Maternal imprinting effect on B chromosome transmission in rye

M. J. Puertas,
M. M. Jiménez,
F. Romera,
J. M. Vega and
M. Díez

Departamento de Genética, Facultad de Biología,
Universidad Complutense, 28040 Madrid, Spain.

A new feature affecting the transmission rate of rye B chromosomes is described. Progenies of $0B \times 2B$, $2B \times 0B$ and $2B \times 2B$ crosses were studied, considering both the B chromosome number of the plants crossed and that of their maternal parents. A remarkably large variation within these progenies was observed. The variation found in $0B \times 2B$ crosses was mainly due to the effect of the maternal parent of the males and females involved: pollen grains formed in 2B plants whose maternal parent had 2Bs transmitted B chromosomes at high frequency; 0B plants whose maternal parent had 2B, accepted pollen with Bs at high frequency. Conversely, 2B males and 0B females with 0B maternal parent, formed progeny with B chromosomes at a much lower frequency.

A very large variation in B chromosome frequency was also observed in progenies of $2B \times 0B$ crosses although in this case the maternal parent of 2B females does not seem to be the cause of such variation.

The observed progenies of $2B \times 2B$ crosses fitted with expectations only when the effect of the maternal parent of the plants used for crosses was considered. The interaction between females having a 2B maternal parent with 2B pollen, and the effect of the male maternal parent was also evident in this case.

It seems that 2B chromosomes produce an imprinting in plants, in such a way that all their descendants can be recognised whether they carry Bs or not. The frequency of transmission of B chromosomes is increased through the plants with a 2B maternal parent. A 0B maternal parent causes the transmission frequency to decrease. We believe that these interactions have an important effect at the population level on the maintenance of the polymorphism for B chromosomes.

INTRODUCTION

In almost all species studied the transmission of B chromosomes is nonMendelian. In rye, the most obvious and best known causes of this behaviour are their peculiar meiosis (Kishikawa, 1965) and the directed nondisjunction of the B chromosome which occurs during gametogenesis (Müntzing, 1946; Håkanson, 1948). However, these features are not the only phenomena which causes the transmission of B chromosomes to deviate from Mendelian expectations.

Table 1 (modified from Matthews, 1981) shows a summary of all crosses, reported to date, involving 0B and 2B rye plants. Two types of variation of the number of Bs among the progenies are remarkable: (i) the variation among B classes within crosses and (ii) the variation of the frequency of B classes among crosses. The source of the first type of variation is the non-Mendelian behaviour of B chromosomes during

gametogenesis. The cause of the second type of variation is unknown.

In the present work we have studied progenies obtained in $0B \times 2B$, $2B \times 0B$ and $2B \times 2B$ crosses considering both the B number of the plants used in the crosses, as males or females, and that of their maternal parents. The objective was to investigate whether the transmission of gametes with different numbers of Bs is influenced by the B number of the maternal parent of the plants used in the crosses.

MATERIAL AND METHODS

The material used was *Secale cereale* with B chromosomes from the Puyo population (Korea), which was kindly provided by Dr Lee (Seoul National University).

Chromosome counts were made during two consecutive years in order to obtain plants with 0

Table 1 Progeny obtained in crosses involving 0B and 2B plants

Cross	B frequency in the progeny					No. of plants screened	Variety	Reference
	0B	1B	2B	3B	4B			
0B × 2B	0.50	0.20	0.30			44	Ostgota Grarag	Müntzing, 1945
	0.32	0.18	0.50			60	JNK	*
	0.22	0.22	0.53		0.03	72	Caribou	Puertas and Lacadena, 1974
	0.10	0.11	0.79			63	Wasa II	Müntzing, 1966
2B × 0B	0.55	0.04	0.41			113	Ostgota Grarag	Müntzing, 1945
	0.50	0.10	0.40			20	Ostgota Grarag	Müntzing, 1943
	0.50		0.46	0.03	0.01	97	Caribou	Puertas and Lacadena, 1974
	0.23		0.67	0.01	0.09	86	JNK	*
	0.16	0.03	0.81			31	Wasa II	Müntzing, 1945
	0.07	0.04	0.89			92	Wasa II	Müntzing, 1966
2B × 2B	0.35	0.12	0.35	0.04	0.14	223	Ostgota Grarag	Müntzing, 1943
	0.26		0.56	0.04	0.14	50	Ostgota Grarag	Müntzing, 1954
	0.25	0.07	0.42	0.10	0.16	96	JNK	*
	0.23	0.06	0.42	0.04	0.25	129	Ostgota Grarag	Müntzing, 1954
	0.21	0.10	0.33	0.04	0.32	79	Ostgota Grarag	Müntzing, 1954
	0.15	0.02	0.53		0.30	86	Turkey I	Müntzing, 1954
	0.08		0.46		0.46	52	Wasa II	Müntzing, 1945
	0.06	0.04	0.43	0.07	0.40	119	Afghanistan	Müntzing, 1954
	0.04	0.11	0.46	0.12	0.27	74	Turkey III	Müntzing, 1954
	0.04	0.02	0.29	0.04	0.61	48	Transbaikal	Müntzing, 1954
	0.02	0.02	0.18	0.13	0.65	125	Turkey II	Müntzing, 1950
	0.01	0.04	0.23	0.15	0.57	376	Caribou	Puertas and Lacadena, 1974
	0.01	0.04	0.21	0.11	0.63	196	Turkey II	Müntzing, 1954
	0.00		0.32	0.11	0.57	28	Turkey IV	Müntzing, 1954
	0.00		0.20	0.06	0.74	83	Wasa II	Müntzing, 1954
	0.00		0.17	0.03	0.80	59	Wasa II	Müntzing, 1966

* Data of our laboratory.

or 2B chromosomes from either 0 or 2B maternal parents, in all possible combinations. The plants were named as follows:

0/0: plant with 0B, whose maternal parent had 0B

2/0: plant with 0B, whose maternal parent had 2B

0/2: plant with 2B, whose maternal parent had 0B

2/2: plant with 2B, whose maternal parent had 2B.

The number on the right is the number of Bs of the plant used in the crosses, either as male or female, and the number on the left is the number of Bs of its maternal parent.

The plants were crossed in all the 12 possible combinations involving 0B × 2B, 2B × 0B or 2B × 2B crosses (0/0 × 0/2, 0/2 × 0/0, etc.). Four plots were made. In each plot a single type of plant was used as males (5 plants), and two (0/2 and 2/2, when males had 0B) or four (0/0, 2/0, 0/2 and 2/2, when males had 2B) types of plant were used as females, with 5 plants of each type. In every case the plants used as females were emasculated, and pollen from the male plants pollinated the females at random. All the plots were isolated in

such a way that open pollination within the plots was allowed, but cross-pollination among plots was prevented.

Offspring were individually collected from the females. About 25 seeds per female were screened for the B number; *i.e.*, about 125 per cross.

As three different generations are involved in these experiments, it is worth bearing in mind that we refer to *maternal parents* as the plants from which we obtained the *males* and *females* used in the crosses; and *offspring* or *progenies* as the descendants obtained from the crosses.

Chromosome numbers were counted in root tips following fixation in 1:3 acetic alcohol and staining by the Feulgen method.

RESULTS

The progeny obtained from the 0B × 2B, 2B × 0B and 2B × 2B groups of crosses are shown in tables 2, 3 and 4, respectively. The 16 different types of crosses were each made with five females, although in some cases the progeny of only four of them

Table 2 Progeny obtained from 0B × 2B crosses

Cross			B frequency in the progeny					Number of seeds screened	Mean number of Bs per plant
Female	Male	Female	0B	1B	2B	3B	4B		
0/0	0/2	1	0.48	0.04	0.48			25	1.00
		2	0.38	0.06	0.56			16	1.19
		3	0.32		0.68			25	1.36
		4	0.31	0.19	0.50			25	1.19
		5	0.13	0.07	0.78		0.02	46	1.71
2/0	0/2	1	0.32	0.12	0.56			25	1.24
		2	0.24	0.44	0.32			25	1.08
		3	0.20	0.16	0.64			25	1.44
		4	0.16		0.84			25	1.68
		5	0.08	0.24	0.68			25	1.60
0/0	2/2	1	0.20		0.80			25	1.60
		2	0.16	0.16	0.68			25	1.52
		3	0.16	0.08	0.76			25	1.60
		4	0.12	0.16	0.68		0.04	25	1.68
		5	0.12	0.08	0.80			25	1.68
2/0	2/2	1	0.05	0.05	0.90			21	1.86
		2			0.78		0.22	36	2.50
		3			0.53		0.47	30	2.93
		4			0.33	0.12	0.55	33	3.21

Table 3 Progeny obtained from 2B × 0B crosses

Cross			B frequency in the progeny					Number of seeds screened	Mean number of Bs per plant
Female	Male	Female	0B	1B	2B	3B	4B		
0/2	0/0	1	0.56		0.40		0.04	25	0.96
		2	0.33		0.67			15	1.33
		3	0.20		0.80			25	1.60
		4	0.16		0.84			25	1.68
0/2	2/0	1	0.48		0.52			25	1.04
		2	0.28		0.72			25	1.44
		3	0.12		0.88			25	1.76
		4	0.04		0.84		0.12	25	2.16
2/2	0/0	1	0.75		0.25			20	0.50
		2	0.45		0.55			20	1.10
		3	0.28		0.60	0.04	0.08	25	1.64
		4	0.21		0.79			24	1.58
		5	0.16	0.04	0.80			25	1.64
2/2	2/0	1	0.76		0.24			25	0.48
		2	0.48		0.52			25	1.04
		3	0.31		0.69			26	1.38
		4	0.18	0.04	0.78			23	1.61
		5	0.13		0.80	0.07		15	1.80

are shown, this is because counts made on less than 15 descendants of a cross were not considered. Chromosome counts were generally made in 25 seeds per female; occasionally, we made more counts in an attempt to decrease the large variance observed.

ANOVAS were made in order to compare the mean number of Bs per plant obtained in all crosses

(table 5). First we compared the four types of crosses within each one of 0B × 2B, 2B × 0B or 2B × 2B groups. Significant differences were obtained in two out of the three cases.

Since the maternal parent is the only known source of variation to account for the differences, we made ANOVAS to estimate whether the number of Bs of the maternal parent, both on the female

Table 4 Progeny obtained from 2B×2B crosses

Cross			B frequency in the progeny						Number of seed screened	Mean number of Bs per plant
Female	Male	Female	0B	1B	2B	3B	4B	6B		
0/2	0/2	1	0.32	0.24	0.28	0.12	0.04		25	1.28
		2	0.20	0.04	0.56	0.08	0.12		25	1.76
		3	0.12	0.12	0.32	0.04	0.40		25	2.48
		4	0.08		0.44		0.44	0.04	25	2.88
		5			0.48		0.52		25	3.04
0/2	2/2	1	0.05		0.36		0.59		22	3.08
		2	0.04	0.04	0.44		0.48		25	2.84
		3			0.42		0.58		24	3.50
		4			0.40		0.60		25	3.20
		5			0.29		0.67	0.04	24	3.16
2/2	0/2	1	0.16	0.04	0.36		0.44		25	2.52
		2	0.12		0.40		0.48		25	2.72
		3	0.08		0.44	0.04	0.40	0.04	25	2.84
		4			0.32		0.64		25	3.20
2/2	2/2	1	0.06	0.03	0.55		0.36		33	2.47
		2	0.04		0.44	0.04	0.48		25	2.92
		3	0.02		0.32		0.64	0.02	41	3.32
		4		0.04	0.48	0.04	0.44		25	2.84
		5			0.56		0.44		25	2.88

and male side, had any effect on the distribution of Bs in the offspring. We first compared the mean number of Bs obtained in 0B×2B, 2B×0B or 2B×2B crosses, distributed in groups with different maternal parents of the females, irrespective the maternal parent of the males, and second comparing the crosses with different maternal parents of

males, irrespective the maternal parents of the females. The results were significant in several cases (table 5).

Table 6 shows the average mean of Bs per plant in all crosses. It is remarkable how large a variance there is within crosses in some cases, as shown by the mean errors; specially, the 2B×0B crosses

Table 5 ANOVAS comparing the offspring obtained according to their maternal parents

Variable	Source of variation	Crosses compared	Level of significance
Bs per plant	Number of Bs of the maternal parent	all 0B×2B	***
		all 2B×0B	ns
		all 2B×2B	*
Bs per plant	Number of Bs of the maternal parent on the female side	0/0×-2	***
		2/0×-2	
		0/2×-0	ns
		2/2×-0	
		0/2×-2	ns
		2/2×-2	
	Number of Bs of the maternal parent on the male side	-0×0/2	***
		-0×2/2	
		-2×0/0	ns
		-2×2/0	
		-2×0/2	***
		-2×2/2	

* 0.10 > P > 0.05.

*** significant at 0.01.

ns = nonsignificant.

Table 6 Mean number of Bs per plant observed in all crosses

Males				
2/2	1.62 ± 0.03	2.62 ± 0.25	3.16 ± 0.09	2.89 ± 0.12
0/2	1.29 ± 0.11	1.41 ± 0.10	2.29 ± 0.30	2.82 ± 0.12
2/0			1.60 ± 0.21	1.26 ± 0.21
0/0			1.39 ± 0.14	1.29 ± 0.20
0/0	2/0	0/2	2/2	females

which did not significantly differ when they were compared by an ANOVA, but the progenies obtained within each cross are highly variable (see also table 3).

It could be assumed that the frequency of B chromosomes transmitted by 2B plants as females, could be estimated from the progeny of a 2B × 0B cross, and similarly, that male gametes could be estimated from a 0B × 2B cross. However, our results (tables 2, 3 and 6) indicate that such assumptions could be wrong if the results of particular 2B × 0B and 0B × 2B crosses are considered, due to the large variation within crosses. In order to calculate the expected offspring in a 2B × 2B cross, we considered all possible combinations among these ranges of frequencies, to obtain the distributions of Bs expected in all possible types of crosses (all possible gametes transmitted by 2B females × male gametes transmitted in a 0/0 × 0/2 cross; all possible gametes transmitted by 2B females × male gametes transmitted in a 0/0 × 2/2 cross, and so on). These distributions were considered in various combinations and the values were grouped in two intervals (1 to 3Bs and 3Bs or more) and compared with the observed distributions (table 7). Only two intervals were made since we have only 19 observed results in 2B × 2B crosses and the χ^2 value could not be calculated if more classes were considered. Even with only two classes, some χ^2 values could not be calculated, although in those cases similarities of dissimilarities between the observed and expected distributions are obvious.

DISCUSSION

Rye is one of the species in which many results of controlled crosses to study the transmission of B chromosomes have been reported. However, the genetic system of B chromosomes is still poorly

understood, due to its uniqueness and wide departure from Mendelian rules.

Table 1 (modified from Matthews, 1981) shows all the results, reported to date, of crosses involving 0B and 2B plants. It is arranged with the progenies ordered from higher to lower frequency of 0B plants, and shows several remarkable features of the data.

Table 7 Distribution of the observed and expected frequencies of B chromosomes in 2B × 2B crosses

Crosses compared	Intervals	Observed	Expected	χ^2
Obs.: 2B × 2B	1 to 3Bs	12	9.55	ns
Exp.: 2B × 2B	3 or more	7	9.45	
Obs.: 2B × 2B	1 to 3Bs	12	11.40	ns
Exp.: 0/0 × -2	3 or more	7	7.60	
Obs.: 2B × 2B	1 to 3Bs	12	7.50	*
Exp.: 2/0 × -2	3 or more	7	11.50	
Obs.: 2B × 0/2	1 to 3Bs	7	7.10	†
Exp.: 0/0 × 0/2	3 or more	2	1.90	
Obs.: 2B × 0/2	1 to 3Bs	7	5.60	ns
Exp.: 2/0 × 0/2	3 or more	2	4.40	
Obs.: 2B × 0/2	1 to 3Bs	7	6.35	†
Exp.: -0 × 0/2	3 or more	2	2.65	
Obs.: 2B × 0/2	1 to 3Bs	7	2.5	‡
Exp.: -0 × 2/2	3 or more	2	6.5	
Obs.: 2B × 2/2	1 to 3 Bs	5	4.11	ns
Exp.: 0/0 × 2/2	3 or more	5	5.89	
Obs.: 2B × 2/2	1 to 3Bs	5	1.11	‡
Exp.: 2/0 × 2/2	3 or more	5	8.89	
Obs.: 2B × 2/2	1 to 3Bs	5	2.77	‡
Exp.: -0 × 2/2	3 or more	5	7.22	
Obs.: 2B × 2/2	1 to 3Bs	5	7.05	‡
Exp.: -0 × 0/2	3 or more	5	2.95	

ns = nonsignificant.

* significant at 0.05.

† χ^2 cannot be calculated, but observed and expected distributions are very similar.

‡ χ^2 cannot be calculated, but observed and expected distributions are very different.

- (i) Very large differences in B chromosome frequency are observed within the offspring obtained from $0B \times 2B$, $2B \times 0B$ and $2B \times 2B$ crosses. It can be concluded that 2B plants transmit B chromosomes with very variable frequencies.
- (ii) It seems that there are rye varieties which tend to form many 0B plants (*i.e.*, Ostgota Grarag), while others tend to transmit many Bs (Wasa II). However, this relation with the variety is not complete; for example, the results with Turkey and Wasa II are rather variable in $2B \times 2B$ crosses.
- (iii) Theoretically, the frequency of the gametes carrying B chromosomes transmitted by a 2B plant either as male or female, can be estimated from the progenies obtained in $0B \times 2B$ and $2B \times 0B$ crosses respectively; and in this way, the expected progeny of a $2B \times 2B$ cross can be calculated. Let m_0 , m_1 and m_2 be the gametes with 0, 1 or 2Bs transmitted by a 2B plant in a $0B \times 2B$ cross, and f_0 , f_1 and f_2 the gametes with 0, 1 or 2Bs transmitted by a 2B plant in a $2B \times 0B$ cross. The expected distribution of Bs in a $2B \times 2B$ cross is $m_0 \times f_0$ of 0B, $(m_0 \times f_1 + m_1 \times f_0)$ of 1B, and so on. As a matter of fact, the progeny observed in $2B \times 2B$ crosses significantly differ from expected in all cases. This can be verified in the varieties Ostgota Grarag, JNK, Caribou and Wasa II which appear in the three groups of table 1. For example, in Caribou 53 per cent of gametes with 2B were transmitted in the $0B \times 2B$ cross, and 46 per cent in the $2B \times 0B$. So that 24 per cent of 4B plants are expected in the $2B \times 2B$ cross, while more than double this frequency were observed. The deviation between observed and expected distributions in $2B \times 2B$ progenies does not follow any apparent pattern. For example, in Ostgota Grarag and Caribou about double the number of 4B plants were observed than expected, in JNK it was just the opposite, while in Wasa II there was no difference for this class.

In the crosses made in this work we observe a remarkably large variation of B chromosome frequency obtained in the progenies (tables 2, 3, and 4). For example, the frequency of 0B plants varies from 0 to 48 per cent in $0B \times 2B$ crosses, from 4 to 76 per cent in $2B \times 0B$, and from 0 to 32 per cent in $2B \times 2B$. It should also be noted that the variation observed in the crosses made in this work, with a single variety, is of the same magnitude to that observed in table 1 for many different varieties. This variation cannot be explained by

any of the features of rye B chromosomes known to date.

We shall demonstrate that one of the causes of this variation in the transmission rate of B chromosomes is whether or not the maternal parent of the plants involved in the crosses carried B chromosomes. In addition, we shall see that another still unknown cause must be also involved.

0B × 2B crosses (table 2)

It is our repeated experience that the frequency of pollen grain types expected to participate in pollination, based on direct observation of chromosome numbers at pollen mitosis, differs markedly from the progeny types observed in a $0B \times 2B$ cross (Puertas *et al.*, 1986, 1988). In all the rye varieties in which we have studied pollen grain mitosis (Puertas *et al.*, 1979; Romera *et al.*, 1989), a remarkable similarity was observed, irrespective of the variety: a 2B plant forms about 5 per cent of pollen grains with 0, 1, 3 or 4Bs and the remaining 80–85 per cent have 2Bs. These frequencies of pollen types formed differ greatly from the frequencies obtained in the offspring of $0B \times 2B$ crosses.

It is remarkable that in the crosses $0/0 \times 0/2$, $2/0 \times 0/2$ and $0/0 \times 2/2$ the frequency of descendants with 0B was high, while in the cross $2/0 \times 2/2$ the frequency of 4B plants was high with almost no 0B plants. All these results are far from expected, according to the frequencies of pollen formed, and indicate that the difference, in all probability, is due to differential fertilisation and not to differential viability of zygotes carrying different number of B chromosomes.

In spite of the high frequency of 0B plants in some cases, the mean number of Bs per plant transmitted in all crosses is higher than 1 (only in one case is equal to 1), indicating a preferential transmission of B chromosomes to the offspring.

It is evident that the four groups of crosses shown in table 2 are different, as demonstrated by the corresponding ANOVA (table 5). It is also evident that the B status of the maternal parent, on both the male and female side, has an essential role in accounting for the differences: $2/2$ males produce more Bs in the offspring than $0/2$ males on any 0B female, and more Bs were produced in $2/0$ females than in $0/0$ ones (table 6). The joint effect of $0/2$ males and $0/0$ females produces a remarkable increase of 0B descendants, while the joint effect of $2/2$ males and $2/0$ females produces a remarkable increase of 4B descendants. It should be noted that only about 3 per cent 0B and 2 per cent 4B pollen grains are produced, while about

50 per cent were transmitted in some cases. As was mentioned above, this increase of 0B and 4B descendants has been observed in previous works, although these results are so unexpected, particularly the increase of 4B plants, that we consider that these crosses should be further investigated.

In previous works (Puertas *et al.*, 1986, 1988) we have hypothesised the existence of pollen-style interactions to explain the competition of different types of pollen grains on 0B stigmas. The results of this work show that at least one of the causes of such differential fertilisation is the B status of the maternal parent of plants involved in $0B \times 2B$ crosses: (i) when pollen grains are formed in 2B plants whose maternal parent had 2B, they transmit Bs with high efficiency; (ii) when stigmas belong to 0B plants whose maternal parent had 2B, they accept Bs with high efficiency; (iii) when these two effects occur together, the number of Bs transmitted to offspring is very high; (iv) the opposite is also true, when pollen is formed in 2B plants whose maternal parent had 0B it transmits the Bs with low efficiency, when the styles belong to 0B plants whose maternal parent had 0Bs they better accept 0B pollen, and therefore, the minimum frequency of Bs is obtained in $0/0 \times 0/2$ crosses.

2B × 0B crosses (table 3)

In this case the transmission data cannot be compared with the cytological data, since female gametogenesis is difficult to observe, specially in a quantitative analysis. However, according to the results of crosses, we can deduce that nondisjunction of B chromosomes occurred in all cases since 1B descendants were almost never observed. This small number of 1B descendants has also been observed in other rye varieties (table 1).

It is also interesting to note that in several cases the mean number of Bs transmitted was less than 1 B per plant, which means that the transmission of Bs is not always preferential on the female side, indicating a less efficient mechanism of transmission than on the male side; however, the average mean of Bs transmitted was higher than 1 in all crosses (table 6).

A very large variation within crosses, with double or triple differences, was observed. The ANOVA (table 5) indicate that there are not significant differences among the four types of $2B \times 0B$ crosses. The slight interaction between 2B females and 0B males was also noted in previous works (Puertas *et al.*, 1988), in which we did not detect competition between 0B and 2B pollen on 2B

females. However, this does not mean that the maternal parent on the female side does not affect the transmission of Bs, as it does on the male side. When crosses are compared according to the number of Bs of the maternal parent on the female side (table 5) there are significant differences between $0/0$ and $2/0$ females.

We think that the variation within each type of $2B \times 0B$ cross is produced by another cause, which is still unknown. Genotypes affecting the rate of transmission of B chromosomes in females could account for this variation, but this possibility has to be further investigated. Such hypothetical genotypes would have a stronger effect than that of the maternal parent of females, producing this large variance and obscuring its effect. It is possible that on the female side two effects occur together, one gametophytic and depending on the genotype of the female, and the other sporophytic and depending on the maternal parent. In the two animal species *Myrmeleotettix maculatus* and *Pseudococcus affinis*, genotypes affecting the transmission rate of B chromosomes have been reported (Shaw, and Hewitt, 1985; Shaw *et al.*, 1985; Nur and Brett, 1987, 1988).

2B × 2B crosses (table 4)

The intra-cross variation, together with the interactions that we have just discussed, make it impossible to estimate the expected distribution of progenies of $2B \times 2B$ crosses, according to the observations of one or a few $2B \times 0B$ and $0B \times 2B$ particular crosses. This is, in all probability the reason why the $2B \times 2B$ crosses shown in table 1 do not fit with expectations in any case or variety. A 2B plant in a $0B \times 2B$ cross transmits different male gametes according to its maternal parent and the maternal parent of the female that it fertilises (table 2), and in $2B \times 0B$ crosses it transmits female gametes with a mean number of Bs ranging from 0.48 to 2.16 (table 3). Therefore, in order to calculate the expected distributions, all of this variation has to be taken into account. Table 7 shows the comparisons made between the observed results and the expected distributions calculated on the basis of the explanation given above.

The distribution of the mean number of Bs per plant observed in $2B \times 2B$ crosses does not differ significantly from either the total expected, or from that expected considering only the male gametes transmitted by a 2B male on a $0/0$ female, while it does differ significantly when we consider the pollen transmitted by a 2B male on a $2/0$ female. This shows again the interaction of $2/0$ females

with 2B pollen and the effect of the male maternal parent on the transmission of B chromosomes.

In addition to the total observed in $2B \times 2B$ crosses, we have considered the partial results of either $2B \times 0/2$ or $2B \times 2/2$. In many of these cases χ^2 value could not be calculated, but similarities or dissimilarities between the observed and expected distributions are so obvious that we can obtain some conclusions.

The observed distribution of $2B \times 0/2$ crosses fits well with any expectation in which a $0/2$ male is considered, irrespective of the female. However, the distribution is very different when a $2/2$ male is considered. This again indicates the strong interaction produced due to the 2B maternal parent.

The observed distribution of $2B \times 2/2$ crosses only fits with expectations calculated with $0/0 \times 2/2$ crosses, indicating again the strong interaction of $2/2$ pollen with any female having a 2B maternal parent.

We can finally conclude that 2B plants produce an imprinting which affects their descendants in such a way that first, all their descendants can be recognised whether they carry Bs or not and second, they increase the frequency of transmission of B chromosomes through $2/2$ males on $2/0$ females, while a 0B maternal parent makes for a decrease in frequency through $0/2$ males on $0/0$ females. This interaction seems to be both sporophytic and gametophytic since on one hand pollen-style recognitions occur according to the maternal parent, and on the other hand particular types of pollen (0B or 4B) are greatly increased in frequency over the level at which they are produced in some cases.

The results obtained in this work are unexpected in several respects, and they show how far the behaviour of B chromosomes is from Mendelian rules. They also show that the maintenance of B chromosomes in populations depends on very complicated interactions that should be further investigated. We believe that these interactions have an important effect at the population level on the maintenance of the polymorphism for B chromosomes.

Acknowledgements This work was supported by the grants 609.2-803.002 from the Complutense University, and PB88-1022 from the CICYT of Spain

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