of unreduced gametes, considerable numbers of unreduced egg cells are produced in even small populations or populations of moderate size in *Hieracium pilosella.*

On the Isle of Vlieland the author found two adjacent populations of *Hieracium pilosella*, one with tetraploid sexual plants, one with pentaploid apomictic plants. The tetraploid population had 66 capitula, the pentaploid had 82 flowerheads. Sexual capitula form 98·95 achenia on average, and 97·87 apomictic. This means that 6530 and 8025 florets, respectively, could be pollinated at the same time, either by pollen of the same cytotype or of the other cytotype. Apomictic plants are able to fertilize sexual plants (Gadella 1987). If in these populations 1·26% of all fertilized achenes had an unreduced chromosome number, 82 and 101 addition hybrid-achenes could have been formed, respectively. The fact that the author never found mixed populations in which tetraploids, pentaploids and higher polyploids were growing intermingled shows that addition hybrids are apparently very rare or adaptively inferior. Some addition hybrids, however, form dense mats in nature, e.g. the large heptaploid population ($2n=63$) on the island of Vlieland. This population is very successful and grows in an isolated locality at a certain distance from other tetraploid and pentaploid populations. In the present study heptaploid plants were formed in two different ways, first by crossing sexual tetraploid plants that form reduced egg cells with 18 chromosomes and apomictic pentaploid plants that produce unreduced pollen grains with 45 chromosomes, and second by fertilizing unreduced egg cells (with 36 chromosomes) of a tetraploid sexual plant with reduced pollen (with 27 chromosomes) of a hexaploid plant. It seems likely that the heptaploid addition hybrid on the island of Vlieland was formed by the former. This view is based on the fact that, after a careful analysis of more than 50 local clones, only tetraploid and pentaploid plants were found. This is the only case of a successful higher natural polyploid. In other areas of sympatric contact between different cytotypes/species under natural conditions, other addition hybrids may also be formed. The fact that addition hybrids are rare in natural populations may also be explained by pollen competition or by the formation of precocious embryos in apomicts. These aspects require further study.

The present study has made it clear that the production of such higher polyploids is by no means rare but, nevertheless, a realistic process. The success of the addition hybrids under natural conditions, however, is probably limited.

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