

Evidence for autotetraploidy in *Plantago media* and comparisons between natural and artificial cytotypes concerning cell size and fertility*

Peter Van Dijk† and
Wilke Van Delden

Department of Genetics, University of Groningen,
P.O. Box 14, 9750 AA Haren, The Netherlands

The hypothesis of autotetraploidy in *Plantago media* ($2n = 12$ and $2n = 24$) was tested. The two cytotypes are morphologically very similar. Rare 4X and 3X hybrids were obtained in inter-ploidy crosses. The reproductive isolation between 2X and 4X is post-zygotic and can be broken down and introduced by colchicine treatment. The studied diploid and tetraploid populations shared the same alleles for nine allozyme loci. Together this provides strong evidence for autotetraploid origin of tetraploid *P. media*. Fertility of colchicine tetraploids was comparable to natural tetraploids and slightly lower than natural diploids. In contrast to colchicine tetraploids, natural tetraploids did not show *gigas*-characteristics. Tetraploid hybrids between diploids and natural tetraploids showed partial *gigas*-characteristics. The possible reasons for the differences in cell size between cytotypes are discussed. Differentiating characters between the natural cytotypes were not affected by colchicine treatment and are therefore not a direct consequence of polyploidization.

INTRODUCTION

Polyploidy is a remarkably wide-spread phenomenon in plants. Grant (1981) estimated that 47 per cent of the angiosperms are probably of polyploid origin. Polyploidy is even more prominent in the genus *Plantago*. In more than two thirds of the 122 *Plantago* species listed in Dietrich (1975) and (1980) polyploid chromosome numbers have been counted. In spite of more than 60 years of polyploid research, the reasons for the evolutionary success still remain largely obscure (Stebbins and Dawe, 1987).

The classification of polyploids is difficult and much discussed (Stebbins, 1971, 1980, 1985; Lewis, 1980; Grant, 1981; Soltis and Rieseberg, 1986). In addition the importance of autopolyploidy in plant evolution is still a matter of much dispute. Clausen, Keck and Hiesey (1945) considered only a few species as true autopolyploids, an opinion shared by Grant (1981). In contrast, Gottschalk (1976) and Lewis (1980) give many examples of supposed autopolyploids to underscore the significance of

autopolyploid evolution. According to Stebbins (1971) genome doubling by itself is not a help but a hindrance to evolutionary success. However, Levin (1983) and Cavalier-Smith (1985) consider genome doubling itself a source of evolutionary novelty.

To provide a reliable polyploid phylogeny many different sources of evidence are necessary (Stebbins, 1971). Experimental resynthesis can be seen as strong evidence, but interpretation may be hampered by evolutionary divergence after polyploidization. Here we use the term autopolyploidy to refer to intraspecific polyploidy and allopolyploidy for interspecific polyploidy (Lewis, 1980).

The most universal effects of artificial autopolyploidization are reduced fertility and increased cell and organ sizes (*gigas*-effects) (Stebbins, 1971; Lewis, 1980). Low fertility, primarily caused by multivalent formation, is expected to constrain autopolyploid evolution. It has been suggested that selection for increased fertility may lead to cytological diploidization in polyploids (DeWet, 1980; Watanabe, 1983). It is therefore of interest to investigate fertility in successful natural autopolyploids. Solbrig (1980) proposed that *gigas*-characteristics could be of adaptive value for new polyploids. However in a review

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† Present address: Department of Plant Ecology, Institute for Ecological Research, Boterhoeksestraat 22, 6666 GA Heteren, The Netherlands.

Tanaka (1953) concluded that natural polyploids in general had smaller stomatal guard cells than artificial polyploids. This difference may be interpreted as a difference between new and established polyploids (Trombetta, 1942; Gottschalk, 1976). However, comparisons between diploids and polyploids are often obscured by uncertain phylogenies and by effects of hybridization in the case of allopolyploids. Intra-specific comparisons between natural and artificial autopolyploids are rare; Tanaka (1953) lists only two cases. Direct evidence for reduction of cell size after polyploidization comes from Von Wettstein (1937) who observed a reduction of the cell volumes of artificial autodiploid *Bryum* to the original haploid level, over a period of 11 years, without changing the chromosome content. Little is known about the selective forces on cell size, the mechanisms and the genetics of cell- and organ-size regulation.

In the taxon *Plantago media* L. (including *P. stepposa* Kuprian., Tutin *et al.*, 1976), two cytotypes have been reported: diploids ($2n = 12$) and tetraploids ($2n = 24$) (Rahn, 1954; 1957). In Europe tetraploids have a wider geographical distribution than diploids (authors observations). Diploid and tetraploid *P. media* are morphologically very similar, but differ in number of ovules per capsule and length of the scape (Rahn, 1957). Rahn (1954) reported a regular meiosis in tetraploids with 12 bivalents, although occasional bridges were observed.

In this article we provide evidence for autotetraploidy in *P. media* from experimental hybridization, artificial polyploidization by colchicine treatment, and comparisons of allozyme pools. In addition natural and artificial cytotypes have been compared to determine how fertility and cell size are affected by autopolyploidization.

MATERIALS AND METHODS

Plant material. *P. media* is a wind pollinated, perennial species from dry, basic grasslands in Eurasia (Sagar and Harper, 1964). *P. media* is normally strongly self-incompatible, but occasionally self-compatible plants occur (authors observations). Samples from the following populations were used: Diploid populations: Pyrenees (Gavarnie: 42.45N, 0.15W); Col de Portalet: 42.59N, 0.27W), Soviet Union (Orol region: 53.00N, 36.05E). Sympatric diploid-tetraploid population: Pyrenees (Formigal: 42.48N, 0.24W). Tetraploid populations: Netherlands (Westervoort: 52.00N, 6.00E), Yugoslavia (Sarajevo: 43.50N, 18.25E), Pyrenees

(Andorra: 42.35N, 1.40E). From the Orol population, the sampling history is not known. All other samples were randomly-collected from natural populations (mature spikes collected from approximately 20 plants, growing at least one metre apart).

Cultivation conditions. Plants were grown in pots in a glasshouse (18 h light, 21°C/6 h dark, 18°C). To prevent selfing, only female flowering (protogynous) parts of the spikes were used in the crosses. Seeds were harvested five weeks after crossing.

Colchicine treatment. A diverse seed sample from the diploid Gavarnie population was used. Colchicine (0.25 per cent in a 2 per cent dimethylsulfoxide solution) was applied as a drip with a syringe between the cotyledons of one week old seedlings. Colchicine was applied once a day for one, two or three successive days (treatment A, B or C, each 40 plants). Forty plants were treated with water as controls. Because only the shoot apex was affected, mixoploids with tetraploid shoots and diploid roots were obtained (=C₀2X/4X). From the roots, diploid grafts were made. Isogenic diploid and colchicine tetraploid F₁ families (C4X) were made by crossing the same parents on the diploid and the tetraploid level to maximize heterozygosity (fig. 1).

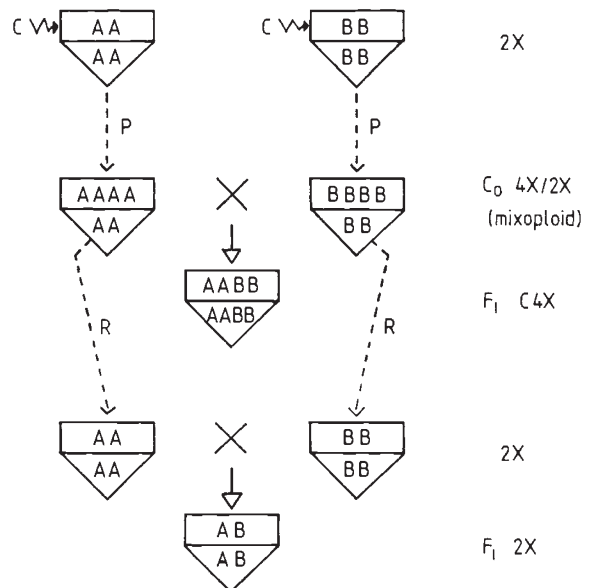


Figure 1 Derivation of isogenic diploid-colchicine tetraploid families. The upper part of the symbol represents the shoot, the lower part the root. (C: colchicine treatment P: polyploidization, R: root graft).

Isogenic 2X and C4X families have identical gene frequencies. Chromosome numbers were counted in squashed pollen mother cells and root-tip cells stained by 2 per cent acetic orcein or the Feulgen method. Root-tips were pretreated with saturated aqueous 1-bromonaphthalene for 4 h at 4°C and fixed in Carnoy.

Measurements. Different cytotypes were compared for fertility and size characteristics in a randomized growth experiment in the glasshouse. Four different cytotypes were compared: natural 2X, natural 4X, colchicine 4X and tetraploid cytotype hybrids. Tetraploid hybrids had been occasionally obtained in 2X-4X crosses. Sixteen plants were used per cytotype, except for tetraploid hybrids in which only ten plants were available. All tetraploids were pollinated by natural tetraploids. Seed set was measured as the seed-ovule ratio. The average number of ovules per capsule was estimated from samples of 25 capsules per spike. Average pollen diameter and stomatal guard cell length of full grown scapes was estimated from samples of 50 cells in a 1:1 aceto orcein: glycerol mixture. Scape stomata were preferred to leaf stomata because of less size variation within plants. The percentage of filled pollen grains was used as an estimate of the male fertility (approximately 200 grains per sample).

Electrophoresis. Polyacrylamide gel electrophoresis was applied as described by Van Dijk and Van Delden (1981). Seven enzymes were selected: glutamate-oxaloacetate-transaminase (GOT), 6-phosphogluconate dehydrogenase (6PGD), shikimate dehydrogenase (SHDH), leucine aminopeptidase (LAP), malic enzyme (ME), isocitrate dehydrogenase (IDH) and glyceraldehyde-3-phosphate dehydrogenase (GA-3-PD) (all according to Van Dijk and Van Delden (1981)). For GOT the lithium borate (pH 8.3) buffer system of Roose and Gottlieb (1978) was used.

RESULTS

Synthesis of colchicine tetraploids

Colchicine strongly retarded growth and reduced survival in all treatments. Survival after 30 days was negatively correlated with the duration of the colchicine treatment (Treatment A: 95 per cent, B: 71 per cent and C: 24 per cent compared to the control). Flowering was also delayed (the first C₀4X/2X plant flowered 18 days later than the control). Of 70 investigated plants from treatments

A and B, shoots of 30 plants were still 2X, 36 were 4X and four were 8X ($2n = 8X = 48$). Octaploids had leathery, deformed, thick leaves, more delayed flowering and were completely sterile when crossed *inter se*. Many plants from treatment C showed these characteristics but since these plants did not flower, no chromosome counts were made. Artificial polyploidization did not affect self-incompatibility: no self-compatible C₀4X/2X or C4X were found. C₀4X/2X mixoploids remained mixoploid during three years of cultivation in an experimental garden.

Crossability

The results from the crosses are given in table 1. Great variation in seed number is caused by large differences in protogyny between plants (see materials and methods). Within both 2X and 4X ploidy levels, seed production in wide crosses was no lower than in within population crosses (table 1(a, b)).

Even although diploids from the Pyrenees and the Soviet Union differ markedly in morphology (pubescence, leaf dimensions, seed weight) their

Table 1 Crossabilities in *Plantago media* in relation to ploidy level and geographical origin. 2X×4X crosses and reciprocal crosses are combined (PYR: Pyrenees (Andorra), SU: Soviet Union, NETH: The Netherlands, YUG: Yugoslavia)

Populations	Mean number of seeds per spike ± s.d.	Number of crosses
(a) within populations, within ploidy levels		
PYR (2X)	81 ± 29	20
SU (2X)	100 ± 24	11
PYR (4X)	38 ± 22	3
NETH (4X)	98 ± 54	8
YUG (4X)	107 ± 25	5
(b) Between populations, within ploidy levels		
PYR (2X) × SU (2X)	111 ± 32	3
SU (2X) × PYR (2X)	73 ± 26	3
PYR (4X) × NETH (4X)	50	1
NETH (4X) × YUG (4X)	110 ± 36	3
YUG (4X) × NETH (4X)	58 ± 14	3
YUG (4X) × PYR (4X)	78 ± 20	3
(c) Between populations, between ploidy levels		
PYR (2X) × NETH (4X)	0.6 ± 0.9	5
PYR (2X) × PYR (4X)	3.8 ± 3.1	13
PYR (2X) × YUG (4X)	2.0 ± 2.8	2
SU (2X) × NETH (4X)	0.3 ± 0.5	4
SU (2X) × PYR (4X)	1.3 ± 2.5	4
SU (2X) × YUG (4X)	0.0 ± 0.0	2

hybrids show no reduction in fitness and can easily be back-crossed to either parent.

In crosses between ploidy levels however, the production of viable seeds was extremely low, irrespective of geographical origin (table 1(c)). Many seeds were shrivelled and empty and a high proportion of the filled seeds from these crosses did not germinate.

Table 2 shows the chromosome numbers from viable offspring of interploidy crosses. Most of the seeds from diploid seed parents were diploid and probably result from contamination with outcross haploid pollen. However 12 tetraploid offspring from diploid seed parents (all from the Pyrenees) were found, presumably from unreduced 2X eggs.

Table 2 Analysis of chromosome numbers of viable offspring from crosses between different ploidy levels

CROSS	2X = 12	3X = 18	4X = 24
2X × 4X*			
LA 5 × AN 14	8	0	0
LA 16 × AN 22	6	0	0
LA 30 × FO 2	2	0	0
LA 12 × FO 4	1	0	2
LA 7 × FO 3	0	0	5
FO 18 × FO 7	2	0	5
Total	19	0	12
4X × 2X†			
FO 2 × GG 6	0	3	1
S 29 × LA 28	0	2	1
Total	0	5	2

* Five additional crosses without viable offspring.

† Nine additional crosses without viable offspring.

Hybrid production was markedly unevenly distributed among crosses. No triploids were found among the offspring of diploid seed parents. However, two 4X-2X crosses produced some 3X seedlings, together with occasional 4X seedlings. Since no genetic markers were used, it cannot be ascertained whether these 4X seeds resulted from fertilization by unreduced 2X pollen or by contamination with reduced 2X pollen from 4X plants. Comparisons of viable seed production in intra- and inter-ploidy crosses gives rough estimations of cytotype hybrid frequencies: 0.0123 for 4X hybrids in 2X × 4X, 0.0084 for 3X hybrids in 4X × 2X and 0.0034 (maximal) for 4X hybrids in 4X × 2X (assuming all 4X offspring were hybrids). Both triploid and tetraploid cytotype hybrids were vigorous. Tetraploid hybrids can readily be back-crossed to natural tetraploids. Triploid hybrids however were almost completely sterile.

No seeds were obtained from crosses between diploids and colchicine tetraploids. Since unrelated plants were crossed, effects of the self-incompatibility system can be excluded. Thus the colchicine treatment must have induced reproductive isolation between diploids and artificial tetraploids. By contrast, crosses between C4X and natural 4X plants produced many seeds, most of them with 24 chromosomes although occasional aneuploids were observed. These hybrids showed normal vigour. The original reproductive isolation between natural 2X and 4X was therefore bypassed by colchicine treatment.

Characteristics of natural and artificial cytotypes

In fig. 2 the mean values per cytotype are shown as percentages of the diploid means. The following values were measured in the diploids: seed set: 81.3 per cent; pollen stainability: 91.2 per cent; seed weight: 0.70 mg; stomatal guard cell length: 25.8 µm; pollen grain diameter; 21.9 µm; number of ovules per capsule: 2.41 and scape length: 21.7 cm.

From fig. 2(a) and 2(b) it can be seen that colchicine tetraploids and tetraploid hybrids (=2X × 4X) had high seed set and high percentages of filled pollen. The seed set of colchicine tetraploids is 79 per cent of the diploids and does not differ significantly from natural tetraploids. There were no significant differences between the cytotypes in pollen stainability.

Natural tetraploids had slightly but non-significant higher average seed weights, while seeds from tetraploid hybrids and colchicine tetraploids were respectively 15 and 38 per cent heavier (fig. 2(c)). The ranking for pollen grain diameter was the same, but in this case all the differences were statistically significant ($P < 0.05$) (fig. 2(e)). Size differences are even more obvious in the estimated pollen volume (fig. 2(f)). It is significant that tetraploid hybrids were intermediate between natural tetraploids and colchicine tetraploids in both seed weight and pollen size.

Stomatal guard cell length was lower in diploids than in the other cytotypes, which did not differ significantly from each other (fig. 2(d)). The number of ovules per capsule and the scape length differed between natural diploids and natural tetraploids (fig. 2(g), (h)). Both characters were not affected by colchicine treatment, whereas tetraploid hybrids were intermediate between the parental cytotypes.

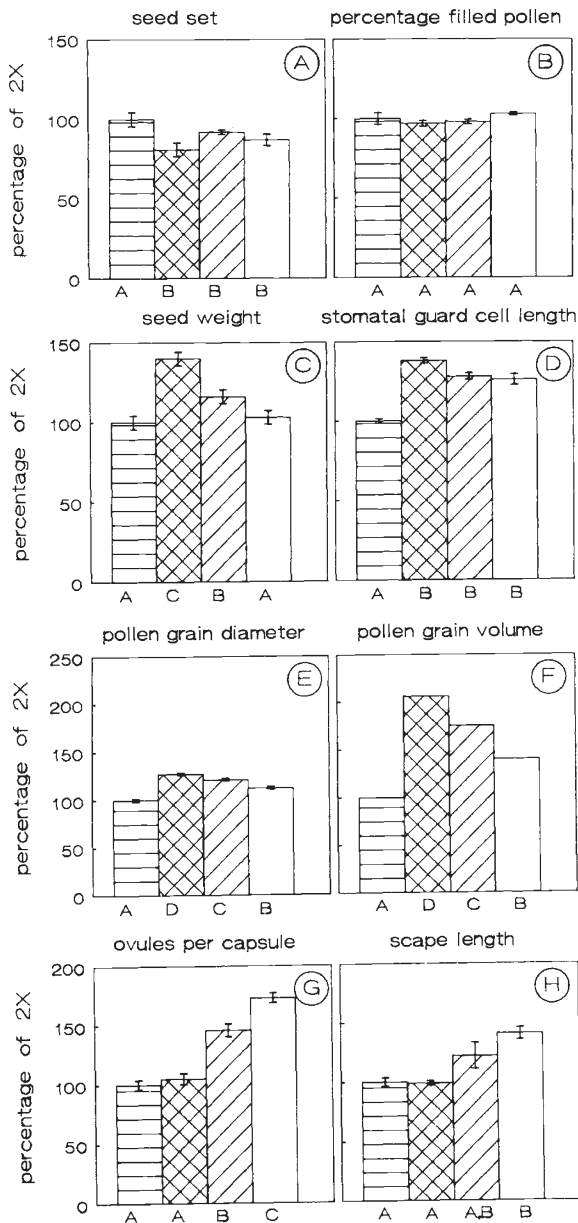


Figure 2 Comparisons between different cytotypes of *Plantago media*. Bars from left to right represent natural 2X, colchicine 4X, 4X (2X-4X) hybrid and natural 4X respectively (means as percentage of natural 2X \pm S.E.). Cytotypes that do not differ significantly are indicated by the same capital (ANOVA, $P < 0.05$).

Electrophoresis

The results from the isozyme electrophoresis are shown in table 3. Allelic segregation for five polymorphic loci was demonstrated in both diploids and tetraploids (data not shown). However in the

4X crosses it was not possible to discriminate between disomic and tetrasomic inheritance.

Tetraploids showed typical asymmetric heterozygotes, but no fixed heterozygosity was observed. In the tetraploids only *Got-2* genotypic deduction was reliable enough to calculate gene frequencies in natural populations. In other loci the different heterozygotes could not be scored with certainty in most cases. Problems of interpretation were due to variation in allozyme activity and presence of null-alleles. In the case of *Shdh* allozymes were triple banded and varied in activity. Therefore for these loci only presence or absence of allozymes is indicated. At the diploid level, all common alleles were found in the Soviet Union population as well as in the Pyrenean populations. Both *Got* loci showed marked differences in gene frequencies between the Soviet Union population and the Pyrenean 2X populations but since the sampling history of the Soviet Union population is not known it is uncertain whether this represents a real difference. All alleles found in the tetraploid populations were also present in the diploid populations although some rare alleles in diploid populations were not observed in tetraploids. All 4X populations were biallelic; no new tri- or quadri-allelic genotypes were seen. Monomorphic loci were identical in the diploid and tetraploid material.

DISCUSSION

Evidence for autotetraploidy

All together the results strongly suggest that *P. media* is an autopolyploid species-complex. Diploids and tetraploids are morphologically very similar but distinct from other *Plantago* species. No barriers to wide crosses were found within ploidy levels. Strong reproductive barriers exist between ploidy levels, however occasionally vigorous 3X and 4X hybrids were produced. The reproductive barriers between the cytotypes could be broken down as well as introduced by chromosome doubling by colchicine treatment. Finally no allozymes were found in the 4X samples that did not occur in the 2X samples.

Recent detailed cytogenetic investigations fully support the autotetraploid origin of *P. media* (Dr J. S. Parker, personal communication). Quadri-valents are found at metaphase I and more significantly, at pachytene, chromosomes are usually associated as quadrivalents with numerous pairing partner exchanges. In viable triploid hybrids observations up to six trivalents in pollen mother

Table 3 Presence and frequencies of allozymes in some 2X and 4X populations of *Plantago media* (SU: Soviet Union, GAV: Gavarnie, POR: Portalet, FOR: Formigal, AN: Andorra, NETH: Netherlands, YUG: Yugoslavia). Rm is the relative migration of allozymes (Bromophenol blue or borate = 100)

	Rm	2X				4X			
		SU	GAV	POR	FOR 2X	FOR 4X	AN	NETH	YUG
Got 1	22	0.00	0.00	0.22	0.06	–	–	–	–
	25	0.06	1.00	0.78	0.94	+	+	+	+
	28	0.94	0.00	0.00	0.00	+	+	+	+
Got 2	16	0.00	0.02	0.02	0.06	0.00	0.00	0.00	0.00
	18	0.09	0.98	0.98	0.94	0.96	1.00	0.69	0.69
	21	0.91	0.00	0.00	0.00	0.04	0.00	0.31	0.31
6Pgd 1	35	?	0.34	0.20	0.50	+	+	+	+
	38	?	0.65	0.80	0.50	+	+	–	+
	40	?	0.01	0.00	0.00	–	–	–	–
6Pgd3	22	0.00	0.07	0.00	0.18	+	+	+	–
	24	1.00	0.93	1.00	0.82	+	+	+	+
Shdh	20	0.00	0.04	0.00	0.00	–	–	–	–
	26	0.27	0.48	0.42	0.20	+	+	+	+
	32	0.63	0.48	0.58	0.80	+	+	+	+
Lap	21	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Me	17	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Idh	26	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
Ga3pd	14	1.00	1.00	1.00	1.00	1.00	1.00	1.00	1.00
N (plants)		41	66	32	17	17	42	30	30

cells indicate strong homology of the 2X and 4X genomes. Also RFLP analysis shows close relatedness between 2X and 4X CpDNA (Dr R. Lumaret and Dr K. Wolff, personal communication). Finally reproductive barriers between *P. media* and other *Plantago* species are strong: Rahn (1957) failed to hybridise *P. media* (both 2X and 4X) with *P. lanceolata*; *P. major*; *P. coronopus*; *P. maxima* or *P. camtschatica*.

Recently a number of other well documented cases of autopolyploidy have been reported (e.g., *Dactylis glomerata* (Lumaret, 1988); *Tolmiea menziesii* (Soltis and Rieseberg, 1986); *Lathyrus pratensis* (Brunsberg, 1977); *Heuchera micrantha* (Ness *et al.*, 1989; Soltis and Soltis, 1989); *Vaccinium corymbosum* (Krebs and Hancock, 1989)). This suggests that autopolyploidy is more common than once thought.

Mechanism of reproductive isolation

The reproductive isolation between cytotypes is due to differences in ploidy level *per se* and not to genome incompatibility, since hybrids between natural and colchicine tetraploids have

high vigour. The ontogeny of the fertilization process shows that the barrier is post-zygotic. No abnormalities are observed until about ten days after pollination. At this time the endosperm development stops, strongly indicating that seed abortion is caused by endosperm collapse. This phenomenon ("triploid block") is well known in inter-ploidy crosses (Levin 1978; Johnston *et al.*, 1980; Parrot and Smith, 1986). The reason for abnormal endosperm development in inter-ploidy crosses is probably a deviation from the maternal:paternal genome ratio of 2:1 in the endosperm ("endosperm balance number hypothesis", Johnston *et al.*, 1980). In 2X–4X crosses this ratio is 1:1 and in 4X×2X crosses it is 4:1. However for unreduced eggs in 2X–4X crosses, the 2:1 ratio is restored and endosperm and embryos develop normally. The endosperm genome ratio may also explain why rare viable 3X hybrids are only found in 4X–2X crosses: failure of fusion of polar nuclei, would restore the viable parental genome ratio. This would not be so in the reciprocal cross. The presence of cytotype hybrids indicates that some gene flow between cytotypes is possible in sympatric populations.

Effects of polyploidization on fertility

Seed set (the number of ovules becoming seeds) was significantly lower in natural tetraploids than in diploids. A similar difference was observed in previous experiments. This difference may be caused by meiotic aberrations in natural tetraploids, but other factors like stronger zygote competition may also be involved. Total seed production however is higher in natural tetraploids since reduced seed set is over-compensated by a higher number of ovules per capsule.

Seed set in colchicine tetraploids of *P. media* is high in comparison to colchicine tetraploids in other plant species (Gottschalk, 1976). Quadri-valents in colchicine tetraploids are found at metaphase I, although at a rather low frequency due to the low chromosome number and low chiasmata frequency (Dr J. S. Parker, personal communication). Although natural tetraploids have a significantly lower quadrivalent frequency than colchicine tetraploids this difference is not reflected in seed set and pollen stainability.

Effect of polyploidization on cell volume

Pollen grain volume of colchicine tetraploids was doubled compared to diploids, whereas pollen grain volume of natural tetraploids was only 40 per cent larger. A similar difference was observed by Rahn (1957). Stomatal guard cell length is difficult to compare between cytotypes, since scape length itself differs between cytotypes. Colchicine tetraploids however show clear size increase compared to diploids. Seeds from colchicine were on average 40 per cent heavier than seeds from either diploids or natural tetraploids. It is not known whether the difference in seed weight between natural and artificial tetraploids is caused by differences in cell number or cell size. Other *gigas*-effects could be seen in the larger leaves and flowers as well as thicker spikes of colchicine tetraploids. Natural tetraploids do not show these *gigas*-effects.

Tetraploid hybrids showed partial *gigas*-characteristics. These hybrids resulted from a non-reduced 2X egg cell of the 2X mother and a reduced 2X male gamete from a natural 4X father. The unreduced 2X genomes have a similar cell size effect to the genomes doubled by colchicine treatment. These results indicate that natural 2X and 4X differ in genetic cell size factors and that the effects are additive.

In two other *Plantago*-species, *P. major* and *P. coronopus*, no differences in cell size were found between diploids and natural polyploids (Favarger

and Vasudevan, 1972; Gorenflot, 1960). However in *P. coronopus* artificial doubling by colchicine treatment markedly increased cell size of the 4X (derived from 2X) and of the 8X (derived from natural 4X) (Gorenflot, 1960; 1963). At the inter-specific level, North American Chasmogamous *Plantago* species show no differences between 2X and 4X in pollen grain diameter (Bassett and Crompton, 1968). Thus differences in cell size between natural and artificial polyploids seem to be a general phenomenon within the genus *Plantago*. Similar differences have also been found in other genera: in both *Gymnaster savatieri* and *Chrysanthemum* sp. natural polyploids had smaller stomatal guard cell length than artificial polyploids (Tanaka, 1953).

Both number of ovules per capsule and scape length, two discriminating characters between 2X and 4X *P. media* (Rahn, 1957), were not affected by artificial polyploidization. Therefore it can be concluded that these differences are not a direct consequences of genome multiplication. This is supported by the fact that 2X-4X hybrids lie between their parental types for these characters, in contrast to cell size characteristics.

Selection against large cells after polyploidization?

The differences found between artificial and natural polyploids suggest that natural selection has been operating against large cells. Clearly increase in cell volume has important functional consequences (Lewis, 1980; Levin, 1983; Cavalier-Smith, 1985). Cell volume is under both nucleotypic and genetic control, both of which may be modified (Cavalier-Smith, 1985; Baer, and Schrader, 1985). In *P. media* natural 4X and colchicine 4X have the same DNA content per cell (Dr J. S. Parker, personal communication). It has been suggested that increased gene dosage rather than total DNA content may be the cause of the volume increase in artificial autopolyploids (Cavalier Smith, 1985). Our results indicate that cell size in natural tetraploid *P. media* is regulated by additive genes. Modifications of gene expression may be expected to parallel cell volume changes in order to maintain optimal metabolic concentrations in cells. There are indications that amounts of cellular constituents are reduced in natural polyploids compared to artificial polyploids (e.g., rRNA cistron number, (Cullis and Davies, 1974) and amount of ribulose-1,5-bisphosphate-carboxylase, Leech *et al.*, 1985)).

In *P. media* natural tetraploids have smaller pollen grains than artificial tetraploids, but longer scapes and scape stomatal guard cells. Successful wind pollination depends on large numbers of small pollen grains and exposed reproductive structures (Whitehead, 1983). In *Plantago* chasmogamous species have smaller pollen grains and higher pollen numbers than cleistogamous species (Primack, 1978). Primack (1978) showed that wind dispersal of large pollen grains was reduced. In addition the costs of pollination in terms of biomass are higher in larger pollen grains. Selection for increased pollination efficiency may be strong in an out-crossing species like *P. media*. This could involve selection both for smaller pollen grains as well as longer scapes (and longer scape cells).

Different cell types may be subjected to different selection regimes. On the other hand cell sizes are not independent, and this may cause correlated selection responses. This may explain why natural diploid and tetraploid *P. media* have the same seed size, although *gigas*-seed itself might be adaptive (Stebbins, 1971; Solbrig, 1980).

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