Genetic control of B chromosome transmission in maize and rye

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The B chromosome system in rye and maize

B chromosomes (Bs) are dispensable genetic elements which lack homology with any members of the basic set and have non-Mendelian modes of inheritance. They are widespread in many animal and plant species and our main interest is to understand the genetic mechanisms underlying their polymorphisms.

Our experiments to investigate the genetic control of B chromosome transmission in maize and rye were basically the same. It is interesting to compare both species because we can see whether the Bs have a common strategy for establishing themselves as a particular part of the genome, or if the evolutionary story of the Bs is different in each species.

In rye and maize the number of Bs varies from plant to plant but it is constant in all somatic cells within a plant. We carried out fluorescence *in situ* hybridization (FISH) with the D1100 rye B specific probe [1, 2] in sections of various tissues, both in seedlings and mature plants, and all nuclei showed a similar signal corresponding to the Bs. It seems that the Bs are repressed in the somatic tissues because there is no significant effect of rye Bs on the phenotype at the somatic level [3, 4]. The stability of maize Bs in the sporophyte was documented following a B-A translocation [5].

Rye Bs display a complicated behaviour during male meiosis and gametogenesis. At pachytene they form bivalents or multivalents [6]. At metaphase I, they can form univalents, bivalents or multivalents; the univalents may be lost as micronuclei. This instability creates a permanent variation in B number which results in a polymorphism at the population level. The maize Bs are more stable than the Bs of rye. When the plant has only one B chromosome it is frequently included in one pole at anaphase I and so the loss of the B univalent is

prevented. When there are more Bs, they usually pair and the microspores show few Bs lost as micronuclei.

A main event occurs during postmeiotic mitosis. In rye, at anaphase of the first pollen grain mitosis, the B chromosome undergoes non-disjunction and migrates to the generative pole from which the gametes will form. The second mitosis is normal and both sperm nuclei carry two Bs. In this way the Bs are accumulated and driven to the next generation. In maize, non-disjunction typically occurs during the second pollen mitosis. If the microspore begins with a single B chromosome, non-disjunction will result in one sperm nucleus possessing two B chromosomes and the other containing no Bs. Few data are available on the behaviour of the Bs on the female side, but the progeny of the appropriate crosses show that the Bs of rye have a mechanism of accumulation; actually, rye is the only known plant species where the Bs are accumulated on both sides. In maize the inheritance through the female parent is conventional (reviews in [7, 8]).

All these peculiar events do not occur in all cells, but there is a variation within and between plants. Figure 1 summarizes B chromosome behaviour outlining the moments where variation of the processes may result in variation of B transmission rate. The main purpose of our work is to determine the genetic control of these sources of variation. This is important to understand the evolution of B polymorphisms.

The first source of variation is the proportion of bivalents to univalents at metaphase I. In rye, the loss of univalents is the most important event in determining B transmission rate. Univalent conservation depends on their ability to divide reductionally since all equationally dividing univalents will be lost as micronuclei. At male meiosis the maize Bs are more stable, showing normal meiosis in general terms.

The second source of variation is the proportion of non-disjunction. In rye, directed non-disjunction to the generative pole is almost fixed [3]. In maize, non-disjunction frequency varies among lines [8]. In a line carrying a B/A translocation, it has been shown using a B specific probe [9, 10], that it occurs at about 60% of the pollen grains and it can occasionally also occur during the first microspore division. However, in the native maize population studied in this paper, non-disjunction is almost fixed. Non-disjunction would not accumulate the Bs if it were not coupled with preferential distribution of the Bs towards the nuclei forming or acting as gametes. In rye the non-disjoined B chromosome is included in the generative nucleus which will divide once more giving rise to the sperm nuclei. We have never observed the reverse distribution, so it seems that this process does not vary.

In maize, the two sperm nuclei differ in B chromosome content, in this situation transmission rate depends on the frequency with which the sperm containing B chromosomes fertilizes the egg. We will see below that this is the main source of variation in determining B chromosome transmission rate in this species.
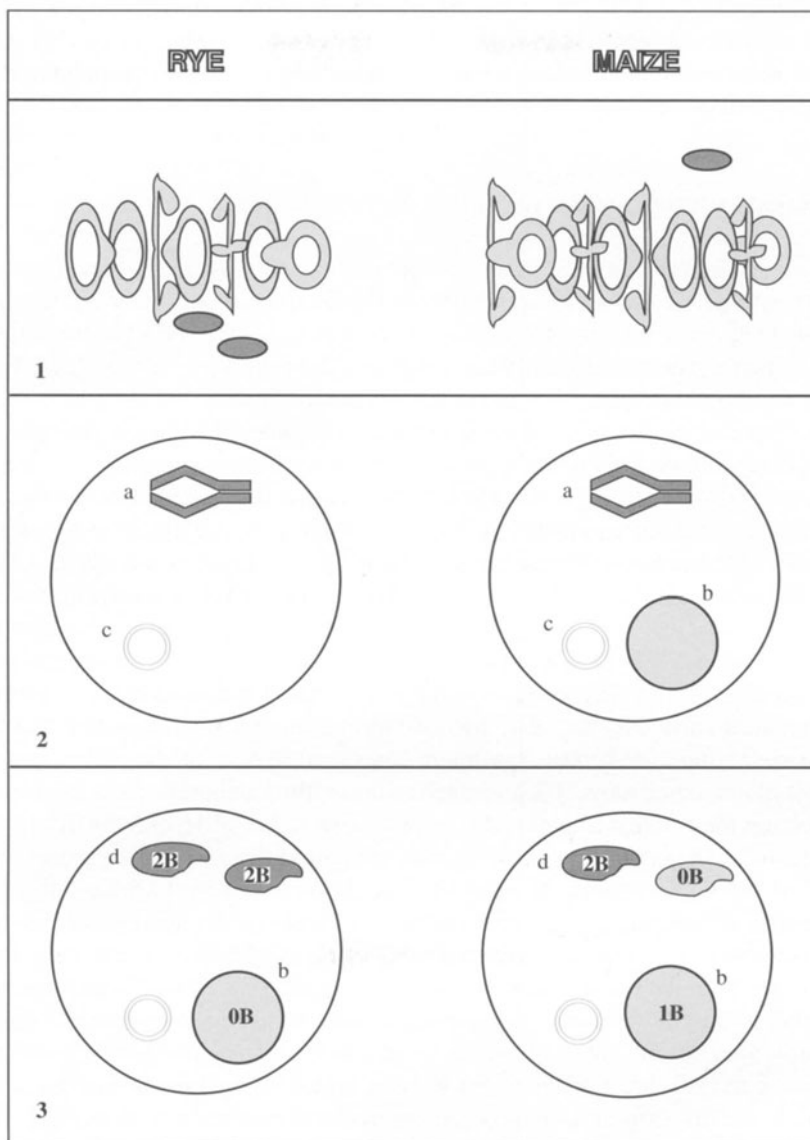
Figure 1. Sources of variation affecting B transmission rate (B TR) in rye and maize.
1. At first meiotic division B univalents may not be included in the poles. In rye the proportion of uni-valents/bivalents at metaphase I is the main factor determining B TR.
2. The B standard chromosome undergoes non-disjunction at pollen grain mitosis in nearly 100% of the cases. In rye, directed non-disjunction towards the generative pole occurs at the 1st mitosis. In maize non-disjunction occurs at the 2nd mitosis. a = B chromosome undergoing non-disjunction; b = tube nucleus; c = pollen grain pore
3. In rye the sperm nuclei (d) coming from the generative pole contain a doubled B number. In maize the two sperm nuclei differ in B content and B TR depends on the frequency with which the sperm containing B chromosomes fertilizes the egg.

It should be noted that there are other sources of variation affecting B chromosome transmission due to competition between pollen grains carrying different B chromosome content, but our discussion in this paper is limited to the stages outlined in Figure 1.

Selection experiments in rye

B chromosome transmission rate (B TR) in 2B × 0B crosses of non-selected plants is highly variable. In some crosses the Bs tend to be lost, whereas others show B accumulation. In previous papers [11, 12] we reported the selection of genotypes which significantly differ in their high (H) or low (L) B TR, demonstrating the heritability of the H or L character.

We have ample evidence that the difference between the lines is not related to a differential non-disjunction ability, firstly because the proportion of plants with odd numbers of Bs is always low; that is, the difference between the H and L lines is the proportion of 0B *vs.* 2B or 4B plants in the progeny of all types of crosses. Secondly, because we have observed pollen mitosis directly, and in both lines the frequency of Bs undergoing non-disjunction at pollen anaphase coincides with the frequency of Bs present at pollen metaphase.

The differential behaviour of B chromosomes at meiosis and gametogenesis accounts for the differences in B TR in the H and L lines. 2B plants of the L line form bivalents in only 20% of the pollen mother cells (PMCs) at metaphase I, and the B chromosome is conserved in 45% of the pollen grains at first pollen metaphase, whereas the remaining Bs are eliminated as micronuclei when they divide equationally at anaphase I. In the H line the Bs form bivalents in nearly 90% of the PMCs at metaphase I and they are present in 85% of the pollen grains. The different transmission in the two lines results from their differential ability to form uni- or bivalents at metaphase I, which determines their loss or conservation in the gametes (Fig. 2) [13]. On the other hand, the Bs of 2B plants regularly form a bivalent at pachytene in all cases as shown by the synaptonemal complexes studied by the whole mount surface spreading technique. This allows us to conclude that the difference between the Bs of the two lines is the ability to have bound arms at metaphase I.

We made 2B × 0B crosses between rye plants of both lines to determine the location of the genes controlling B chromosome transmission rate. Our results show that the transmission of the Bs depends on the H or L source of the Bs whereas the 0B parent has no significant effect on the progeny. B TR is low when the Bs come from the L line, and high when the Bs come from the H line, irrespective of the 0B parent. This demonstrates that the genetic control of B TR is located on the Bs [14]. Statistical analyses of the observed revealed the significance of other factors during transmission, suggesting that the A chromosomes also affect B transmission, but to a relatively small extent.
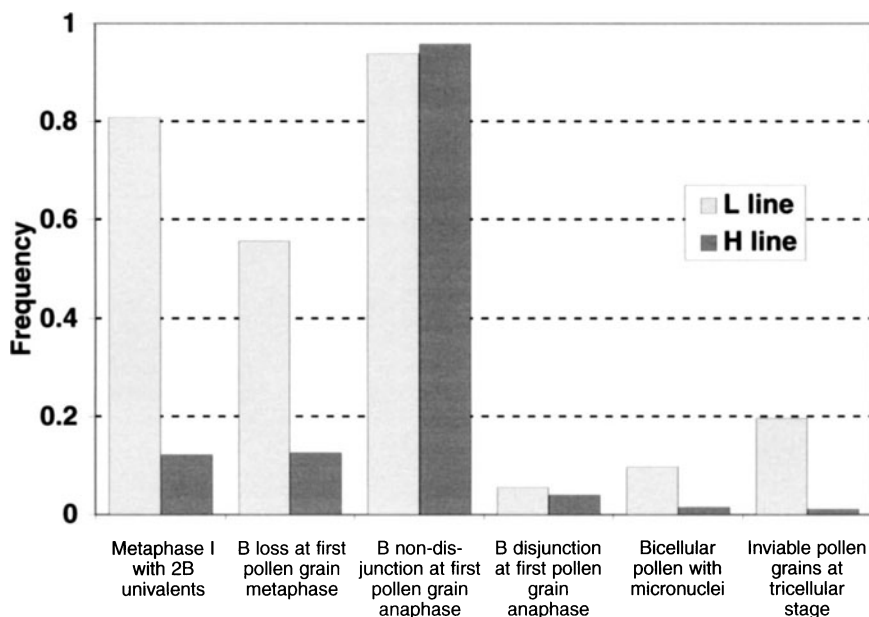
Figure 2. Differences in 2B chromosome behaviour in the L and H B transmission rate rye lines in relevant stages of male meiosis and gametogenesis. The main difference between the two lines is the frequency of cells showing two B univalents at metaphase I. Many of these univalents are lost as micronuclei which are degraded during pollen maturation. The frequency of non-disjunction in the pollen grains with the B is nearly the same in both lines. The frequency of inviable pollen grains is higher in the L line.

According to all our data, the simplest assumption is that the genes for high and low transmission rate selected in our lines actually are sites for chiasma formation, or at least B homologous arm binding sites, located on the B chromosomes.

It is interesting to know the behaviour of the H and L Bs under heterozygous conditions, to study their interaction when they are together in the same cell. In rye, it is not possible to obtain 2B plants heterozygous for the Bs because the two Bs always come from non-disjunction. Therefore, we made 2B × 2B crosses to obtain 4B plants in all possible combinations: HxH, LxL where the four Bs are of the same type, and HxL and LxH where there is heterozygosity for the Bs and crosses to obtain 4B plants were reciprocal.

We studied the behaviour of the four classes at pachytene and metaphase I. At pachytene, the four Bs may form either two bivalents or one quadrivalent (Fig. 3); the frequency of each type is shown in Table 1. In contrast to the 2B plants, where the Bs always form a bivalent at pachytene irrespective of their H or L status, 4B plants differ in synapsis behaviour. According to Sved's model [15], when four homologues are present, one quadrivalent is expected
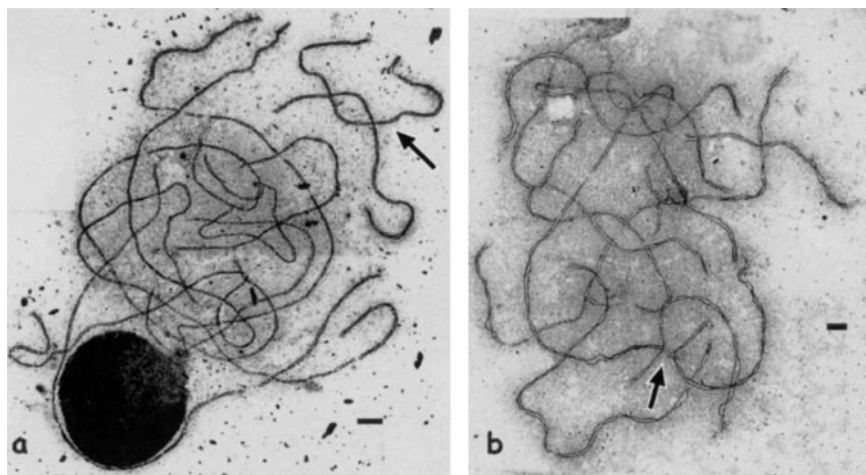
Figure 3. Electron micrographs of synaptonemal complexes in 4B rye nuclei obtained by the surface spreading technique. a. 7 A bivalents and 2 B bivalents (arrow); b. 7 A bivalents and 1 B quadrivalent (arrow). Scale bar represents 2 μm.

Table 1. Meiotic configurations of 4B chromosomes at pachytene (Pch) and metaphase I (MI)

Type of cross		2 II	1 IV	4 I	1 II + 2 I	1 III + 1 I
2BH × 2BH	Pch	21 (70.00)	9 (30.00)			
	MI	137 (62.55)	6 (2.7)	10 (4.56)	66 (30.11)	0
2BL × 2BL	Pch	17 (44.73)	21 (55.26)			
	MI	60 (20.34)	11 (3.73)	69 (23.39)	149 (50.51)	6 (2.03)
2BH × 2BL	Pch	17 (54.84)	14 (45.16)			
	MI	167 (45.38)	17 (4.62)	50 (13.59)	130 (35.33)	4 (1.08)
2BL × 2BH	Pch	13 (48.15)	14 (51.85)			
	MI	57 (26.51)	2 (0.93)	22 (10.23)	129 (60.00)	5 (2.33)

in 2/3 of the cells and two bivalents in the other 1/3. This prediction is based on the assumption that there are two independent initiation sites at the telomeres and that the beginning of synapsis is at random. In our case, it was found that both LL and LH 4B plants fitted the 2/3, 1/3 distribution, indicating random pairing. However, HL and especially HH, significantly differed since the number of bivalents was higher than expected. It seems that these two lines

have restricted pairing towards the formation of bivalents. We do not know how such restriction takes place, but we can hypothesize that there is a maternal effect since H B chromosomes coming from a H female parent form more bivalents than the other types.

The results at metaphase I (Tab. 1) show, firstly, that the proportion of cells with 2 II is correlated with those at pachytene showing also 2II, indicating that synapsis at pachytene influences further associations of the Bs at metaphase I in 4B plants. Metaphase I data of the HL and LH classes are of special interest because they are significantly different. This indicates a lack of complementation in the hybrid between the H and L Bs at this level. This result is in agreement with the hypothesis that chiasma formation depends on the B chromosome itself, because if a product produced by the normal A chromosomes determined chiasma formation in the Bs, the HL and LH would have a similar behaviour. On the other hand, since LH has the same distribution of bivalents to non-bivalents as LL, and HL is closer to HH, a maternal effect on chiasma formation seems evident.

We also studied the correlation between B transmission rate and meiotic configurations at metaphase I. The only significant correlation (r = 0.993, P = 0.003) is between the frequency of PMCs with 2II and transmission rate. Therefore, our final conclusion is that both in 2B or 4B plants the main feature which determines the B transmission rate is the ability to form bivalents at metaphase I.

Selection experiments in maize

Similar experiments on selection for the character H or L B transmission rate were carried out in maize. The parental population was the native race Pisingallo from northwest Argentina, with a frequency of plants with Bs of 44% [16].

To study B TR on the male side, twenty 0B × 1B crosses were made. They produced progeny with either 0B or 2B due to non-disjunction, which occurred regularly in nearly all cases. The variation of B TR was large, from 0.17 to 0.98, the mean being Mendelian (0.5). The progenies showing the highest and nearly the lowest B TR were selected (Fig. 4). In the H line the mean B TR was 0.65 with a proportion of 2B plants significantly higher than the Mendelian expectation. The mean B TR was higher than 0.5 in all crosses. In the L line the mean B TR was 0.4 with a proportion of 2B plants and a B TR significantly lower than the Mendelian expectation. B TR was significantly different in the L and H lines, indicating a selection gain and therefore a genetic component of the variation [17]. Selection was repeated in four generations. The results were similar for the H line, whereas in the L line B TR resulted in a Mendelian distribution.
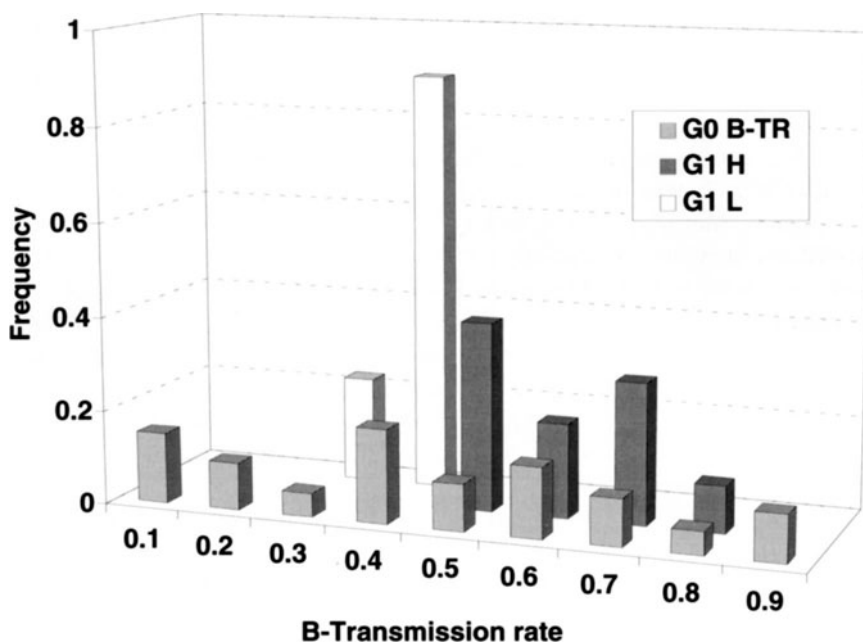
Figure 4. Male B transmission rate (B TR) distribution in 0B × 1B crosses of the native race Pisingallo of maize (G0) and in 0B × 2B crosses (G1) selected for high (H) or low (L) male B TR.

The same type of crosses were made to select the Bs transmitted on the female side [17]. In this case, the distribution of B TR in twenty 1B × 0B crosses in the non-selected population was less variable (0.3 to 0.6) than in the former reciprocal cross 0B × 1B. The average mean was 0.47, again Mendelian (Fig. 5). In these crosses the progeny had either 1B or 0B due to the lack of a mechanism of accumulation on the female side. In this case, significant selection gain was obtained only in the line selected for low B TR, with a mean of 0.4 whereas in the line selected for high B TR the mean remained Mendelian. Similar results were found in further generations of selection. It is possible that the B univalent tends to be lost or to be preferentially directed to the non-functional megaspore, or both.

We studied male meiosis in the selected maize plants to see if there is a correlation between meiotic behaviour and B TR as occurs in rye. In 2B plants, the Bs formed bivalents in about 90% of the metaphase I cells, irrespective of their H or L status and the generation of selection studied. On the other hand, the number of micronuclei at the tetrad stage was lower than 5% in all cases. It was concluded that the differential transmission of the Bs on the male side is not due to a differential B loss at meiosis [18]. Therefore, rye and maize are different in this respect.
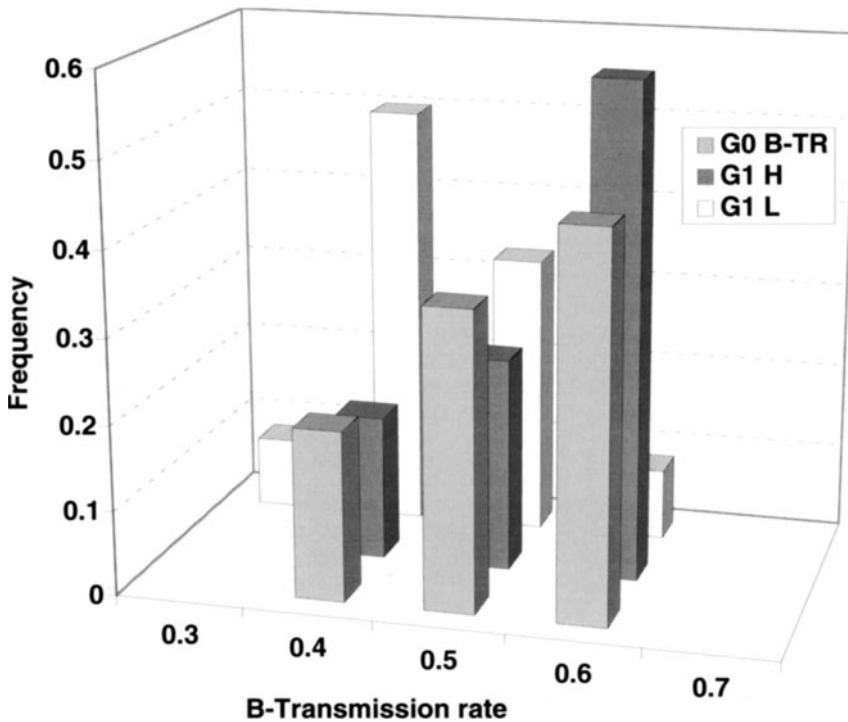
Figure 5. Female B transmission rate (B TR) distribution in 1B × 0B crosses of the native race Pisingallo of maize (G0) and in 1B × 0B crosses (G1) selected for high (H) or low (L) male B TR.

0B × 2B crosses were made between and within the H and L lines in the third generation of selection to determine the location of genes controlling male B TR in maize [19]. We observed, firstly, that during the three generations of selection the H line transmitted the Bs with a rate of about 0.7 whereas the L line transmits the Bs at about a Mendelian rate. Secondly, that B transmission rate depends on the 0B female parent, because the transmission rate is low when the 0B parent is L, and high when the 0B female parent is H, irrespective of the Bs. Therefore, the genes controlling B TR are located on the A chromosomes.

Bearing in mind that the sperm nuclei carry 0B or 2B by means of non-disjunction, our results indicate that the genotype of the 0B female determines the rate at which the egg cell is fertilized by the sperm nucleus carrying the Bs. This means that a 0B female parent of the H genotype selects the 2B sperm to fertilize the egg; in contrast, the 0B L female plant does not select any sperm nucleus because fertilization is random in this case, resulting in a Mendelian B TR.

In the next generation of selection we made 2B × 2B crosses, that is, in this case the female also carries B chromosomes. B TR in these crosses was the same as before, indicating that the presence of Bs in the egg cell does not affect male B TR. This result further supports the previous conclusion that it is the genotype of the A chromosomes in the female which controls male B TR, and that this is irrespective of the presence of Bs in the egg cell.

B chromosome evolution

In our opinion, the differences between the genetic systems for the maintenance of B polymorphism in rye and maize are only secondarily important, whereas the similarities are more important and may help us to understand the evolution of B chromosomes.

We should accept that B chromosomes have evolved separately in both species, but the similarity of the strategies to establish the polymorphism is remarkable. We might ask whether the Bs of these two species have a very ancient common origin, or if the similarities are due to an obligated common strategy for any supernumerary B chromosome to establish itself in the genetic system of the gramineae.

It is difficult to hypothesize on the origin of Bs in maize and rye because both are cultivated plants and therefore human migration has had a great influence on their distribution. However, it seems that the origin of Bs is ancient in both cases because the distribution of populations with Bs is widespread and they are present in native races. In addition, the mechanism of non-disjunction at pollen mitosis is common to all related species [7]. It is necessary to perform a molecular study to compare B DNA sequences in these species, although the answer will not be easy due to the high mutability of B chromosomes because of their negligible effects on the phenotype.

It has to be emphasized that the regularity of meiotic segregation is strongly related to B transmission rate. In the varieties of maize reported to date, meiosis is mostly regular and the Bs at male meiosis are conserved even if they are present as univalents. The same is true for the H line of rye, whereas the L line tends to lose the Bs because they are not bound at metaphase I.

The importance of a regular meiosis is further supported because structural variants of the Bs undergoing non-disjunction, but having irregular meiosis, soon disappear after they are formed *de novo,* and only the standard type is maintained at the population level. We can conclude that regular meiotic behaviour is essential for a B chromosome to be maintained in the long term.

The classical hypothesis for parasitic Bs assumed that B chromosome polymorphisms are stable due to antagonistic forces, the equilibrium being the result of B accumulation, and the detrimental effects on the fitness of B carriers [20–22]. A more recent non-equilibrium hypothesis of B chromosome evolution has been proposed by Camacho et al. [23–25]. They propose that the

long term evolution of the Bs can be considered as the outcome of an arms race between the host genome that attempts to eliminate the Bs or suppress their parasitic effects and the Bs themselves that try to escape through generating new selfish variants. This hypothesis views B chromosome evolution as a continuous conflict between parts of the genome with different interests so that B influences may shift back and forth between parasitic to, neutral and possibly beneficial effects.

Our results fit this new hypothesis only partially. We have found H and L B variants in rye, but the variant with the higher transmission rate is not the most parasitic. In fact just the opposite, since in 2B plants the higher transmission corresponds to a regular meiosis and proper pollen development, whereas the presence of univalents in the variant with low transmission influences pollen development thus decreasing its viability. 4 Bs in rye are very parasitic, producing a strong reduction in fertility but, even in this case, the H line is linked to a more regular meiosis and transmission; therefore, it seems that the Bs are protecting themselves by linking higher transmission to less parasitic effects.

On the other hand, the "arms race" hypothesis predicts that the A genome will be the counterpart that will tend to eliminate the Bs. Therefore, only "anti B" genes and not "pro B" genes are expected to exist in the A genome. It is therefore difficult to fit into the hypothesis the existence of alleles that increase B transmission rate in maize. We have found "anti B" genes in the L line of maize which tend to eliminate the Bs during female gametogenesis. This is in agreement with the hypothesis, but we have also found that the female parent of the H line preferentially chooses the sperm carrying B chromosomes to fertilize herself, and this is a "pro B" action of the A chromosomes because this behaviour tends to increase B transmission rate.

Nevertheless, we found the arms race hypothesis very good to explain that the Bs of maize are more evolved than the Bs of rye. In maize, B effects are neutral at the B frequencies normally found in populations and all Bs form chiasmata and segregate normally. In this more evolved situation, the overall B transmission rate is Mendelian.

Our result show that in rye, B transmission rate depends on the Bs themselves, whereas in maize, B TR depends on the normal complement of A. Why the difference? If we assume that rye is a younger system where the A chromosomes have not developed alleles against the Bs, the variation of B transmission rate will depend on the Bs themselves. If we assume that the Bs of maize have reached a more evolved status, the effect of the A chromosomes becomes apparent. Probably the As have developed alleles for B tolerance and the Bs can be maintained at a Mendelian rate although displaying a complicated behaviour as a relict of their previous, more parasitic nature.

We would like to finish by saying that the results reported here correspond to one race of maize and one of rye. We have, however, preliminary results showing that in other races the B polymorphism is established at a different level. Figure 6 shows the distribution of B TR in three different populations:

Puyo corresponding to the experiments reported in the present paper, Paldang, where the average mean B TR is lower than Mendelian, the H Bs do not exist
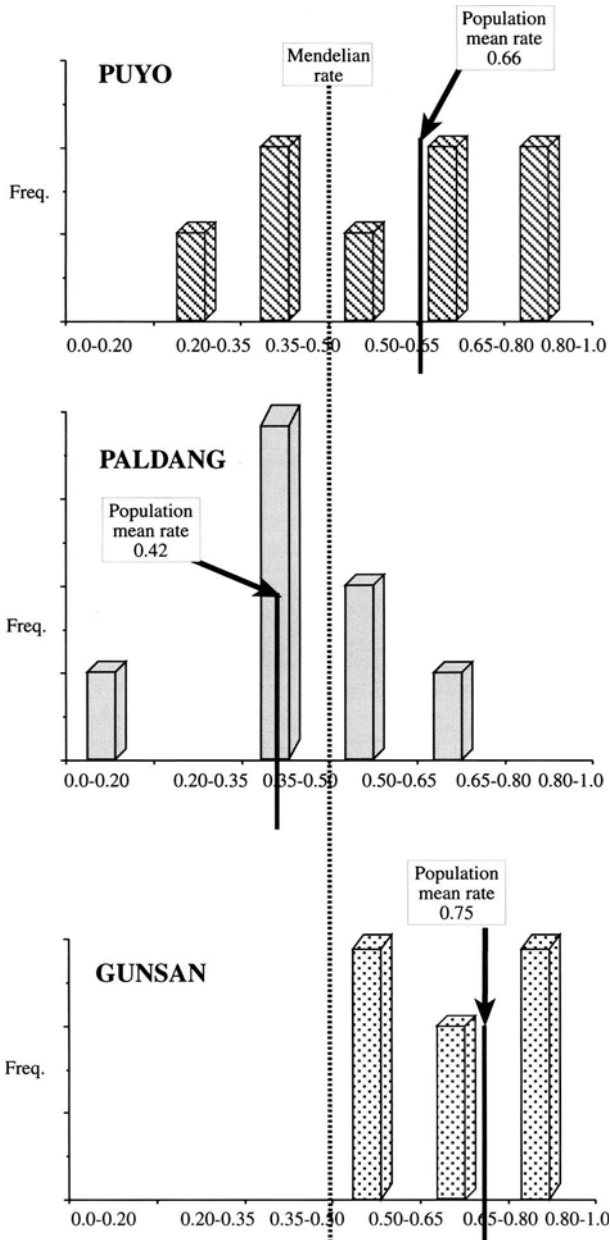

Figure 6. Distribution of B transmission rate in 2B × 0B crosses in three different rye populations.

and B frequency is low (20% of individuals carry Bs), and Gunsan where it seems that L Bs do not exist, but to our surprise B frequency in the population is as low as in Paldang. Fortunately, we have lots of B variants providing interesting data to study B evolution in the future.

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