

Genetic control of the rate of transmission of rye B chromosomes. II. 0B × 2B crosses

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Twenty ♀ 0B × ♂ 2B crosses were made in which a single male was crossed with four different females, and the character 'mean number of Bs per plant in the progeny' was analysed. The 2B plants used as males and the 0B females belonged either to low or to high transmission rate lines, previously selected. The genetic basis of the difference between the high and low transmission rate lines was statistically tested. The male group (high or low class of the 2B male) has a significant effect on the progeny, while the female group (high or low class of the 0B female) has no significant effect. The genes controlling the transmission rate of B chromosomes affect the proportion of 0B vs. 2B plants in the 0B × 2B cross, but this is not a result of any effect on the nondisjunction mechanism, since the proportion of 1B plants is low in all cases. Structural variants of B chromosomes appeared *de novo* at a high frequency.

Keywords: B chromosomes, B transmission control, *Secale cereale*.

Introduction

The transmission of B chromosomes (Bs) is usually non-Mendelian. They tend naturally to increase in number because of various mechanisms of drive which vary in different species (see Jones, 1991 for a review). In rye the drive is postmeiotic, following nondisjunction at the first pollen grain anaphase and preferential segregation to the generative nucleus. However, in crosses involving plants with and without Bs, large and continuous variation is found for the mean number of Bs per plant in the progenies, ranging from progenies which tend to lose the Bs to others which tend to gain them.

In the first part of this work (Romera *et al.*, 1991), we carried out selection for the character *mean number of Bs per plant in the progeny* of ♀ 2B × ♂ 0B crosses, and demonstrated the existence of genotypes which affect this character. The plants from the low transmission rate class tended to lose the Bs, with a mean number of 0.55 Bs per plant in the progeny, while the average mean of the high transmission rate class was 1.85 Bs per plant.

In the present work we made ♀ 0B × ♂ 2B crosses in which the males and females belonged either to the low or to the high transmission rate classes. Therefore, in this selection experiment we can detect genetically controlled differences in B transmission rate on the male side.

Materials and methods

The material used was *Secale cereale* from the Puyo population in which about 60 per cent of the plants naturally carry Bs.

Previously (Romera *et al.*, 1991), we reported ♀ 2B × ♂ 0B crosses, that we here call 'the parental population' or 'G₀', from which we selected seed of those crosses in which B transmission rate was either low or high, and then again made ♀ 2B × ♂ 0B crosses to obtain both the 'low class G₁' (LG₁) and the 'high class G₁' (HG₁).

Ten 2B plants from the LG₁ and ten from the HG₁ were used as males for the ♀ 0B × ♂ 2B crosses of the present work. Table 1 shows the distribution of Bs and the mean number of Bs per plant in the progenies of ♀ 2B × ♂ 0B crosses, from which these males were obtained. Males 1–10 belonged to families with a mean number of Bs per plant ranging from 0.32 to 0.64 (LG₁) and males 11–20 belonged to families with a mean number of Bs per plant ranging from 1.76 to 2.84 (HG₁).

Eighty 0B plants were used as females: 40 from LG₁ and 40 from HG₁. The LG₁ 0B plants were obtained from the same crosses shown in Table 1, and the HG₁ from these and also from other crosses.

These plants were distributed in 20 plots of 1 m² each. In every plot, a single plant was used as male (pollinator) and four plants were emasculated and therefore used as females. In 10 of the plots the male was of the low transmission rate class and in the other

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10 it was of the high transmission rate class. In every plot there were four females, two each of the types described above (Fig. 1). The male open-pollinated the four females of his plot freely whereas cross-pollination among plots was prevented by covering every plot with a white cotton fabric during anthesis. The 20 plots were distributed in two rows side-by-side: 10 plots with the male of the L line in one row and ten plots with the male of the H line in the other.

The progeny were collected separately from every female and about 25 seedlings from each female were screened for the presence of Bs. These progenies are therefore the second generation (G_2) of the process of selection, although the Bs were transmitted this time by the pollen grains.

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

Table 1 Distribution of B chromosomes in ♀ 2B × ♂ 0B crosses of LG1 and HG1, from which 2B and 0B plants were selected for the ♀ 0B × ♂ 2B crosses reported in the present work

	2B male used in the 0B × 2B cross	Distribution of Bs in the 2B × 0B cross						Mean no. of Bs/plant
		0	1	2	3	4	6	
Low class	1	21		4				0.32
	2	20	1	4				0.36
	3	20	1	2		2		0.52
	4	18		7				0.56
	5-10	17		8				0.64
High class	11-12	4		20		1		1.76
	13-14	3		22				1.76
	15-16	3		21		1		1.84
	17	0		24		1		2.08
	18-19	2		17		6		2.32
	20	2		11	1	10	1	2.84

Results

Tables 2 and 3 show the results of the ♀ 0B × ♂ 2B crosses with plants of the low and high transmission rate classes, respectively.

The structural variants (B isochromosomes, etc.) are shown in the tables for completeness of information on the progenies, but they were not included in the analysis.

In the last row of Tables 2 and 3 we see that the mean number of Bs per plant based on a total of about 900 plants screened in each group is 1.02 for the low class and 1.31 for the high class. The distributions of the mean numbers of Bs per plant in the progenies of the males of the low and high transmission rate classes are shown in Fig. 2.

The first selection generation was carried out in ♀ 2B × ♂ 0B crosses whereas the generation reported in the present paper was obtained from ♀ 0B × ♂ 2B crosses. It seems, therefore, inappropriate to calculate the heritability of the character from the selection gain between both generations as B transmission might vary depending on whether the transmission is by the male or the female. Our results, in fact, indicate that the genetic control of high or low transmission rate does not have the same effect in the male as in the female.

We compared the character under selection (mean number of Bs in the progeny) in the high and low transmission rate classes by a two-way ANOVA. The sources of variation were male group (H or L class) and female group (H or L class). The effect of male group was highly significant ($F_{1,67} = 8.68$; $P = 0.0044$) whereas that of the female group was not significant ($F_{1,67} = 0.172$; $P = 0.68$). The factor interactions were also nonsignificant ($P = 0.77$). In other words, 2B males 1-10 (L class, Table 2) transmitted significantly fewer Bs to their progenies than did males 11-20 (H class, Table 3), while the 0B females belonging to either of the H or L classes did not influence the B transmission (Tables 2 and 3).

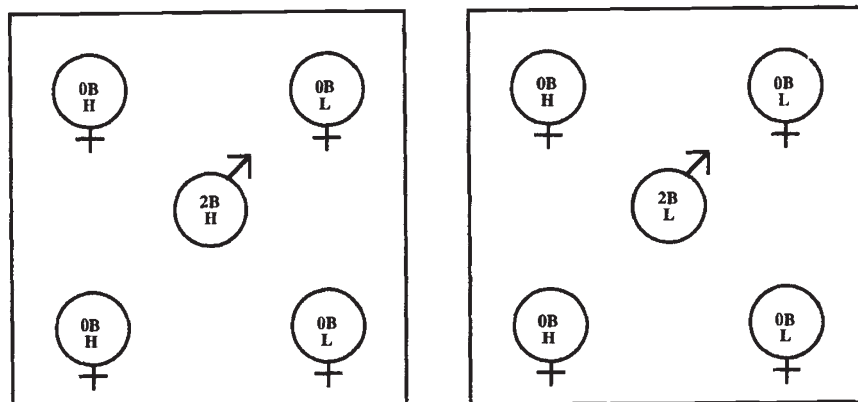


Fig. 1 Types of plots. In every plot one 2B male open-pollinated four 0B females belonging either to the high (H) or to the low (L) transmission classes. Ten plots had males of the H class and 10 had males of the L class.

Table 2 ♀ 0B × ♂ 2B crosses involving males of the low transmission line

Male	Parents			Progeny								
	Bs/plant in the male	Female	Bs/plant in the female	Number of Bs in the progeny					Total 0B + B st	Mean number of Bs/plant		
				0	1	2	3	4			Others	
1	0.32	H	2.32	13		9				1 ₍₁₎	22	0.82
		H		15	1	10					26	0.81
		L	0.32	17	1	6					24	0.54
		L		16	2	10					28	0.79
		Total male 1			61	4	35			1	100	0.74
2	0.36	H	1.74	16	1	7				2 ₍₂₎	24	0.63
		H		16		7					23	0.61
		L	0.36	20		4					24	0.33
		L		29		16					45	0.71
		Total male 2			81	1	34			2	116	0.59
3	0.52	H	1.74	15	3	7	1				26	0.77
		H		15	5	5				1 ₍₁₎	25	0.60
		L	0.36	7	2	10					21	1.43
		L		16	3	4	1	1	1 ₍₉₎	25	0.72	
		Total male 3			53	13	26	2	3	2	97	0.86
4	0.56	H	2.08	21	2	30				2 ₍₁₎	53	1.17
		H		21		14		1	1 ₍₇₎	36	0.89	
		L	0.36	13		11		1		25	1.04	
		L		9	3	15			3 ₍₁₎	27	1.22	
		Total male 4			64	5	70		2	6	141	1.08
5	0.64	H	1.84	11	3	11					25	1.00
		H		13	2	10				1 ₍₂₎	25	0.88
		L	0.32	15	1	9					25	0.76
		L										
		Total male 5			39	6	30			1	75	0.88
6	0.64	H	1.40	3		10					13	1.54
		H		13		9		1		23	0.96	
		L	0.32	11	1	14		1		27	1.22	
		L		15	1	12				28	0.89	
		Total male 6			42	2	45		2		91	1.09
7	0.64	H		6		16		2			24	1.67
		H		11	1	13			1 ₍₁₎	25	1.08	
		L	0.32	12		13				25	1.04	
		L										
		Total male 7			29	1	42		2	1	74	1.26
8	0.64	H		2		24					26	1.85
		H		5		19			1 ₍₈₎	24	1.69	
		L	0.52									
		L										
		Total male 8			7		43			1	50	1.77
9	0.64	H	1.40	7	1	17					25	1.40
		H		9		17					26	1.31
		L	0.52	12		13				1 ₍₂₎	25	1.04
		L		7		19				26	1.46	
		Total male 9			35	1	66			1	102	1.30
10	0.64	H	1.68	8		15		1			24	1.42
		H		10		14		1	1 ₍₅₎	25	1.12	
		L	0.36	16		8		1		25	0.80	
		L		14		11			25	0.88		
		Total male 10			48		48		3	1	99	1.09
Grand total				459	34	439	2	12	16	945	1.02	

As the selected character is measured in the progenies of the crosses, the column 'Bs per plant in the male' refers to the mean number of Bs transmitted by the mother of each male (because they were selected from ♀ 2B × ♂ 0B crosses). Similarly, the column 'Bs per plant in the female' refers to the mean number of Bs transmitted by the mother of each female.

Other types of B are as follows. 1: 1 small iso-B; 2: 1 large iso-B; 3: 1 deficient-B; 4: 1 dicentric B; 5: 1B standard and 1 small iso-B; 6: 1B standard and 1 deficient B; 7: 2B standard and 1 small iso-B; 8: triploid plant with 4 B standard; 9: 1 telocentric B.

Table 3 ♀ 0B × ♂ 2B crosses involving males of the high transmission line

Male	Parents			Progeny							Total 0B + B st	Mean number of Bs/plant	
	Bs/plant in the male	Female	Bs/plant in the female	Number of Bs in the progeny					Others				
				0	1	2	3	4					
11	1.76	H	1.84	1		18	1	5			25	2.36	
		H		16		7					23	0.61	
		L		0.32	4		21				25	1.68	
		L		7	1	16				1 ₍₁₎	24	1.37	
		Total male 11		28	1	62	1	5		1	97	1.53	
12	1.76	H	0.32										
		H											
		L		1		19				3(1 ₍₃₎ ,2 ₍₁₎)	23	1.65	
		L		2		8				10	1.60		
		Total male 12		3		27				3	33	1.64	
13	1.76	H	0.32										
		H											
		L		28	3	13				1 ₍₁₎	44	0.66	
		L		18	1	13		1			33	0.94	
		Total male 13		46	4	26		1		1	77	0.78	
14	1.76	H	1.68	7	4	16				1 ₍₁₎	27	1.33	
		H		11	3	10				1 ₍₁₎	24	0.96	
		L		0.64	8	2	14				1 ₍₁₎	24	1.25
		L		16		9					25	0.72	
		Total male 14		42	9	49				3	100	1.07	
15	1.84	H	1.68	4	2	27					33	1.70	
		H		12		11					23	0.96	
		L		0.52	8	1	8				2 ₍₁₎₍₂₎	17	1.00
		L		14		11					25	0.88	
		Total male 15		38	3	57				2	98	1.19	
16	1.84	H	1.40	5	3	17				2 ₍₄₎₍₅₎	25	1.48	
		H		6	3	18					27	1.44	
		L		0.52	5	4	17				26	1.46	
		L		11	3	11				1 ₍₆₎	25	1.00	
		Total male 16		27	13	63				3	103	1.34	
17	2.08	H	2.32	6		21					27	1.56	
		H		8		17					25	1.36	
		L		0.56	6	1	18		1		26	1.58	
		L		3	4	18				2 ₍₁₎₍₅₎	25	1.60	
		Total male 17		23	5	74		1		2	103	1.52	
18	2.32	H	1.40	9	2	11				2 ₍₂₎	23	1.04	
		H		11	1	12				1 ₍₂₎	24	1.04	
		L		0.64	2	1	19		1		2 ₍₂₎₍₆₎	23	1.87
		L		5	1	17		1		1 ₍₇₎	24	1.62	
		Total male 18		27	5	59		2		6	94	1.39	
19	2.32	H	1.40	17	3	7		1			28	0.75	
		H		9	1	17				1 ₍₆₎	27	1.30	
		L		0.36	6		20				26	1.54	
		L		2	2	20				1 ₍₂₎	24	1.75	
		Total male 19		34	6	64		1		2	105	1.31	
20	2.84	H	1.40	6	4	15					25	1.36	
		H											
		L		0.36	5	2	18				25	1.52	
		L		6		19					25	1.52	
		Total male 20		17	6	52					75	1.47	
Grand total				285	52	533	1	10	23	885	1.31		

See footnote to Table 1.

Other types of B are as follows: 1: 1 small iso-B; 2: 1 large iso-B; 3: 1 deficient-B; 4: 1 dicentric B; 5: 1B standard and 1 small iso-B; 6: 1B standard and 1 deficient B; 7: 2B standard and 1 small iso-B.

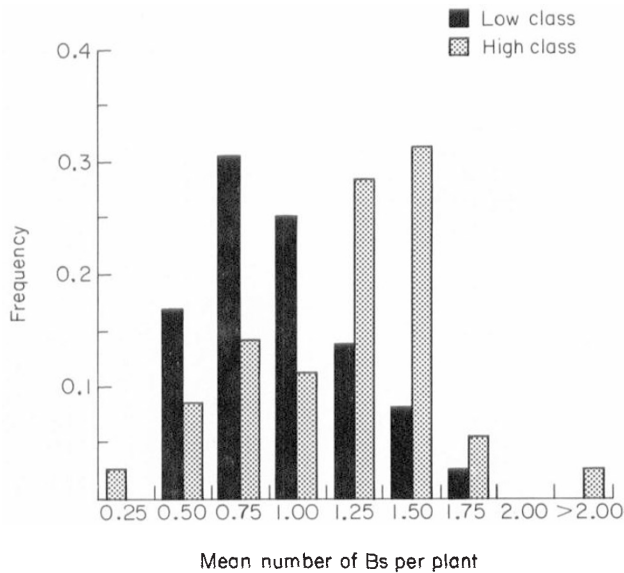


Fig. 2 Distributions of the mean numbers of Bs per plant in progenies of ♀ 0B × ♂ 2B crosses of both the low and high transmission rate classes.

Discussion

The variation of the mean number of Bs per plant in the progeny of crosses involving 0B and 2B plants has a genetic component, as demonstrated by the existence of high and low transmission rate lines obtained by selection. The low line transmitted 0.55 Bs per plant in ♀ 2B × ♂ 0B crosses (Romera *et al.*, 1991) whereas it transmitted 1.02 in ♀ 0B × ♂ 2B ones. This mean transmission rate on the male side is low compared with the level of accumulation in unselected plants, but about double the value obtained when Bs are transmitted through the female in the selected line. The high line transmitted 1.85 Bs per plant in ♀ 2B × ♂ 0B crosses (Romera *et al.*, 1991) whereas we obtained 1.31 in this work. More generations of selection are needed to determine if these shifts result from an intrinsic difference in the behaviour of Bs on the male and female sides or whether they result simply from random drift.

The ANOVA shows that the progenies of ♀ 0B × ♂ 2B crosses are mainly influenced by the male transmission class, indicating that the two lines that we had separated by selection in ♀ 2B × ♂ 0B crosses also behave differently in ♀ 0B × ♂ 2B ones. The simplest explanation is that the alleles influencing the B transmission on the female side also influence the transmission by the male.

The variation observed among the four females crossed with the same male is remarkable because it accounts for about 60 per cent of the variance. This variation may, at least partially, result from differences

among the 0B to 4B gametes produced by a single male. It is worth mentioning that such large variation was not previously found when five 2B males pollinated the same 0B female (Puertas *et al.*, 1990); therefore, there must be a different pattern of pollination when few or many pollen grains are competing for the same female flower. It is probable that the lower numbers of pollen grains available in the crosses reported in this work (one male to four females) increased the environmental variance, and that this effect is randomized when pollen from many males is available at the same time. A differential ability in the female to accept the different types of male gametes is also possible. Carlson (1969) found an inbred line of maize which suppressed preferential fertilization of the egg and suggested that a simple recessive allele was responsible for blocking the acceptance of B chromosomes.

From the results obtained it is evident that the selected gene (or genes) affects the proportion of 0B vs. 2B progeny, all other B types (1B, 4B, etc.) being formed only at low frequencies as by-products of the intrinsic instability of Bs. For example, the high mean value of some males (males 18–20, Table 3) which arose from the large number of 4B plants among their full sibs, was not conserved in their progenies, which range from 0.75 to 1.75 Bs per plant.

It should be noted that the B chromosome always tends to undergo nondisjunction because the proportion of 1B descendants is always low. Therefore, the low transmission rate class tends to lose the Bs and to produce a high proportion of 0B progeny, but when the B is retained it undergoes nondisjunction resulting in 2B descendants.

Finally, we should mention the large proportion (2.13 per cent) of structural variants (B-isochromosomes, etc.) arising *de novo* in these crosses. 1B or structural variants of the B are almost never obtained in ♀ 2B × ♂ 0B crosses, and this indicates that micro- and megasporogenesis differ in this respect.

Sex-dependent meiotic behaviour of B chromosomes was found in the grasshopper *Eyprepocnemis plorans* (Henriques-Gil *et al.*, 1989) although in this case the transmission of the B is not affected by sex (López-León *et al.*, 1992).

Genes on the A chromosomes controlling the transmission rate of Bs may be important in maintaining the polymorphism for Bs in natural populations. Bs tend naturally to increase in number because of various mechanisms of drive; therefore, those alleles promoting the loss of Bs tend to increase the frequency of 0B individuals and to keep the Bs at a moderate frequency in the population.

Such genes have been reported in two animal species: *Myrmeleotettix maculatus* (Shaw & Hewitt,

1985; Shaw *et al.*, 1985) and *Pseudococcus affinis* (Nur & Brett, 1985, 1987, 1988). In *M. maculatus*, where there is meiotic drive on the female side and drag on the male, with a net overall drive, a modifier of meiotic drive was found that drastically reduced the female transmission rate. A polymorphism in female transmission rate exists in most natural populations. In *P. affinis*, where the Bs are accumulated by meiotic drive in the males, genotypes were isolated that can control and suppress the drive of the Bs, affecting their segregation pattern at meiosis.

Rye is the first plant species where genotypes affecting the transmission rate of B chromosomes have been isolated, although Parker *et al.* (1982) suggested their existence in *Hypochoeris maculata*. Recently, Cebriá *et al.* (1994) reported genetic control of B transmission in *Aegilops speltoides*.

There is no conclusive evidence to determine whether the alleles controlling rye B transmission rate are located on the A chromosomes, on the Bs or both.

Acknowledgements

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References

- CARLSON, W. R. 1969. Factors affecting preferential fertilization in maize. *Genetics*, **62**, 543–554.
- CEBRIÁ, A., NAVARRO, M. L. AND PUERTAS, M. J. 1994. Genetic control of B chromosome transmission in *Aegilops speltoides* (Poaceae). *Am. J. Bot.*, **81**, 1502–1507.
- HENRIQUES-GIL, N., CANO, M. I. AND SANTOS, J. L. 1989. Sex-dependent meiotic behaviour of B chromosomes in the grasshopper *Eyprepocnemis plorans*. *Heredity*, **62**, 217–221.
- JONES, R. N. 1991. B-chromosome drive. *Am. Nat.*, **137**, 430–442.
- LOPEZ-LEON, M. D., CABRERO, J., CAMACHO, J. P. M., CANO, M. I. AND SANTOS, J. L. 1992. A widespread B chromosome polymorphism maintained without apparent drive. *Evolution*, **46**, 529–539.
- NUR, U. AND BRETT, B. L. H. 1985. Genotypes suppressing meiotic drive of a B chromosome in the mealybug, *Pseudococcus obscurus*. *Genetics*, **110**, 73–92.
- NUR, U. AND BRETT, B. L. H. 1987. Control of meiotic drive of B chromosomes in the mealybug *Pseudococcus affinis* (*obscurus*). *Genetics*, **115**, 499–510.
- NUR, U. AND BRETT, B. L. H. 1988. Genotypes affecting the condensation and transmission of heterochromatic B chromosomes in the mealybug *Pseudococcus affinis*. *Chromosoma*, **96**, 205–212.
- PARKER, J. S., TAYLOR, S. AND AINSWORTH, C. C. 1982. The B-chromosome system of *Hypochoeris maculata* III. Variation in B-chromosome transmission rates. *Chromosoma*, **85**, 299–310.
- PUERTAS, M. J., JIMÉNEZ, M. M., ROMERA, F., VEGA, J. M. AND DÍEZ, M. 1990. Maternal imprinting effect on B chromosome transmission in rye. *Heredity*, **64**, 197–204.
- ROMERA, F., JIMÉNEZ, M. M. AND PUERTAS, M. J. 1991. Genetic control of the rate of transmission of rye B-chromosomes. I. Effects in 2B × 0B crosses. *Heredity*, **66**, 61–65.
- SHAW, M. W. AND HEWITT, G. M. 1985. The genetic control of meiotic drive acting on the B-chromosome of *Myrmeleotettix maculatus* (Orthoptera: Acrididae). *Heredity*, **54**, 187–194.
- SHAW, M. W., HEWITT, G. M. AND ANDERSON, D. A. 1985. Polymorphism in the rates of meiotic drive acting on the B-chromosome of *Myrmeleotettix maculatus*. *Heredity*, **55**, 61–68.