Theor Appl Genet (1983) 65:103–111

The use of irradiated pollen for differential gene transfer in wheat (*Triticum aestivum*)

J. W. Snape, B. B. Parker, E. Simpson, C. C. Ainsworth, P. I. Payne and C. N. Law
Plant Breeding Institute, Maris Lane, Trumpington, Cambridge, England

Received January 3, 1983
Communicated by R. Riley

Summary. The use of irradiated pollen to bring about limited gene transfer in wheat has been investigated. Doses of X-rays of 2Kr, 3Kr and 5Kr were used to generate M_1 progeny between maternal and paternal genotypes differing in quantitative and major gene characters. Cytological studies of M_1 plants revealed hybrids with widespread aneuploidy and structural rearrangements in the paternal genome. These effects resulted in phenotypic variation between M_1 progeny and complex multivalent formation at meiosis. All M_1 plants at the 5Kr and 3Kr doses were sterile and all but 2 plants at the 2Kr dose.

Studies of the two M_2 families from these plants revealed disturbances in genotype frequencies for some of the marker loci with an excess of maternal homozygotes and a deficit of paternal homozygotes. This was also reflected in a more maternal appearance for quantitative characters. These results are interpreted as showing that irradiation damage to the paternal genome in M_1 plants results in the differential transmission of maternal alleles.

Key words: Pollen irradiation – Wheat – Differential gene transfer

Introduction

The exposure of seed to ionizing radiation has been widely used to generate genetical variation in important crop species and many breeding programmes have demonstrated the feasibility of irradiation plus selection as a direct method of varietal production (Micke and Donini 1982). Recent work in *Nicotiana* has shown that irradiation of pollen may provide a different but equally valuable tool for plant breeders by being able to affect limited gene transfer between donor and recipient genotypes (Pandey 1975; Jinks et al. 1981).

In wheat the use of pollen irradiation is not new and has been investigated for many years (for example, Katayama 1934). Interest in this approach has centred on the production of haploids for cytogenetical and breeding studies (Natarajan and Swaminathan 1958; Cao et al. 1979). In these investigations haploids have been produced at low frequencies amongst euploid progenies. However in none of these investigations were the euploid progeny examined for the possibility that limited gene transfer from the paternal genome may have taken place, with the obvious advantages that this could have for wheat breeders. This paper reports the first results of a programme to investigate the feasibility of limited gene transfer using irradiated pollen in wheat.

Materials and methods

Parental genotypes

The maternal genotype chosen for these investigations was the variety 'Chinese Spring' which is well characterised genetically and cytologically. The paternal genotype was the 'Hobbit' (*Triticum spelta* 5A) single chromosome substitution line developed at the Plant Breeding Institute by A. J. Worland and C. N. Law. This substitution line differs from 'Chinese Spring' in a wide range of quantitative characters but more importantly for the present investigations, by a number of phenotypic and electrophoretic, single gene markers.

Irradiation and hybridisation techniques

Plants of both parental genotypes were grown in the glasshouse. Prior to anthesis, ears of 'Chinese Spring' were emasculated and enclosed in cellophane bags to prevent accidental

cross pollination. When emasculated ears were mature, irradiation of whole ears of the substitution line was carried out by detaching whole mature tillers and exposing to X-rays generated by a MEL PHILIPS SL75/20 at Addenbrooke's Hospital, Cambridge. Doses of 5Kr, 3Kr and 2Kr were used. Within 1 h of irradiation, fresh pollen was collected from dehiscent anthers and used for pollination.

Examination of M_1 progenies

At the 2Kr and 5Kr doses, small samples of ovules were removed from pollinated ears at 2 days after pollination and fixed in 3:1 ethyl alcohol/acetic acid for examination of early stages in embryo and endosperm development of the M_1 progeny. This was carried out using the Feulgen squash method of Bennett et al. (1973). Seed sets on all pollinated ears were scored at 5 days after pollination.

Because abnormal embryo and endosperm development was anticipated, approximately half of the M_1 progenies at the 2Kr dose and all progenies at the 5Kr dose were excised and cultured on agar 18 days after pollination using the techniques described by Simpson and Snape (1981). Prior to planting these seedlings into soil, root-tips were taken for cytological examination of mitotic chromosomes. The remaining grains were allowed to mature on the maternal plants and then germinated in petri dishes and root tips taken for cytological examination prior to planting into soil. Root-tip chromosome counts were obtained using the Feulgen squash technique.

All M_1 seedlings were grown to maturity in the glasshouse. Prior to heading, anthers containing PMCs at metaphase 1 were fixed from random plants for cytological examination of meiotic chromosomes. At heading all ears were enclosed in cellophane bags to ensure self-pollination. At maturity all M_2 grain was harvested and sample ears taken for phenotypic classification of ear characteristics of the M_1 plants. Meiotic chromosome counts were obtained using the Feulgen squash technique.

Examination of M_2 progenies

All of the M_1 plants at the 5Kr and 3Kr doses were completely sterile and all except 2 plants at the 2Kr dose. Two M_2 families, I19-1 and I30-2, were grown from these in a spring sown field experiment together with the conventional F_1, F_2 and parental genotypes. Because only small amounts of seed of the M_2's were produced only a single plot of each was sown. Each plot consisted of rows of 11 plants spaced 10 cm apart within rows and rows spaced 30 cm apart. Five rows of M_2 family I19-1 and 3 rows of family I30-2 were grown, with adjacent plots of the parents (3 rows), the F_1 (2 rows) and the F_2 (10 rows).

Prior to heading, tillers were removed from random M_2 plants and anthers fixed for meiotic chromosome counts. At maturity all plots were harvested as single plants and a range of quantitative characters measured. Sample ears from each plant were also taken for phenotypic classification of ear characteristics. All M_3 grain was harvested and used for progeny tests to identify the genotype of each M_2 plant for the major gene markers. The marker systems used together with their known chromosome locations are shown in Table 1.

The presence of awns in this cross is determined by the segregation of three dominant inhibitors which act epistatically. Therefore segregation frequencies in the F_2 were not simple ratios and the M_2 was tested against the observed frequencies in the sample of 91 F_2 plants. For all the other marker systems, single-gene segregation is involved. For progeny tests, 6 M_3 plants were used to estimate the M_2 genotype with respect to GA response, whilst for the electrophoretic markers combined test samples from 3 M_3 grains were used.

Table 1. Major gene markers examined

Character	Locus designation	Chromosomal location	Method of genotype classifaction	Reference
A. Phenotypic markers				
1. Presence of awns	*B1, B2, Hd*	5A, 6B, 4B	Awned or awnless glumes	Law and Worland 1974
2. Ear morphology	*q*	5AL	Speltoid or square ear	McIntosh 1973
3. Gibberellic acid response	*Rht2*	4D	Sensitivity to applied GA_3	Gale and Marshall 1976
B. Electrophoretic markers				
1. High molecular weight glutenin sub-units	*Glu-B1*	1BL	SDS-PAGE	Payne et al. 1982
2. ω-Gliadins	*Gli-B1*	1BS	SDS-PAGE	Payne et al. 1982
3. γ-Gliadins/low molecular weight glutenins	*Gli-A1*	1AS	SDS-PAGE	Payne et al. 1982
4. β-amylase	*β-Amy-A2*	5AL	Isoelectric focusing	Ainsworth et al. 1983
5. α-amylase	*α-Amy-B1*	6B	Isoelectric focusing	Gale et al. 1983
6. α-amylase	*α-Amy-B2*	7B	Isoelectric focusing	Gale et al. 1983

Table 2. Frequencies of seeds set at different doses of irradiation

	Dose	No. ears pollinated	No. florets pollinated	No. seeds set	No. embryos/ or grains produced	No. viable seedlings	% of viable seedlings from florets pollinated
	5Kr	39	1154	–	159	19	1.6
	3Kr	37	885	336	323	10	1.1
Excised	2Kr	62	1768	1215	933	125	5.5
Germinated	2Kr	68	1982	1253	1159	238	9.0

Results

Performance of M_1 progenies

Early embryo and endosperm development. Cytological examination of ovules fertilised by pollen irradiated with 2Kr and 5Kr doses revealed nuclear abnormalities in both embryo and endosperm characteristically seen in situations where chromosome elimination occurs, for example in the interspecific cross between *Hordeum vulgare* and *H. bulbosum* (Bennett et al. 1976). There was asynchronous cell division, and mitotic irregularities with laggard chromosomes, mitotic bridges and the presence of micronuclei. In the endosperm this resulted in highly polyploid nuclei. These observations indicate that part or the whole of the paternal genome was being eliminated from the developing embryo. No qualitative differences were observed between the 2Kr and 5Kr doses although the frequency of aberrations was highest at the higher dose.

Seed set and plant growth. The frequencies of grains set and the numbers of viable plants obtained are shown in Table 2. Both seed set and seed germination decreased with increasing irradiation dose. Further the fitness of the plants obtained decreased. At the 5Kr and 3Kr doses all M_1 plants exhibited reduced growth rates, had smaller leaves and ears, and a high mortality rate before maturity. At these two doses the few mature M_1 plants obtained were all sterile.

There was variation in plant vigour of M_1 plants produced from the 2Kr dose with many indistinguishable from F_1 plants whilst others exhibited a reduced vigour. 29% did not survive to maturity. At this dose fewer plants were obtained from culturing 18 day old embryos than from mature seeds. There seems little advantage therefore in using embryo excision techniques to recover M_1 plants at an early stage.

At all dose levels there was variation between M_1 progenies in ear morphology. In addition to typical F_1 phenotypes both maternal and paternal phenotypes were represented. At the 2Kr dose the mature M_1 plants were classified phenotypically for the presence of awns and speltoid or square ear type and compared to the parental and F_1 phenotypes. The frequencies of each phenotypic class are shown in Table 3. For these marker systems the highest frequency was of F_1 types but there were also maternal phenotypes and, in the case of awns, paternal types. Of the 256 plants scored, 38 had the recessive maternal characteristic for both characters suggesting the absence of the dominant paternal genes.

M_1 chromosome constitutions. The mitotic chromosome counts obtained are shown in Table 4. Most of the M_1 plants exhibited aneuploidy. In addition to the loss of whole chromosomes, some chromosomes were represented only by telocentrics showing the loss of whole arms, some plants contained acentric fragments and others had short metacentric chromosomes where distal parts of both arms had been deleted. Structural rearrangements were also present with very long chromosomes, presumably formed by fusion between two chromosomes, and dicentrics. Examples of aneuploidy

Table 3. Phenotypic classification of M_1 progeny

Genotype	No. plants	Awning			Ear morphology	
		Fully awned	Awnletted	Awnless	Speltoid	Square
'Chinese Spring'	10	0	0	10	0	10
'Hobbit' (*T. spelta* 5A)	10	10	0	0	10	0
F_1	10	0	10	0	10	0
2 Kr M_1	256	39	131	86	164	92

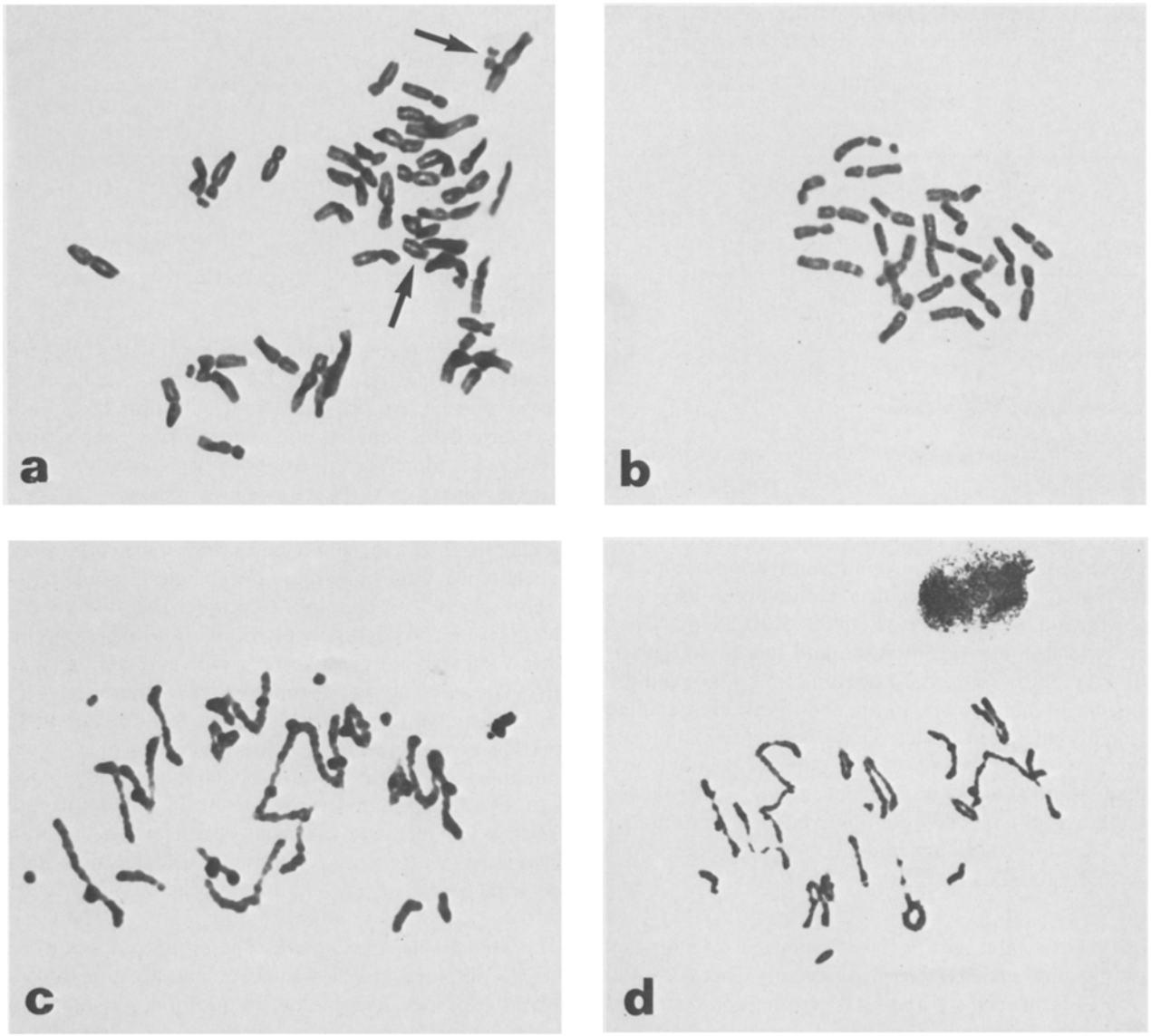

Fig. 1 a – d. Mitotic and meiotic karyotypes of M_1 plants, 2Kr dose. **a** M_1 mitosis 2n = 41, telocentric and small metacentric chromosomes arrowed; **b** M_1 mitosis 2n = 21, maternal haploid; **c** M_1 meiosis 2n = 42 comprising 4 telocentric univalents, 3 univalents, 2 heteromorphic bivalents, 2 rod bivalents, 3 ring bivalents, 2 trivalents, 2 quadrivalents, 1 heptivalent; **d** M_1 meiosis 2n = 41 comprising 1 telocentric univalent, 2 univalents, 5 rod bivalents, 3 ring bivalents, 4 trivalents, 1 quadrivalent, 1 pentivalent

Table 4. Mitotic chromosome counts on M_1 plants

Dose	No. plants scored	Chromosome numbers (telocentrics included)							
		21	37	38	39	40	41	42	43
5 Kr	3	–	–	–	1	–	2	–	–
3 Kr	5	–	1	1	1	–	–	2	–
2 Kr excised	46	1	2	–	7	4	10	19	3
germinated	129	–	–	2	12	26	51	37	1

and structural damage are shown in Fig. 1 a. The abnormalities seen in early development are probably a reflection of the elimination of damaged whole chromosomes or chromosome arms and chromosome fragments which fail to participate in mitosis which then gives rise to aneuploidy. Thus much of the phenotypic variation observed between the M_1 progenies must be attributable to aneuploidy and chromosome rearrangements following irradiation damage, although of course, mutations caused by irradiation may also play a part. Presumably the loss of expression

of the dominant major genes for ear morphology is a consequence of the absence of these genes or of modifiers in adult plants.

In addition to aneuploid plants, one plant at the 2Kr dose had the haploid number of 21 chromosomes (Fig. 1 b). Its morphology indicated it to be a 'Chinese Spring' haploid. This plant was treated with colchicine and the chromosome number doubled to produce a fertile doubled haploid which was indistinguishable from 'Chinese Spring' and did not exhibit any genetic segregation in its selfed progenies. This result confirms the observations of previous workers using irradiated pollen in wheat (Cao 1979). However the previous explanation of haploid production via irradiated pollen has been of female parthenogenesis stimulated by pseudo-fertilisation with irradiated pollen. On the basis of present results it seems more likely that fertilisation occurs to form a zygote but that during early development the complete, damaged, paternal genome is eliminated in the same way as in haploid formation by interspecific hybridisation with *Hordeum bulbosum* (Barclay 1975).

The meiotic configurations of the M_1 plants confirmed their aneuploid nature but also revealed a much wider range of structural rearrangements than could be seen at mitosis. Structural rearrangements were apparent even in plants which had the euploid number of 42 chromosomes and appeared to have a normal karyotype in mitotic preparations, and were in addition to the one, or rarely two, quadrivalents seen in normal F_1 plants. Typical meiotic configurations are shown in Figs. 1 c and d with univalents, heteromorphic bivalents, trivalents, quadrivalents and higher order multivalents. Configurations involving up to nine separate chromosomes were seen and most plants contained four or more multivalents per PMC. The explanation of this behaviour must be that chromosome breakages are caused by pollen irradiation and these can be repaired with non-homologous chromosome segments being combined into a new karyotypic structure. Consequently at meiosis, pairing with the undamaged maternal complement gives rise to a complex array of multivalents.

Performance of M_2 progenies

Measurements of quantitative characters. Of the two M_2 families grown, I19-1 and I30-2, from the 2Kr M_1 progenies, only 42 and 24 plants respectively, survived to maturity in the field. In terms of general morphology it was clear from a visual assessment that the progenies of both M_2's were more maternal in appearance than the corresponding F_2. This was particularly true for ear morphology, plant shape and leaf colour. However for quantitative measurements of individual characters this was not always the case. Data for the mean performance of the M_2, F_2, F_1 and parental genotypes for three characters, plant height, ear length and grain number on the leading tiller are shown in Table 5.

For ear length these data show that both M_2 families were more maternal in mean expression than the F_2, family I19-1 significantly so, although there was genetical variation for this character within the M_2 families. This suggests a greater representation of maternal genes than in the F_2. With respect to plant height, however, the M_2 were more paternal in mean expression than expected from the F_2. This latter result probably indicates a carry-over effect of radiation damage from the M_1 reflected in a reduction in height rather than an excess of paternal genes. This effect is probably also responsible for the reduced grain number per ear for both M_2 families which coincidentally makes them more maternal in performance.

The data for height and grain number appear to highlight a difficulty in assessing the effects of pollen irradiation without the inclusion of controls to separate the effects of irradiation damage from differential genetic transmission. When using a recessive maternal genotype which is lower scoring for fitness characters it would seem necessary to include the reciprocal cross of maternal × irradiated "maternal" progenies as controls

Table 5. Mean field performance of M_2 progenies

Genotype	No. plants	Ear length (mm)	Plant height (cm)	Grain no./ear
'Chinese Spring'	27	70.0	111.8	42.89
'Hobbit' (*T. spelta* 5A)	30	131.5	87.8	62.70
F_1	21	118.8	110.5	54.10
F_2	90	113.3	108.5	49.98
M_2I 19-1	42	89.6**	88.4***	24.70***
M_2I 30-2	24	107.5	97.5***	25.50***

Significance levels for differences between M_2 means and F_2 mean based on within plot variances: ** = 0.01 – 0.001, *** < 0.001

Table 6. Observed phenotype frequencies in M_2 (expected numbers in brackets)

Locus	Chromosome location		Phenotype			χ^2 deviation from F_2
			'Chinese Spring'	Heterozygote	'Hobbit' (*T. spelta* 5A)	
B1, B2, Hd	5A, 4B, 6B	F_2 ratio	12	37	42	
		M_2I 19-1	10	18	14	3.12
		M_2I 30-2	7	10	7	4.23
		Combined	17	28	21	6.26*
q	5AL	F_2 ratio	1		3	
		M_2I 19-1	28 (10.5)		14 (31.5)	38.89***
		M_2I 30-2	11 (6)		13 (18)	5.56*
Rht2	4D	F_2 ratio	1	2	1	
		M_2I 19-1	6 (8)	20 (16)	6 (8)	2.00
		M_2I 30-2	5 (5.75)	11 (11.5)	7 (5.75)	0.39
		Combined	11 (13.95)	31 (27.5)	13 (13.75)	1.04
Glu-B1	1BL	F_2 ratio	3		1	
		M_2I 19-1	30 (27.75)		7 (9.25)	0.73
		M_2I 30-2	18 (17.25)		5 (5.75)	0.13
		Combined	48 (45)		12 (15)	0.80
Gli-B1	1BS	F_2 ratio	1	2	1	
		M_2I 19-1	6 (8.75)	22 (17.5)	7 (8.75)	2.37
		M_2I 30-2	8 (5.75)	9 (11.5)	6 (5.75)	1.43
		Combined	14 (14.5)	31 (29)	13 (14.5)	0.17
Gli-A1	1AS	F_2 ratio	1	2	1	
		M_2I 19-1	8 (8.75)	22 (17.5)	5 (8.75)	2.83
		M_2I 30-2	5 (6)	11 (12)	8 (6)	0.92
		Combined	13 (14.75)	33 (29.5)	13 (14.75)	0.83
β-Amy-A2	5AL	F_2 ratio	1	2	1	
		M_2I 19-1	12 (9.5)	21 (19)	5 (9.5)	3.00
		M_2I 30-2	10 (6)	7 (12)	7 (6)	4.92
α-Amy-B1	6B	F_2 ratio	1	2	1	
		M_2I 19-1	16 (8.75)	12 (17.5)	7 (8.75)	8.09*
		M_2I 30-2	8 (4.75)	7 (9.5)	4 (4.75)	3.00
		Combined	24 (13.5)	19 (27)	11 (13.5)	11.00**
α-Amy-B2	7B	F_2 ratio	1	2	1	
		M_2I 19-1	14 (9.25)	18 (18.5)	5 (9.25)	4.41
		M_2I 30-2	4 (4.75)	10 (9.5)	5 (4.75)	0.16
		Combined	18 (14)	28 (28)	10 (14)	2.29

Significance levels: * = 0.1 – 0.01; ** = 0.01 – 0.001; *** < 0.001

to unambiguously exclude radiation damage or mutation as the effects being observed.

M_2 phenotpye frequencies for marker systems. Disturbances of gene frequencies in the M_2 can be unambiguously assessed by analysing the inheritance of alleles for the marker loci. The observed numbers of each phenotype for each system for the two families separately and, when homogeneous, for the families combined are shown in Table 6, together with the expected numbers from the F_2 ratios and the χ^2 deviation for fit to the F_2 ratio.

A significant difference from the F_2 ratio was exhibited by three out of the nine marker systems examined. In the case of awning, and loci *q* and *α-Amy-B1* the difference is due to a significant excess of maternal phenotypes and a deficit of paternal homozygotes. Further for three other loci, *Glu-B1*, *β-Amy-A2* and *α-Amy-B2* an excess, though not significant, was apparent for one or both families. Three of these loci, *B1*, *q* and *β-Amy-A2* are known to be linked on the long arm of chromosome 5A and, as expected, they show the same pattern of behaviour. Thus for linked and unlinked loci the M_1 parents must have been heterozygous for the alleles concerned but that these were transmitted differentially to the viable M_2 progeny with the maternal allele predominating. There is, however, heterogeneity between the two M_2 families for

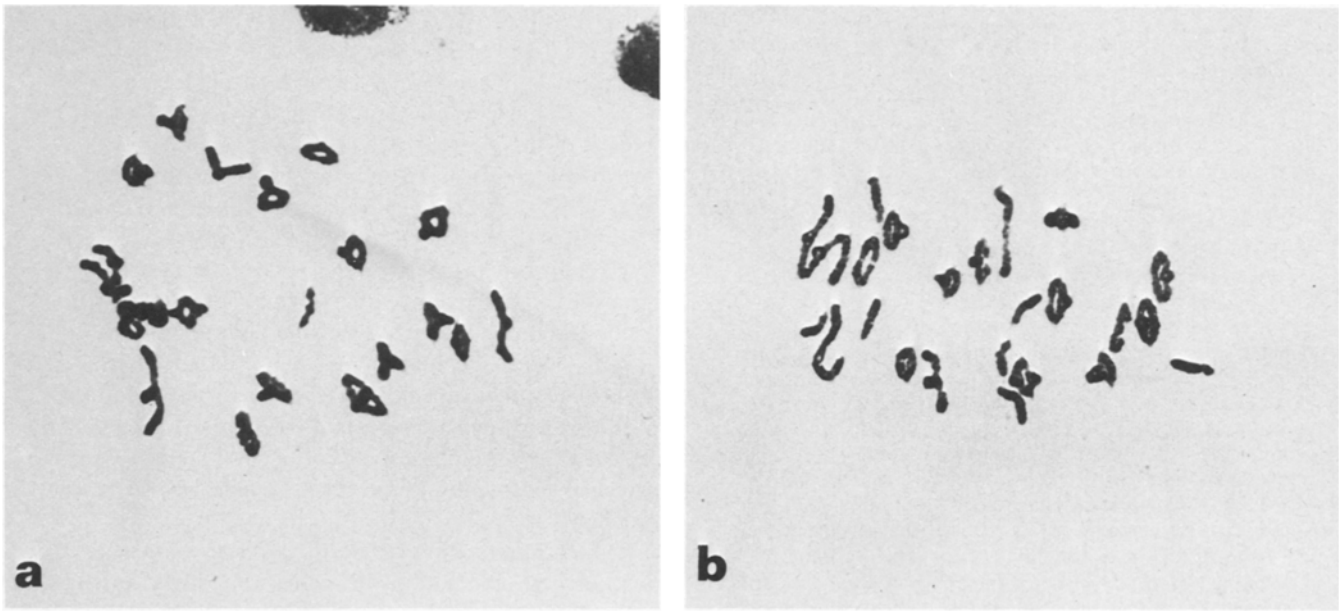

Fig. 2 a, b. Meiotic configurations of M_2 plants, 2Kr dose. **a** 2n = 42 comprising 5 rod bivalents, 15 ring bivalents, 1 telocentric bivalent. **b** 2n = 42, 2 univalents, 4 rod bivalents, 12 ring bivalents, 1 trivalent, 1 quadrivalent

certain loci. Consequently it would appear that this phenomenon is not specific to any particular locus or chromosome and presumably occurs at random throughout the genome.

Overall the results for these marker systems show that the maternal genome is represented to a greater extent than the paternal genome in the M_2 progeny due to, predominantly, differential transmission or survival from heterozygous M_1 plants. This would also explain the more maternal phenotype of the M_2 progenies relative to the F_2 for the quantitative characters examined which are determined by many loci acting together.

M_2 chromosome constitutions. Meiotic chromosome counts were obtained on 19 plants of family I19-1 and on 14 plants of family I30-2. As in the M_1 progenies, aneuploidy and structural rearrangements were present in both families with univalents, whole or telocentric, and one or two trivalents or quadrivalents per plant. Typical configurations are shown in Figs. 2a and b. There was, however, a much higher frequency of 42 chromosome progenies than in the M_1 and a lower frequency of multivalents, generally only one, and occasionally two trivalents or quadrivalents per plant, which would also be expected in unirradiated progeny since the parents differ by two reciprocal translocations. No higher order multivalents were found. It would appear that the M_2 progenies have a much more normal karyotype than that seen in the M_1 plant. However the particular M_1 plants giving rise to these M_2's were not themselves scored meiotically so that a direct comparison was not possible.

Discussion

The phenomenon observed in the present experiments is the same as that observed in interspecific crosses in *Nicotiana* (Pandey 1975; 1978), in intraspecific crosses in *Nicotiana rustica* (Jinks et al. 1981; Caligari et al. 1981) and in intervarietal crosses in *Hordeum vulgare* (Powell et al. 1983). This is the limited transmission of the paternal genome in crosses using irradiated pollen resulting in progeny which have a greater proportion of maternal genes than would result from segregation from the unirradiated cross. In the present experiments in wheat this occurs for two reasons. Firstly a certain proportion of the paternal genome is eliminated early in development resulting in hemizygosity so that subsequent progeny can only contain the maternal allele. Secondly, other loci are heterozygous in the M_1 but in some cases the maternal allele is transmitted to surviving M_2 progeny at a higher frequency than the paternal allele. On the present data these process would appear to be random with respect to the loci and chromosomes which are affected, resulting in variation between M_2 families in the proportion of the maternal contribution and the frequencies of the paternal alleles present.

In the work in *Nicotiana* no definitive evidence for a mechanism for this phenomenon has been found. However, Pandey (1978) has proposed a model of the incorporation of paternal DNA fragments into the egg nucleus by "pseudo-

fertilisation" followed by parthenogenetic development of the egg. This results in M_1 plants which are effectively doubled haploids but contain a triallelic chromosome structure for the transformed loci. Following meiosis normal balanced gametes containing the transformed chromosomes are produced which give rise to normal diploid M_2 progeny. On this model, only a few small fragments of the paternal genome are incorporated into the egg nucleus.

This mechanism is not applicable to these results in wheat. In the present work true fertilisation takes place resulting in a zygote which contains a normal maternal chromosome complement and a damaged paternal complement. During early development certain parts of the paternal genome are eliminated because, presumably, they cannot participate in mitosis. In the extreme case the whole paternal genome is eliminated resulting in a maternal haploid. The surviving M_1 plants contain most and in some cases all of the paternal genome but, however, it has been reorganised into a different karyotype to the maternal genome. At meiosis pairing between the two genomes takes place resulting in multivalent formation. The gametes produced from this disturbed meiosis will undoubtedly have complex combinations of maternal and paternal genes. However since the progeny from this process are predominantly maternal the viable gametes must contain a higher proportion of maternal chromosomes. Hence either paternal chromosomes cannot undergo the mechanical movements during meiosis as effectively, or gametic function has been lost, resulting in pollen or zygote lethality when paternal chromosomes are present. Thus the M_1 meiosis would appear to act as a "meiotic sieve" to limit transmission of paternal genes.

This model is not at variance with the results obtained in *Nicotiana rustica* and in barley. Indeed preliminary studies of meiosis in M_1 plants in barley show the presence of multivalents (Powell et al. 1983). This model may also explain the observation that there is not always a very good correspondence between M_1 performance and their M_2 progenies. This was noted in the present work in barley and in *Nicotiana rustica* (Caligari, pers. comm.). This would occur if alleles present in the M_1 were differentially transmitted to the M_2.

The potential use of irradiated pollen in plant breeding would be to transfer single genes between paternal and maternal genotypes so reducing the time and effort necessary to achieve this transfer by recurrent backcrossing. In wheat the success of this approach will depend on the proportion of the paternal genome which is present in the M_2 progenies. This appears to vary between M_2 families and will also, undoubtedly, vary with the irradiation dose used, since in barley and *Nicotiana rustica* the proportion of the paternal genome eliminated increased with increasing dose. However, a compromise has to be made between using a high dose to cause maximum genome damage and producing viable M_1 progeny which are fertile. In the present work 2Kr would appear to be this dose. At this dose M_2 plants contain a lower frequency of paternal genes than the F_2 but no M_2 plant was homozygous or heterozygous for only one paternal marker gene allele and maternal for all the others. Nevertheless in terms of the small numbers of M_2 plants obtained a considerable difference in genotype frequencies of rare recombinants over the F_2 was observed. For example in family I19-1, 4 plants out of 42 were homozygous for the maternal alleles for the unlinked amylase loci, *β-amy-A2* on chromosome 5A, *α-amy-B1* on chromosome 6B and *α-amy-B2* on chromosome 7B, whereas only 1/64 of the progeny would have this genotype in the F_2. However it should be noted that this is only as effective as one round of backcrossing where 1/8 of the progeny would be homozygous for three maternal alleles. Of these four progeny one was homozygous for the paternal allele at the *Rht* locus and one homozygous for the paternal allele at the *Glu-B1* locus – combinations expected to occur at a frequency of only 1/256 in the F_2. These genotypes would not appear in the first backcross although on selfing they should appear at a frequency of 1/64, which is marginally lower than the observed frequency in the M_2.

In plant breeding terms the present data suggests that pollen irradiation in wheat has only a slight advantage over backcrossing although technically it is quicker and easier to perform. Also it would be particularly useful in transferring recessive alleles which in a backcrossing programme can only be followed by selfing the crossed progeny every generation. Higher radiation doses may allow a greater proportion of the paternal genome to be eliminated in future studies. However the polyploid nature of wheat may also allow the transmission of a greater proportion of genetically and cytologically unbalanced gametes so that "cleaning up" is still necessary to extract stable, homozygous genotypes. This would not be expected to occur in a diploid species such as barley. Consequently lower frequencies of paternal genes in the M_2 may be achieved more readily in a diploid crop species.

Acknowledgements. Thanks are due to the Department of Medical Physics, University of Cambridge, for use of their irradiation facilities and to Mr. R. Gouldstone and Mr. D. Adams fo their expert technical assistance. Thanks are also due to Dr. P. D. S. Caligari for helpful discussions during the conduct of these experiments.

References

Ainsworth CC, Gale MD, Baird S (1983) The genetics of β-amylase isozymes in wheat. 1. Allelic variation amongst hexaploid varieties and intrachromosomal gene locations (in press)

Barclay IR (1975) High frequencies of haploid production in wheat (*Triticum aestivum*) by chromosome elimination. Nature 186:410–411

Bennett MD, Finch RA, Barclay IR (1976) The time, rate and mechanism of chromosome elimination in *Hordeum* hybrids. Chromosoma 54:175–200

Bennett MD, Rao MK, Smith JB, Bayliss MW (1973) Cell development in the anther, the ovule, and the young seed of *Triticum aestivum* L. var. 'Chinese Spring'. Philos Trans R Soc London Ser B 266:39–81

Caligari PDS, Ingram NR, Jinks JL (1981) Gene transfer in *Nicotiana rustica* by means of irradiated pollen. I. Unselected progenies. Heredity 47:17–26

Cao H, Song H, Hu O, He Z, Wei X (1979) Induction and utilisation of parthenogenesis in wheat (*Triticum aestivum*) breeding. 2. Parthenogenesis in *Triticum aestivum* following crosses with x-rayed pollen. Ann Rep Inst Genet Acad Sin 1979, pp 183–185

Gale MD, Law CN, Worland AJ (1975) The chromosomal location of a major dwarfing gene from Norin 10 in new British semi-dwarf wheats. Heredity 35:417–421

Gale MD, Law CN, Chojecki AJ, Kempton RA (1983) Genetic control of α-amylase production in wheat. Theor Appl Genet 64:309–316

Jinks JL, Caligari PDS, Ingram NR (1981) Gene transfer in *Nicotiana rustica* using irradiated pollen. Nature 291: 586–588

Katayama Y (1934) Haploid formation by x-rays in *Triticum monococcum*. Cytologia 5:235–237

Law CN; Worland AJ (1974) Genes affecting awning. European Aneuploid Co-operative Newslett 4:5–7

Natarajan AT, Swaminathan MS (1958) Haploidy induced by radiations in wheat. Experientia 14: 336–337

McIntosh RA (1973) A catalogue of gene symbols for wheat. In: Proc 4th Int Wheat Genet Symp. Columbia, Missouri, Missouri Agr Exp Sta, pp 893–938

Micke A, Donini B (1982) Use of induced mutations in improvement of seed propagated crops. In: Induced variability in plant breeding. Wageningen, The Netherlands, Centre for Agricultural Publishing and Documentation, pp 2–9

Pandey KK (1975) Sexual transfer of specific genes without gametic fusion. Nature 256:310–313

Pandey KK (1978) Gametic gene transfer in *Nicotiana* by means of irradiated pollen. Genetica 49:53–69

Payne PI, Holt LM, Worland AJ, Law CN (1982) Structural and genetical studies on the high molecular weight subunits of wheat glutenin. Part 3. Telocentric mapping of the subunit genes on the long arms of the homologous Group 1 chromosomes. Theor Appl Genet 63:129–138

Powell W, Caligari PDS, Hayter AM (1983) The use of pollen irradiation in barley breeding. Theor Appl Genet 65:73–76

Simpson E, Snape JW (1981) The use of doubled haploids in a winter barley programme. In: Barley genetics IV. Proc 4th Int Barley Genet Symp. Edinburgh, pp 716–720