Cytogenet Genome Res 120:265–280 (2008)
DOI: 10.1159/000121076

Cytogenetic and Genome Research

Chromosomes with a life of their own

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Accepted in revised form for publication by M. Schmid, 05 December 2007.

Abstract. B chromosomes (Bs) can be described as 'passengers in the genome', a term that has been used for the repetitive DNA which comprises the bulk of the genome in large genome species, except that Bs have a life of their own as independent chromosomes. As with retrotransposons they can accumulate in number, but in this case by various processes of mitotic or meiotic drive, based on their own autonomous ways of using spindles, especially in the gametophyte phase of the life cycle of flowering plants. This selfish property of drive ensures their survival and spread in natural populations, even against a gradient of harmful effects on the host plant phenotype. Bs are inhabitants of the nucleus and they are subject to control by 'genes' in the A chromosome (As) complement. This interaction with the As, together with the balance between drive and harmful effects makes a dynamic system in the life of a B chromosome, notwithstanding the fact that we are only now beginning to unravel the story in a few favoured species. In this review we concentrate mainly on recent developments in the Bs of rye and maize, two of the species currently receiving most attention. We focus on their population dynamics and on the molecular basis of their structural organisation and mechanisms of drive, as well as on their mode of origin and potential applications in plant biotechnology.

B chromosomes (Bs) are a unique class of supernumerary chromosomes that are optional extras in the genomes of numerous plant and animal species. They are dispensable, being found in some individuals in populations that carry them, and absent from others, which raises significant biological questions in terms of genome organisation, population cytogenetics and evolution. Bs were first discovered 100 years ago in an insect, in species of the leaf-footed plant bug *Metapodius*, by Edmund Wilson (Wilson, 1907a, b). They were first recognised in plants in the 1920s, when Gotoh (1924) and Kuwada (1925) correctly classified them in rye, and later when Longley (1927) found them in maize. Longley first called them *supernumeraries*, and Randolph (1928), also working with maize, later used the term *B chromosomes*, to distinguish them from the chromosomes of the basic complement called the A chromosomes (As). The term supernumerary B chromosomes is now simplified to Bs.

The essential features of Bs are: (i) they are dispensable; (ii) they pair only among themselves at meiosis (in species where they do pair) and do not recombine with the As; (iii) their inheritance is irregular, due to their polysomic nature and to the occurrence and elimination of univalents, all of which compromises their transmission through meiosis; (iv) meiotic elimination in some species is counter-balanced by processes of drive at mitosis, mainly in the gametophytes, and less frequently at meiosis, leading to equilibrium frequencies in populations; (v) they have adverse and quantitative effects on the phenotype when present in high numbers, especially on fertility which also contributes to their loss; (vi) they lack any known major gene loci, but rDNA se-

This work was supported by the grants BFU 2006-10921 and AGL2005-05104 from the DGICYT-MEC of Spain, and PR27/05-13949-BSCH from the Banco de Santander-UCM.

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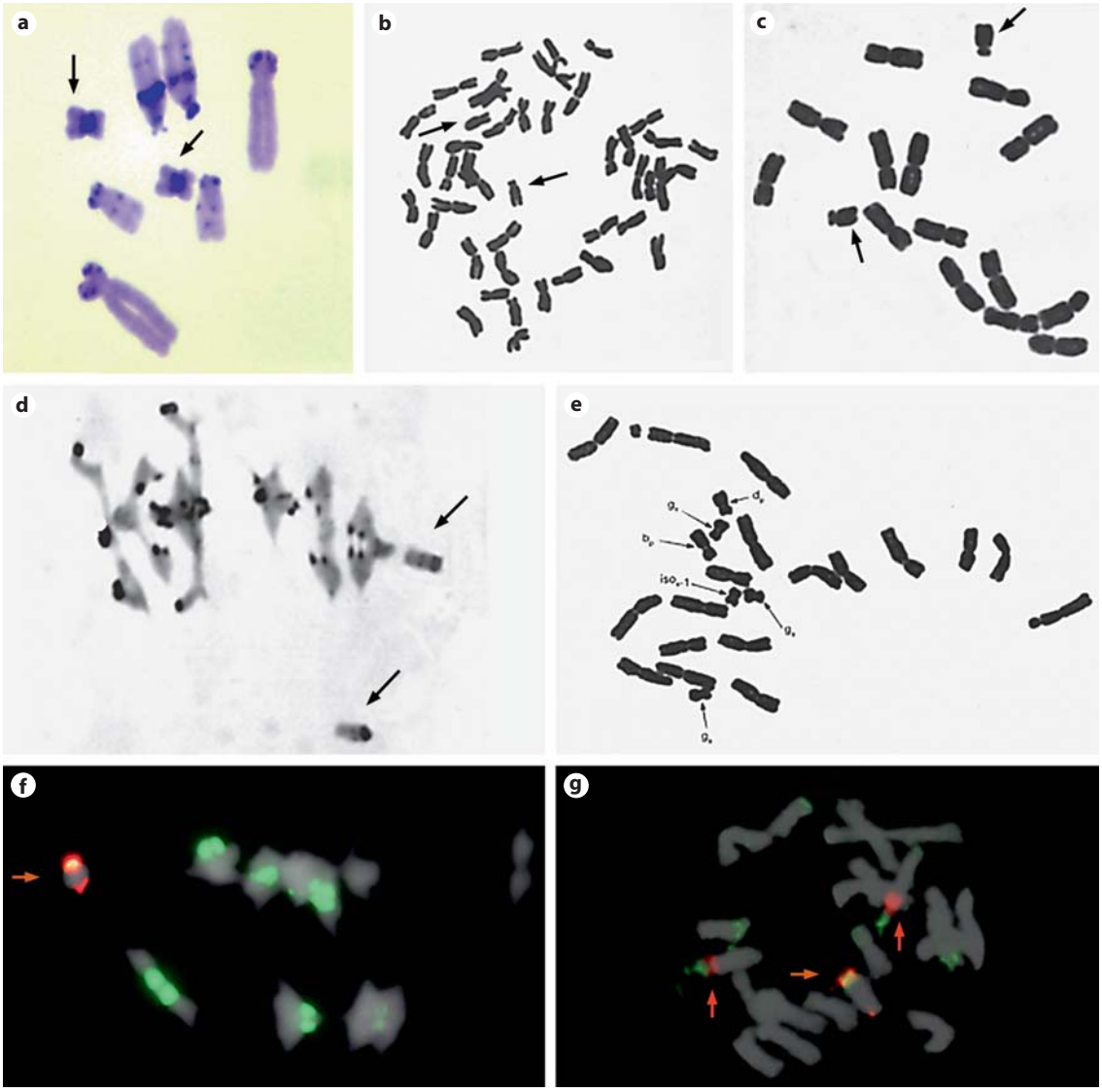

Fig. 1. B chromosome morphology at mitosis and meiosis in various plant species. (**a**) *Crepis capillaris* 2n = 2x = 6 +2B; (**b**) Lindström wheat with 2 rye Bs; (**c**) *Secale cereale* 2n = 2x = 14 +2B; (**d**) *Secale cereale* with 2B at meiosis (Original S. Manzanero); (**e**) *Aster ageratoides* with 6Bs of different forms and sizes; (**f**, **g**) *Zea mays* coloured with FISH using the probes: pZmBs specific to the B chromosome, which labels the centromeric and a subtelomeric region (red); the pZm4-21 probe which labels the 180-bp knobs (green); and ptA71 which labels the NOR (45S) region (red, red arrow). (**f**) Meiosis in the native race Pisingallo (Argentina) with 1B (orange arrow). (**g**) Mitosis in the native race Lluta (Chile) with 1B. The maize B has a knob close to the centromere, giving an orange signal (orange arrow).

quences are known in a few species; (vii) they contribute greatly to intraspecific variation in genome size; (viii) they have no obvious adaptive properties; (ix) their mode of origin remains a mystery; and (x), new information is now accruing on their molecular sequence organisation and expression of some noncoding sequences, and there are early indications that they may be structurally modified (maize) to have useful attributes in plant biotechnology.

Bs in plants have been studied by many authors over the last 80 years, and there is a wealth of information on their occurrence, effects, structure, transmission and origin, with recent work focussing most strongly on their molecular organisation (Jones and Rees, 1982; Jones, 1995; Puertas, 2002; Jones and Houben, 2003; Camacho, 2004, 2005; Burt and Trivers, 2006; Jones et al., 2007).

One of the most striking aspects of the story of B chromosomes is how common they are, being found not only in more than a thousand species of flowering plants, but also in Gymnosperms, Pteridophytes, Bryophytes and fungi (by electrophoretic separation, and where they carry some genes); and in species where they do occur they may be extremely widespread. In rye, for instance, they can be found in every region where the species grows in the wild or under semi-wild conditions (Jones and Puertas, 1993); and like-

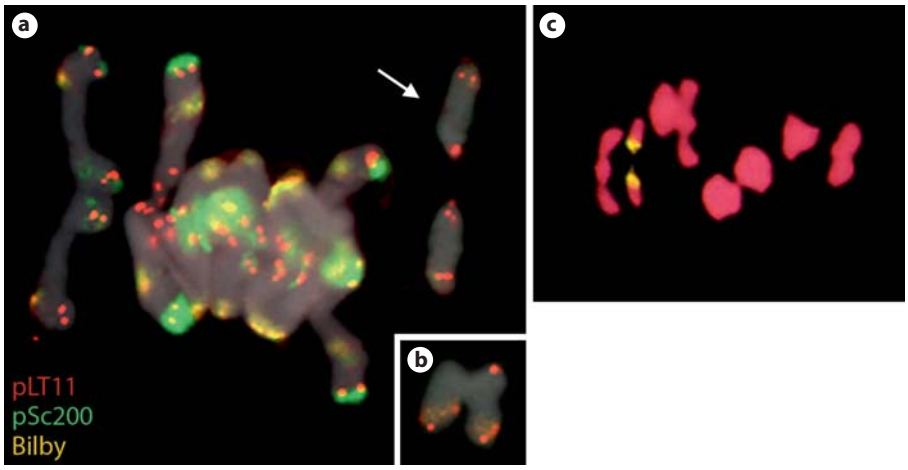

Fig. 2. (**a**) Metaphase I cell of rye with 2B forming bivalent (arrow), bound by the long arm, and coloured with FISH using the rye specific subtelomeric sequence pSc200 (green), the telomeric sequence pLT11 (red), and the rye specific centromeric sequence Bilby (orange). pSc200 is present in all A chromosomes but not in the Bs, whereas pLT11 and Bilby are present in both As and Bs. (**b**) Detail of a B bivalent at diplotene showing a chiasma in the long arm. (**c**) Metaphase I of rye with a 2B bivalent, and coloured with FISH using the E3900 probe which is specific to rye Bs.

wise in maize (Longley, 1938; McClintock, et al., 1981) and in many other cases. They are absent from cultivars, and from inbreeders, due to selection for high fertility and to their mode of inheritance, respectively.

We recognise Bs because they are usually smaller than the As, and can be seen in root cell meristems as additional chromosomes present in only some of the individuals in a sample of a population. Their most diagnostic feature however is their separate identity at meiosis, where they may be found as univalents, or various pairing configurations as bivalents or multivalents, but never pairing with the As. Meiotic analysis is critical to distinguish between Bs and aneuploidy for A chromosomes, especially where there is only a small difference in size between As and Bs (Fig. 1).

Recent studies on distribution suggest that the occurrence of Bs among different groups of angiosperms is not random: it appears that there are 'hot spots', and that their presence is correlated with genome size. In a survey of 979 species (Levin et al., 2005) a large disparity was found between their presence in monocots (8.0%) and eudicots (3.0%). Within the monocots they are known in 27.2% of species in the Commelinales, while in the order Zingiberales they are only found in 4.3% of species. There is much more variability in B-frequency among monocot orders than among eudicots, and they are rare or absent among non-monocot basal angiosperms (Nymphaeaceae, Magnoliales, Laurales). At the family level the largest number of +B species are the highly speciose Poaceae and Asteraceae, and there are also hot spots of occurrence in the Liliales and Commelinales. There is virtually no difference in frequency between diploids and polyploids (Jones and Rees, 1982; Trivers et al., 2004; Palestis et al., 2004), but there is a trend suggesting that Bs have a higher frequency in families with large genomes (Trivers et al., 2004). We may speculate that genomes with larger amounts of noncoding DNA present a more tolerant nuclear environment for the origin of Bs, since as in rye, they share overall similarity with As, except for the B-specific terminal region (Figs. 1, 2) (Timmis et al., 1975; Tsujimoto and Niwa, 1992; Wilkes et al., 1995; Houben et al., 1996). Bs are not known in *Arabidopsis*, but they have been found in rice (Cheng et al., 2000).

Where Bs do occur they are mostly found in low numbers, in the region of 0–5 in natural populations; but high numbers have been found in *Silene maritima* (0–15), *Brachycome lineariloba* (0–22), and *Allium schoenoprasum*, and as many as 34 Bs have been recorded in *Zea mays* in experimental material (see Jones and Rees, 1982 for references). There is no species known where the size of the Bs exceeds that of the largest A chromosome. In a few cases they are equal to and are indistinguishable from the As at mitosis; e.g. in *Clarkia elegans* (Lewis, 1951), *Sorghum nitidum* (Raman and Krishnaswami, 1960) and *Rumex thysiflorus* (Zuk, 1969). Small micro Bs are found in *Hypochoeris maculata* (Parker, 1976), *Brachycome dichromosomatica* (Houben et al., 1997) and *Campanula rotundifolia* (Böcher, 1960).

How do Bs affect the plants that carry them?

B chromosomes generate a numerical chromosome polymorphism for the species which carry them, and impact strongly on the nuclear DNA content. The basic genome of rye, for example, has a 1C nuclear DNA value of 8.28 pg = 8,114 Mbp, and each single B adds a further 800 Mbp which is four times the genome size of *Arabidopsis*. Natural populations of rye carry mostly two and four Bs, which contribute to the population polymorphism accordingly (Jones, 1976). The mean B frequency of +B plants in rye populations ranges from 6.6 to 54.0% (Jones and Rees, 1982), and the additional nuclear DNA in a plant with four Bs is a massive 3,200 Mbp. The B in maize accounts for ~ 4% of the total chromosome volume, and there are correlations between the number of Bs and the size of heterochromatic knobs. The knobs can mask the contribution the Bs make to total genome size (Rosato et al., 1998). We can safely assume that such polymorphisms for nuclear DNA amounts occur in all species which carry B chromosomes.

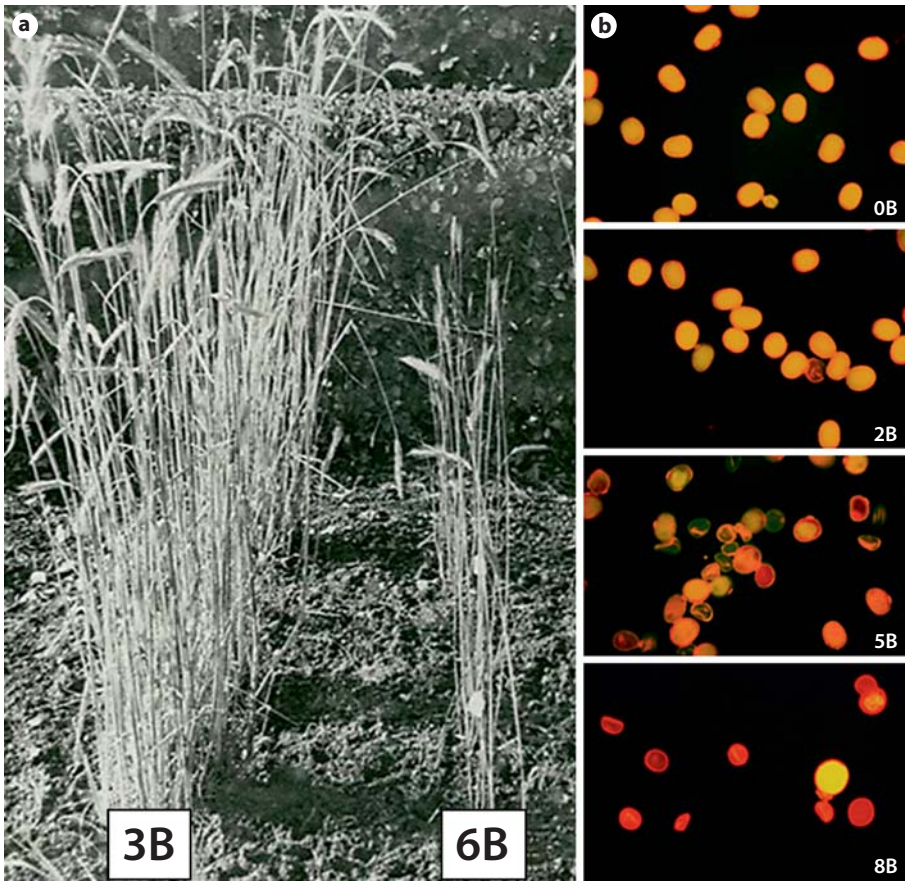

Fig. 3. The harmful effects of B chromosomes on fitness. (**a**) Rye plants with 3Bs are phenotypically indistinguishable from 0B plants (not shown), but 6Bs produce a remarkable decrease in vigour. (**b**) The effect of different doses of Bs on pollen grain viability in *Aegilops speltoides*. Plants with a high number of Bs are virtually sterile. Pollen stained with fluorescein diacetate and propidium iodide. Yellow pollen grains are viable; empty or red pollen grains are inviable. Original A. Cebriá.

The result of these polymorphisms is an effect on the physiology of the nucleus, of the whole plant and the populations in which they occur, as described in detail in Jones and Rees (1982). Essentially the cell cycle is lengthened, cells size is increased and there are declining levels of nuclear proteins and nuclear RNA in proportion to the number of Bs present, as determined by cytochemical methods. Interestingly, the levels of nuclear histone, in isolated nuclei, show the opposite trend, with some inexplicable complexity due to odd and even B numbers. These effects are expressed at the whole plant level, giving reduced vigour and fertility, especially for high numbers of Bs (Fig. 3), and there is a dearth of evidence, other than in experimental situations, to indicate that Bs are related to environmental variables or have any adaptive properties for natural populations – despite the best efforts of a number of workers to show otherwise (e.g. Semple, 1989). The influence that Bs have on the meiotic behaviour of the A chromosomes, in terms of variation in the frequency and distribution of chiasmata, and possibly the release of genetic variation, has also been covered in detail (Jones and Rees, 1982); as well as the way that Bs interact with the A genome (Jiménez et al., 1994).

In most species Bs are stable at mitosis during development, but in certain tissues they may become unstable and induce instabilities in the A chromosomes. This occurs in archesporial mitosis (Alfenito and Birchler, 1990), and in the tapetum in relation to programmed cell death (Chiavarino et al., 2000; González-Sánchez et al., 2004b).

Inheritance and survival

The A and B chromosomes exist in the same cytoplasm, and therefore share the same essential mitotic and meiotic mechanisms (Fig. 4) – such as the microtubule spindle and synaptonemal complex structures. However, the Bs enjoy their own non-Mendelian mechanisms of inheritance, consisting, in general terms, in passing on variable numbers from generation to generation, but with strategies which avoid their loss, which is a problem in diploids with any A chromosome in an aneuploid condition. The mechanisms of inheritance of Bs have been investigated in depth in only a few species. One overall conclusion is that the Bs have evolved conserved mechanisms of transmission in a number of species, indicating that they all fulfil certain general requirements, but the details of the process vary between species. A common strategy in angiosperms is for the number of Bs to increase by non-disjunction during the gametophytic phase of the life cycle (Fig. 5). In maize for example non-disjunction takes place at the second pollen grain mitosis (Roman, 1947, 1948), while in *Aegilops speltoides* it oc-

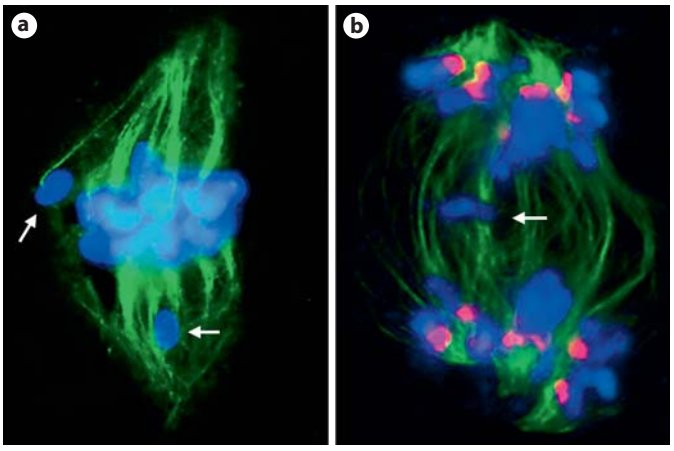

Fig. 4. (**a**) *Secale cereale* with 2Bs at metaphase I of meiosis, in a cell with microtubule immunolocalization with anti-tubulin (green). The 2Bs are the univalents, located outside of the metaphase plate (arrows). A small number of tubulin fibres are joined to the B centromere. (**b**) Anaphase II in a 2B plant of rye. Immunolocalization with anti-tubulin (green) and anti-phosphorylated histone H3 (red). Chromosomes are counterstained with DAPI (blue). The unlabeled laggard is a B chromatid (arrow), which results from a univalent B that divided equationally at anaphase I. The laggard will fail to reach the poles, and the B will be lost as a micronucleus. Figure 3b was originally published in Manzanero et al. (2000).

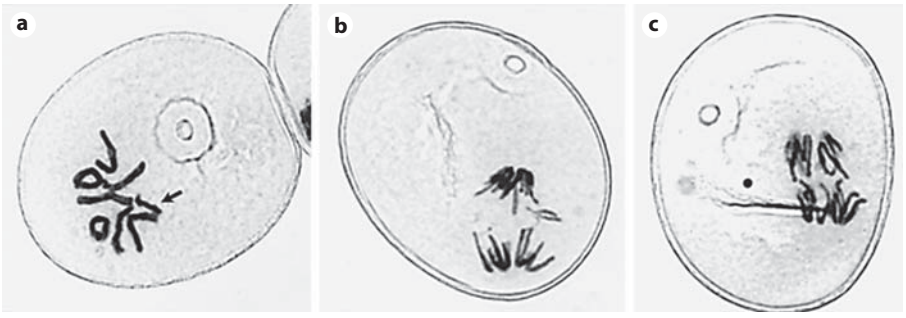

Fig. 5. Pollen mitosis in rye with Bs. (**a**) First pollen metaphase, B arrowed. (**b**) First pollen anaphase with the B undergoing directed nondisjunction to the generative pole. (**c**) Bs that were lost at meiosis form micronuclei in the pollen grains.

curs at the first pollen grain mitosis (Mendelson and Zohary, 1972), and in rye it happens during the first division of the gametophyte on both the male and female sides (Hasegawa, 1934; Hakansson, 1948). In *Aegilops* there is an additional odd phenomenon: at a certain time in development, and by means unknown, there is a directional mitotic division of the Bs in such a way that they pass into the shoots, and into the germ line, but are excluded from the roots (Mendelson and Zohary, 1972).

This species-specific pattern of behaviour of the B has important consequences for the carrier. In the *Aegilops* model both sperm nuclei in a pollen grain carry Bs in the same number, and the Bs will be transmitted with the same efficiency. In maize both sperm nuclei carry different B numbers (Fig. 6), giving rise to the possibility that the Bs may or may not be transmitted during the fertilization process. In rye, as B non-disjunction occurs on both sides, the number of Bs may highly increase from one generation to the next.

Corollaries of these three examples are: firstly, that every species manages the balance between B presence and negative fitness effects with different evolving strategies; secondly, it is difficult to establish a general model for B inheritance and, consequently, to understand the maintenance of B polymorphisms in natural populations.

In this review we will concentrate our thoughts on rye and maize, which are the two plant species where the mechanisms of B inheritance have been investigated most thoroughly. These studies take two approaches: the physical basis, using cytological and molecular studies, and genetic analysis based on selection experiments for high and low transmission types.

Population dynamics

B population dynamics is a consequence of their peculiar mechanisms of inheritance and fitness effects, and therefore both aspects need to be well known in order to have an understanding of the evolutionary aspects. In most species Bs are of parasitic nature because they lack genes with specific phenotypic function, they have non-Mendelian mechanisms of drive and their presence is deleterious to carriers when they are present at high numbers (Fig. 3).

The cases of non-parasitic, slightly parasitic or highly parasitic Bs of *Allium schoenoprasum*, maize and rye, respectively, have been reviewed in Bougourd and Jones (1997), Puertas et al. (2000) and Puertas (2002). Here we will present a review of only the most recent data for rye and maize.

Rye – Secale cereale

Rye is the only plant species known where the Bs undergo nondisjunction at the postmeiotic mitoses in both the

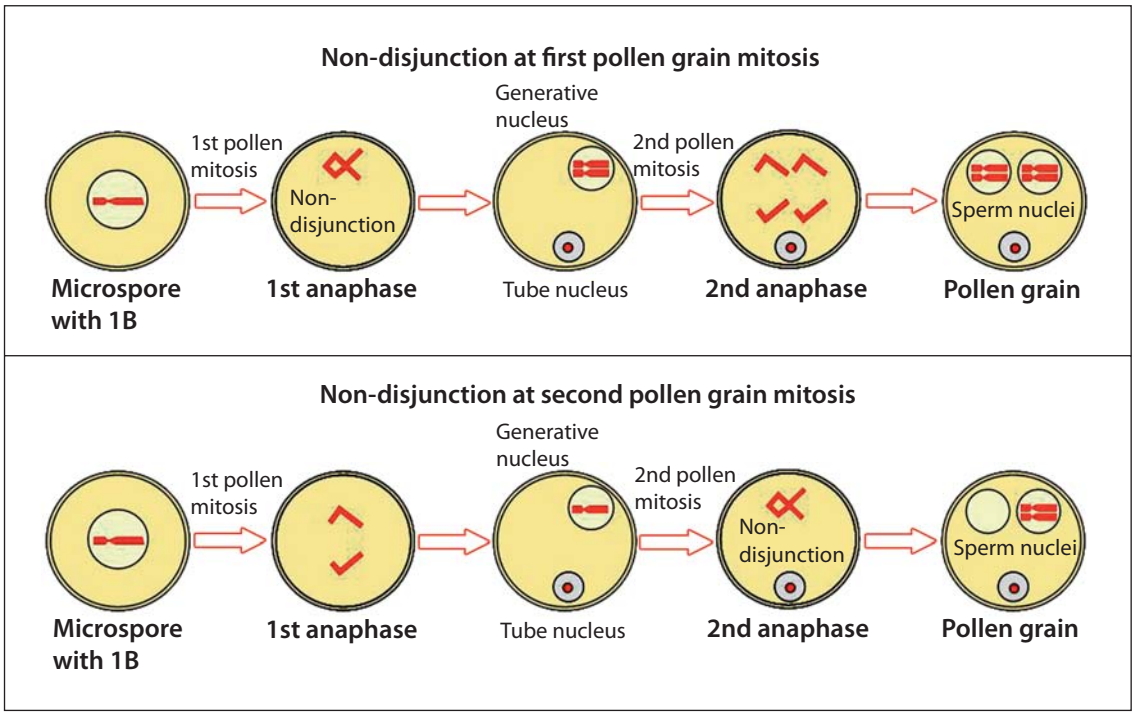

Fig. 6. Diagrams depicting non-disjunction of B chromosomes in gametophytes. When non-disjunction occurs at the first pollen grain mitosis both sperm nuclei duplicate the B number, and when it occurs at the second mitosis only one of the sperm nuclei carries the Bs. In maize the sperm with Bs preferentially fertilises the egg with about 70% efficiency. The A chromosomes (not shown) follow a normal mitosis with the reduced chromosome number.

female and male gametophytes, followed by preferential distribution to the gametes. Non-disjunction occurs almost in 100% of the cases, as shown in +B × 0B crosses (Jiménez et al., 1995). This strong drive is counteracted firstly because the Bs have harmful effects on fertility (Romera et al., 1989; Jiménez et al., 1994), and secondly because Bs are frequently lost at meiosis when they are present as univalents (Fig. 4) (Jiménez et al., 1997). Detailed studies on meiosis show that at pachytene they form bivalents or multivalents. At metaphase I they can form univalents, bivalents or multivalents, but regular meiotic behaviour is the most important event in determining B transmission (Jiménez et al., 2000).

González-Sánchez et al. (2004a) showed that rye Bs have a dosage effect interfering with grain formation, because 2B plants reduce fertility by about 25% and 4B by about 75% compared with 0B plants. They reported two factors influencing fertility reduction. One is the high (H) or low (L) transmission status of the plant, the other is the B number of the maternal parent.

Low B transmission is associated with higher fitness. L plants have a slighter lower parasitic effect than the H plants, both on the female and male sides. For example, the frequency of aborted pollen grains in H plants is nearly double that of L plants.

The B number of the mother plant significantly affects fertility; for example, 0B H plants coming from a 4B mother are the most fertile. Since 4B plants produce few seeds, and those produced are the most fertile in the next generation, the Bs could act as selective factors of the best genotypes for fertility. In this way, alleles in the A chromosomes tolerating or resisting the deleterious B effects may be selected. On the contrary 4B plants with 0B mothers are highly sterile. These plants must be formed from 0B egg cell and 4B pollen, which always comes from an abnormal meiosis and/or gametogenesis. The general conclusion is that B instabilities occurring during male and female meiosis and gametogenesis affect not only the gametes formed from these processes themselves, but also the next generation.

Genetic analysis of transmission properties have involved selection of rye genotypes for high (H) and low (L) B transmission rates. The Bs of 2B L plants form bivalents in only 20% of the metaphase I cells, and B univalents divide equationally at anaphase I, and are then subsequently eliminated as micronuclei (Fig. 7). On the contrary, Bs in the H line form bivalents in nearly 90% of the pollen mother cells, and are present in 85% of the pollen grains (Jiménez et al., 1997). It was determined that the genes controlling rye B transmission rate are located on the Bs, because in the F1 HL hybrids the behaviour at meiosis depends on the H or L status of the B carrying parent (Puertas et al., 1998). It seems that rye B transmission and population polymorphism mainly depends on the Bs themselves, and that regular meiotic behaviour is essential for a B chromosome to be maintained in the long term.

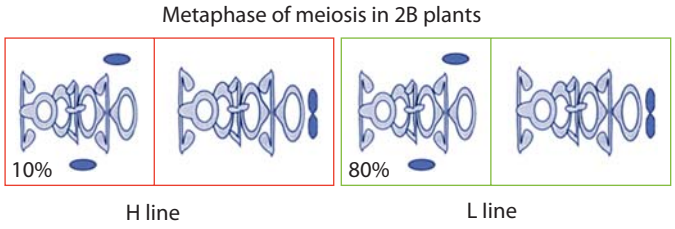

Fig. 7. Diagram showing transmission genotypes in rye lines selected for high (H) and low (L) transmission rates of Bs. In the H line the Bs form univalents in only 10% of the metaphase I cells, whereas in the L line the frequency of univalents rises up to 80%. In rye the feature of forming univalents or bivalents depends on the Bs themselves.

To determine whether the A genotype also influences B transmission, isogenic rye Bs were introduced by hybridization and backcrossing in eleven inbred lines (Ortiz et al., 1996). Pairing of the Bs was greatly influenced by background genotypes, although the exact nature of such influence was not determined.

Maize – Zea mays

There are three main processes controlling the inheritance of Bs in maize: (i) B non-disjunction at the second pollen grain mitosis (Fig. 6) (Roman, 1947; Carlson, 1978; Carlson and Chou, 1981); (ii) preferential fertilisation by the sperm nucleus carrying the Bs after the non-disjunction process (Roman, 1948; Carlson, 1969); (iii) the suppression of meiotic loss when the Bs are unpaired (Carlson and Roseman, 1992).

Maize Bs in their native form regularly undergo non-disjunction in the male gametophyte, as shown in the progeny of 0B × 2B crosses which has 0B or 2Bs in more than 99% of the cases (Rosato et al., 1996; Chiavarino et al., 2001). Using deletions created by translocation breakpoints in the TB-10 L18 B-A translocation line it was demonstrated that the genetic control of the non-disjunction process is due to a region in the B chromosome itself (Lin, 1978, 1979; Carlson, 1978; Carlson and Chou, 1981) (Fig. 8).

The genetic control of preferential fertilization when the Bs are transmitted on the male side has been determined by selection for genotypes of high (H^m) and low (L^m) B transmission rate (B-TR) (Rosato et al., 1996). It was demonstrated that the gene controlling B-TR on the male side is located on the A complement, because in 0B × 2B crosses the B-TR depends on the H^m or L^m status of the 0B parent (Chiavarino et al., 1998). Interestingly, the 0B female parent controls preferential fertilisation, because the 0B H^m female is preferentially fertilized by the sperm nucleus carrying 2Bs. On the contrary, the 0B L^m female is fertilized by the 0B or 2B sperm nuclei at random. The H^mL^m F1 hybrids show intermediate B-TR (Chiavarino et al., 2001) and the F2 segregation demonstrated that the H^m and L^m lines differ in a single major gene called *mBt* (male B transmission) (González-Sánchez et al., 2003).

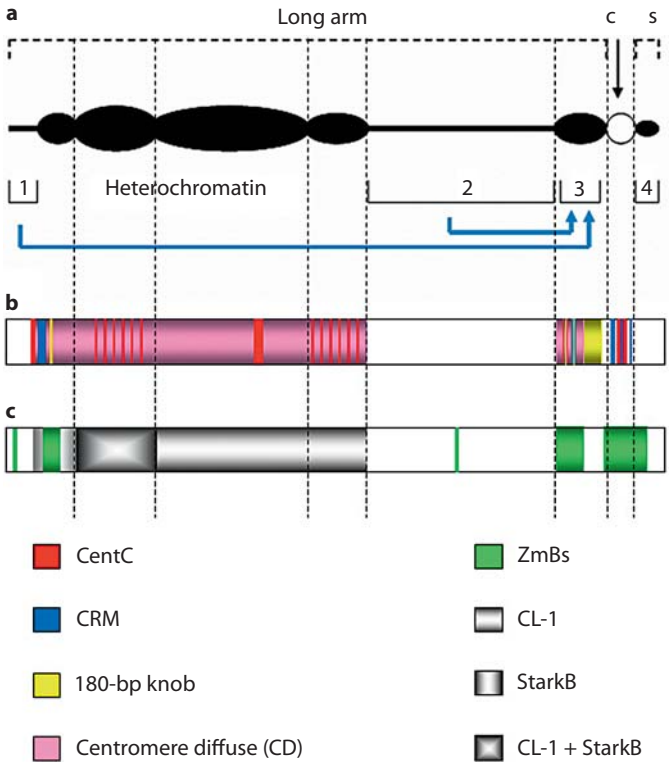

Fig. 8. (**a**) Structure of the maize B in relation to non-disjunction according to Carlson and Chou (1981). Regions 1 and 2 are *trans* acting, and are thought to signal the sensitive sticking sites of essential region 3. Deletion of regions 1 or 2 causes complete elimination of non-disjunction. Region 4 modifies the rate of non-disjunction, but its loss does not eliminate it. According to Han et al. (2007), the essential region for non-disjunction is only the centromere. C: centromere; S: short arm. (**b**, **c**) Schemes showing the molecular composition of the maize B. (**b**) DNA sequences shared with the A chromosomes. (**c**) B-specific sequences.

Selection for high (H^f) and low (L^f) B-TR lines was also carried out when the Bs were transmitted on the female side in 1B × 0B crosses (Rosato et al., 1996). In the L^f line, a significant loss of the B univalent occurs at meiosis. On the contrary, in the H^f line the B is present in the microspores at a Mendelian rate (Gonzalez-Sanchez et al., 2003). In the H^fL^f F1 hybrids it was determined that the gene/genes controlling B-TR on the female side called *fBt* (female B transmission) are located on the As, acting at diploid level, with the fBt^l allele(s) for low B transmission being dominant.

The B chromosome itself also has control over the preferential fertilization process as shown using deletion derivatives of the B-9 translocated chromosome which lack the centric heterochromatin and possibly some adjacent euchromatin as well (Carlson, 2007).

The lack of meiotic loss of B univalents is a special property of maize Bs that has not been reported in other species. This suggests a unique structure of the maize B allowing its proper migration at anaphase I in spite of forming univalents. Carlson (1986) reported that certain deletion stocks of the translocated chromosome B^9 were much more suscep-

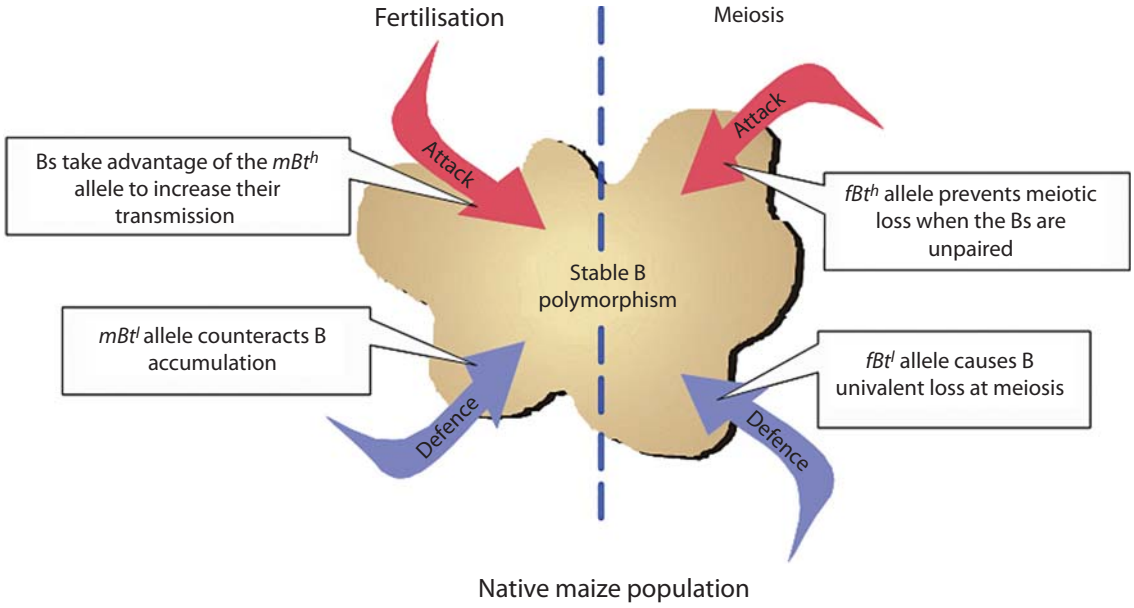

Fig. 9. Model of coevolution of B attack and A defence in a native maize population. At meiosis the loss of unpaired Bs is prevented in plants homozygous for the fBt^h fBt^h alleles. A genome defence is provided by the fBt^l dominant allele which causes loss of univalent Bs. At fertilisation B attack occurs in mBt^h egg cells, which are preferentially fertilised by B-carrying male gametes. Egg cells with the mBt^l allele tend to counteract B accumulation, because they are fertilised by 0B or 2B sperm nuclei at random. Originally published in González-Sánchez et al. (2003).

tible to loss of the univalent than the standard translocation. Carlson and Roseman (1992) studied five different deletion types and showed that these deletions removed components of the B that serve to suppress meiotic loss. It has been recently suggested (González-Sánchez et al., 2007) that the subtelomeric region of the B-specific ZmBs sequence might be involved in the proper orientation of the B univalent, because in a number of metaphase I cells both the centromeric and the subtelomeric portion are stretched to the poles and co-oriented (Fig. 1f).

Maize Bs are an example of intragenome conflict (Frank, 2000), because the *mBt* and *fBt* loci, controlling B transmission through preferential fertilisation and transmission are located on the A chromosomes (González-Sánchez et al., 2003), and constitute a polymorphic system of attack and defence between As and Bs. Maize Bs have a mechanism of drive and impose only slightly deleterious phenotypic effects on the carrier: they can therefore be classified as moderately parasitic, according to Camacho et al. (2000). The Bs should be maintained at low numbers and, therefore, alleles in the A genome providing defence against the B attack are expected to increase in frequency.

González-Sánchez et al. (2003) proposed that the main function of the *mBt* gene, controlling male B transmission, is involved in the normal maize fertilisation process, but the Bs take advantage of the mBt^h function to increase their own transmission, allowing B accumulation and attack. The fBt^l allele could be the response of the host A genome to get rid of the B effects, suppressing B accumulation and providing A chromosome defence.

Another system complements this function acting at the diploid level during meiosis. The *fBt* locus/loci acts in such a way that in the fBt^h fBt^h homozygote the unpaired Bs are not lost, providing B attack, whereas in all plants carrying the dominant fBt^l allele the unpaired Bs tend to be lost, providing an efficient A chromosome defence. This model is represented in Fig. 9.

The coevolution of As and Bs in maize is in agreement with the nonequilibrium model proposed for the grasshopper *Eyprepocnemis plorans*, where the Bs have been neutralised by the A genome (Camacho et al., 1997a, b; Zurita et al., 1998; reviewed in Camacho et al., 2000).

Molecular organisation

Rye – Secale cereale

In general the rye B is comprised of repetitive DNA which shares its properties with that of the bulk of the A chromosomes (Rimpau and Flavell, 1975; Timmis et al., 1975), but the distinctive feature of the B lies in two kinds of B-specific sequences located in the distal region at the end of the long arm. The molecular analysis of these particular sequences is now attracting the greatest level of attention, since they cover the region of the B that is implicated in the control of the non-disjunction process, as described below.

When Hasegawa (1934) first described directed nondisjunction (ndj) in rye pollen he was able to observe that a key factor in the process was the way in which the sister chro-

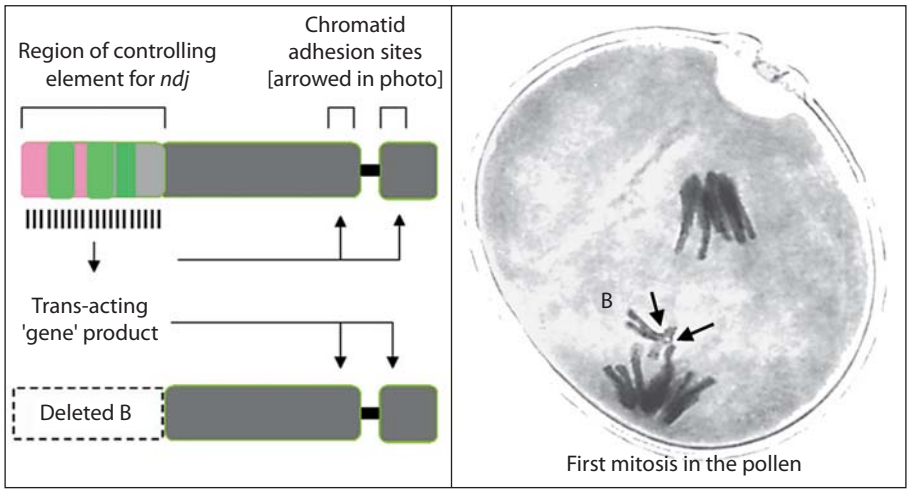

Fig. 10. Control of non-disjunction for rye B chromosomes. A deleted B does not undergo non-disjunction (njd) unless there is a normal B present in the same nucleus, showing that the controlling element is *trans*-acting. The B-specific E3900 sequences are shown in pink and the D1100 in green.

matids of a single B were held together at two sticking sites on either side of the centromere, which then resulted in both chromatids ending up in the generative nucleus. To this day it is not known if this is an active process, involving movement of the undivided Bs to the pole concerned, or whether it is passive. The passive argument is acceptable, albeit speculative, on the grounds that the spindle at first pollen mitosis is asymmetrical, and the equator is nearer to the generative than to the tube nucleus pole (Fig. 5). Transient sticking of the chromatids may thus be all that is required to hold them together until they become included in the generative nucleus, in up to 86% of pollen grains (Matthews and Jones, 1983).

A major advance in our knowledge of the mechanism of drive in the Bs of rye came when it was discovered that there was genetic control of the process, based on a controlling element located in the heterochromatic block in the distal part of the long arm of the standard B. Deficient Bs which are missing the distal part of the long arm, including small isochromosomes, do not undergo non-disjunction when present as the only forms of the B, but when a standard B is also present it provides the necessary *trans*-acting function to enable the process to occur in the deficient Bs as well as the standard B itself (Fig. 10) (Lima-De-Faria, 1962). The question as to what this controlling element may be has long exercised the minds of researchers, especially as the B is lacking in genes.

More breakthroughs came with the discovery that the controlling element region is comprised of two families of B-specific sequences, E3900 and D1100 (Sandery et al., 1990; Blunden et al., 1993; Houben et al., 1996; Langdon et al., 2000), in contrast with the rest of the B which has identity with the dispersed repetitive sequences of the A genome, as determined by GISH. These B-specific sequences were described in detail by Langdon et al. (2000): they appear to be complex, longer than typical satellites, of high copy number, lacking in ORFs and have been assembled from fragments of a variety of sequence elements, suggesting their de novo origin. The relationship between the two elements, and the complexity of the domain, in terms of distinct subdomains, is shown in Fig. 10. Neither family can be profiled as a single monotonous tandem array: they contain more than one size class of *Eco*RI fragments, and they also contain some sequences common to both the As and Bs. In addition, their chromatin organisation is unusual, and differs from the typical constitutively condensed subtelomeric regions of the A chromosomes.

This region, which can also be visualized as a C band, undergoes decondensation during interphase, unlike the subtelomeric regions of the A chromosomes (Langdon et al., 2000; Carchilan et al., 2007), and is also marked by trimethylated H3K4 and trimethylated H3K27 lysine residues, which are conflicting epigenetic histone modifications. H3K4me promotes transcriptionally competent chromatin whereas H3K27 is typical for heterochromatin in rye (Carchilan et al., 2007). These recent studies raise significant biological questions about the way in which epigenetic marks for chromatin compaction occur in a region where the chromatin is decondensed at interphase, in various cell types, in both in rye and in hexaploid wheat carrying rye Bs as an addition line. Furthermore, it is revealed that both the E3900 and the D1100 sequences are transcribed, and produce a heterogeneous collection of transcripts of noncoding RNA (Carchilan et al., 2007). Here we have yet another B chromosome enigma, but in this case providing a real handle with which to explore the relationship between this specialised region of the rye B, which acts as a controlling genetic element for non-disjunction, and its inconsistent transcriptionally active heterochromatin.

This physical/molecular story is not to be confounded with the effects of transmission genotypes, since nondisjunction at pollen mitosis operates at a constant level, and whereas transmission genotypes depend on the pairing properties of the B at meiosis.

Maize – Zea mays

The molecular analysis of the maize Bs is giving new insights into their origin, and into the molecular basis of the

mechanisms that allow them to survive. As in the case of rye, all studies indicate that the DNA on the maize Bs has high homology with that of the A chromosomes. The maize B is mainly composed by highly repetitive DNA in the form of tandem repeated satellites and retroelement related sequences. There is enrichment for repetitive elements that are found at or near the A chromosome centromeres. Interestingly, these elements are not confined to the B centromeric region but are scattered throughout the length of this chromosome, and are the major components of the heterochromatic blocks on the maize B (Lamb et al., 2005). In addition to the DNA common with the As, three high copy number repetitive elements specific to the Bs have been identified and characterized in detail (Alfenito and Birchler, 1993; Kaszás and Birchler, 1996; Stark et al., 1996; Cheng and Lin, 2003, 2004; Lamb et al., 2007).

We describe here the sequences identified on the maize B according to their distribution on the five regions that characterize this chromosome at pachytene: centromere, proximal heterochromatin, proximal euchromatin, distal heterochromatin and distal euchromatin (Fig. 8).

Centromere

The centromere of the maize B has been extensively characterized by J.A. Birchler and colleagues. Two aspects were crucial in this characterization. First, the use of B centromere misdivision derivatives has allowed the study of a centromere deletion series, making it possible to define which centromeric regions are retained as being essential for regular transmission of the B (Kaszás and Birchler, 1996, 1998; Jin et al., 2005). Second, the presence at the B centromere of the repetitive element ZmBs, that is not found on the A chromosomes (Alfenito and Birchler, 1993). This specificity has allowed cytological and molecular analysis of centromere organisation at the level of a single chromosome in the maize genome (Kaszás and Birchler, 1996; Jin et al., 2005).

ZmBs is a degenerated tandem array, with a repeat unit of 1.4 kb (Alfenito and Birchler, 1993), that expands over 9 Mb of DNA interspersed with other repetitive sequences (Kaszás and Birchler, 1996; Theuri et al., 2005). A region within this 9 Mb is part of the functional B centromere, because all the misdivison derivatives analysed retained a portion of the ZmBs repeat (Kaszás and Birchler, 1996). Moreover, the size of this portion correlates with altered meiotic transmission of the resulting B derivatives (Kaszás and Birchler, 1998). These findings were supported when the fine organisation of the B centromere became apparent by fibre-FISH analysis of ZmBs and two repetitive elements found in maize A centromeres, CentC and CRM (Jin et al., 2005). CentC is a 156-bp satellite repeat specific to the centromere region on maize A chromosomes (Ananiev et al., 1998). CRM are retrotransposons from the CR family of Ty3/Gypsy retroelements that are present only at centromeres of grass species (Miller et al., 1998; Ananiev et al., 1998; Zhong et al., 2002). CentC and CRM elements are located at the functional core of the A centromeres in maize (Jin et al., 2004). Fibre-FISH mapping on the intact B centromere revealed that CentC and CRM are restricted to a 700-kb domain that is embedded within the much larger array of the ZmBs repeat (Jin et al., 2005). Interestingly, the breakpoints of the misdivision derivatives analyzed are mapped within this domain. This is also the binding domain of CENH3 on the B centromere (Jin et al., 2005). CENH3 replaces the regular H3 histone in kinetochore specific nucleosomes, and is considered to be an epigenetic mark for functional centromeric chromatin (Henikoff et al., 2001; Sullivan et al., 2001). In addition, the amount of CENH3 in misdivided B centromeres correlates with the size of the DNA fragment derived from the 700-kb domain (Jin et al., 2005). Taken together, these results demonstrated that the functional boundaries of the maize B centromere are mapped to a relatively small 'core' consisting of interspersed CentC, CRM and ZmBs elements (Jin et al., 2005).

The two blocks of ZmBs flanking the B centromere core are megabases in length (Jin et al., 2005). This is a vast excess of ZmBs over that which is required for centromere function, and may suggest that this repeat presumably plays a role in other centromeric functions. One of the accumulation mechanisms of the maize B involves non-disjunction at the second pollen mitosis (Roman, 1948; Carlson, 1978). The analysis of an intact B centromere translocated into maize chromosome 9 has shown that the nondisjunction of the maize B involves strong attachment of the two sister chromatids at a site spanned by the ZmBs sequence array, including the centromere core region (Han et al., 2007). These authors argue that since the other two DNA elements located at the B centromere core (CentC and CRM) are also present at the centromeres of the A chromosomes, the unique molecular feature that correlates with non-disjunction is the ZmBs repeat. The ZmBs repeat unit of 1.4 kb has a region of homology with the tandemly arrayed 180-bp knob repeat found at the heterochromatic blocks on the A chromosomes (Alfenito and Birchler, 1993). The knobs are the last sites to replicate, compared with other types of chromatin in the maize genome (Pryor et al., 1980). Given the sequence relationship between the knob and the ZmBs repeats, it is possible that throughout the ZmBs array region the replication is delayed beyond the time of anaphase movement during the second pollen mitosis. The different replication timing of ZmBs would account for nondisjunction (Han et al., 2007).

The B-specific ZmBs repeat also has homology with a chromosome 4 specific repeat, Cent 4, that is present as tandem arrays in the centromere of this chromosome (Page et al., 2001). The areas of homology of these two sequences overlap with the knob-homologous area in the ZmBs sequence. Based on this homology, Page et al. (2001) suggested that the chromosome 4 centromere is the most likely donor of the B centromere. If this is the case, the ZmBs repeat has evolved in the B chromosome to gain a number of functions not found in Cent4. First, Cent4 is outside of the functional centromere 4 core region, and does not associate with CENH3 (Jin et al., 2004). In contrast, ZmBs is found interspersed with CentC and CRM at the B centromere core, and is associated with CENH3 (Jin et al., 2005). This would sug-

gest that ZmBs have apparently invaded the centromeric functional core and probably reinforces B centromere function. Second, while the Cent4 locus spans about 350 kb DNA (Jin et al., 2004) the ZmBs repeat expands over 9 Mb of DNA (Kaszás and Birchler, 1996). The large expansion of ZmBs in the regions flanking the B centromere core is probably responsible for the nondisjunction accumulation mechanism of the maize B, which evolved independently of the centromere function (Han et al., 2007).

Proximal heterochromatin

The proximal half of the proximal heterochromatin is characterized by the presence of a distinct knob. This knob hybridizes to the 180-bp knob repeat (Lamb et al., 2005) that is also present in the A chromosome knobs. There are contradictory results about the hybridization at this same location of the TR1 element, the other repeat found at the A knobs, although in most reports TR1 has not been found on the Bs (Hsu et al., 2003; Lamb et al., 2005; Gonzalez-Sanchez et al., 2007).

The distal half of the proximal heterochromatin is composed of a mixture of ZmBs, CentC, CRM and 180-bp knob repeats (Lamb et al., 2005). In this region there are also located repetitive elements that have been designated as 'Centromere Diffuse' (CD) elements because of their distinct pattern of hybridization on the A chromosomes (Kato et al., 2004). These elements include retroelements (such as Cinful1), and other DNA elements of unknown origin (Nagaki et al., 2003; Kato et al., 2004), which hybridize along the length of the A chromosomes but show a pattern of enrichment in the pericentromeric regions. Also, in the case of the B chromosome, the CD element hybridization is more abundant in the proximal heterochromatin and declines in the proximal euchromatin.

Proximal euchromatin

Distal to the proximal heterochromatin there is a euchromatic region which makes up about one-third of the length of the pachytene chromosome. No genes have been mapped to this euchromatic region. There is a minor hybridization site for ZmBs, clearly outside the centromere or pericentromeric areas where this repeat is mainly found (Lamb et al., 2005). The Huck retroelement is located primarily in this euchromatic region, and hybridises weakly to the rest of the B (Lamb et al., 2005). Huck is one of the most represented retroelements in the maize genome, and it is highly abundant in the euchromatic regions of the A chromosomes.

Distal heterochromatin

Distal to the euchromatic region there is a large heterochromatic region which makes up about half of the pachytene length of the B. This region is usually divided into four blocks.

The DNA elements present at the centromere and proximal heterochromatin of the maize B are also the most abundant elements at the heterochromatic blocks, but they are arranged in a different way (Lamb et al., 2005). CD elements show strong hybridization throughout the four blocks of heterochromatin. The centromeric satellite, Cent C, hybridizes throughout the first and third blocks, and in two distinct sites on the second and fourth blocks. The intensity of CentC hybridization signals in each heterochromatic block is greater than the intensity on the A centromeres (Lamb et al., 2005), which may suggest that CentC could support centromere function throughout the long arm of the B. However, the organisation of CentC in the heterochromatin is different from that at the centromere, and only at the centromere core is CentC interspersed with CRM and ZmBs. In the heterochromatic blocks, CRM and ZmBs are restricted to the fourth block. CRM hybridizes to a minor site proximal to the most distal CentC signal of the fourth block, and ZmBs hybridize to a major site proximal to the CRM site. To determine whether the centromeric elements located outside the centromere recruit kinetochore specific nucleosomes Lamb et al. (2005) labeled mitotic and meiotic cells with antibodies against CENH3. The labeling was observed only at the functional centromere. This finding indicates that DNA sequence alone is insufficient to cause centromere formation. Instead, centromere identity may require the intermingled pattern of CentC, CRM and ZmBs observed at the core of the B centromere, and the presence of epigenetic factors including chromatin structure.

González-Sánchez et al. (2007) suggested that the ZmBs region in the distal half of the fourth heterochromatic block may be involved in the passage of the B univalent through meiosis, which is one of the maize B accumulation mechanisms. They found that the centromeric and distal ZmBs signals are conspicuously co-oriented at metaphase I. This feature would facilitate the proper orientation and migration of the B univalent to one of the poles, thus preventing amphitelic orientation of the B univalent and its loss as micronuclei in dyads. Because the B univalent co-orientation requires the centromere and distal ZmBs, it seems that both regions are needed for the suppression of meiotic loss when the Bs are unpaired.

In addition to the DNA common with the A chromosomes, two B-specific repeated sequences have been identified in the distal heterochromatin, CL-1 and StarkB. CL-1 is organized in long tandem arrays with repeat units of 1.6 kb, and it is present in the first three heterochromatic blocks (Cheng and Lin, 2003, 2004). Stark et al. (1996) identified a B-specific RAPD marker that was later used by Lamb et al. (2007) to isolate a DNA element termed StarkB that is distributed throughout the third and fourth blocks of heterochromatin. StarkB is much larger than the other B-specific elements (ZmBs and CL-1), approximately 22 kb, and is not arranged in tandem arrays. It is composed of repetitive sequences known from the A genome, mainly retroelements, as well as novel sequences unique to the B (Lamb et al., 2007). Furthermore, most regions of StarkB are expressed, and the open reading frames (ORF) found have strong similarity to the protein products encoded by transposable elements (Lamb et al., 2007). Interestingly, StarkB and the rye B element E3900 share a number of common features (including that both are transcriptionally active) which may

reflect similarities in how these elements formed and expanded, or requirements for B chromosome survival (Lamb et al., 2007).

Distal euchromatin

A short euchromatic region terminates the maize B chromosome. A *trans*-acting factor required for non-disjunction of the B is located in the distal euchromatin (Carlson, 1978). The only sequence identified in this region is a minor site for ZmBs (Lamb et al., 2005).

The information available suggests that the maize B is built up from centromeric and pericentromeric regions of the A chromosomes. Some sequences from these regions have been subsequently amplified on the B. For instance, the CentC satellite and the CD elements are found throughout the large heterochromatic blocks. Because centromeres are gene poor, they are a good substrate to assemble Bs, which are basically inert. Moreover, the repetitive elements present at the centromeres (satellites and retroelements) have amplification mechanisms, such as unequal crossing-over and targeted transposition, which can account for their subsequent expansion on the B. Lamb et al. (2005) suggested that the maize B could form as result of a rearranged multicentric chromosome undergoing the chromosome type breakage-fusion-bridge (BFB) cycle. In this cycle, the centromeres migrate to opposite poles forming a chromatin bridge that breaks, resulting in deletions of portions of the original chromosome. This process will eliminate genes that are detrimental in greater than 2n copies, and the final outcome will be a minichromosome with little more than the centromere regions. The minichromosome could then be stabilized by inactivation of one of the centromeres. The inactive centromere stage would be reinforced by expansion of the repetitive elements to give rise to a chromosome rich in centromeric elements at a location distinct from the remaining functional centromere (Lamb et al., 2005). Recent observations by Han et al. (2006) provide support for this model. They found that dicentric chromosomes carrying two B centromeres undergo the BFB cycle and give rise to minichromosomes with big chromosomal deletions. Some of the minichromosomes were stabilized by inactivation of one of the centromeres, and only the functional centromere showed labelling by CENH3 (Han et al., 2006).

The repetitive DNA elements found on the maize B may provide an adequate amount of chromatin for efficient chromosome transmission, or may play a role in survival of the B through involvement in the accumulation mechanism. As shown above, the B-specific sequence ZmBs seems to be involved in centromere function (Jin et al., 2005), non-disjunction (Han et al., 2007), and efficient passage of the B through meiosis as a univalent (González-Sánchez et al., 2007). The ZmBs is therefore an excellent example of the functional plasticity that a repetitive DNA sequence can achieve. In addition, the transcription of the B-specific StarkB element in a highly heterochromatic region (Lamb et al., 2007) suggests that RNA could be involved in the *trans*-acting factors required in the accumulation mechanisms.

Origin of Bs – a life of their own

Nobody really knows where B chromosomes come from, other than to say that they originate from the As, although there are some plausible ideas and models (Jamilena et al., 1994; Dhar et al., 2002; Berdnikov et al., 2003; Jones and Houben, 2003).

The most convincing case is that for the fully documented origin of a nascent B in *Plantago lagopus* (2n = 2x = 12) (Dhar et al., 2002). The story began with finding a spontaneous trisomic of chromosome 2 in 1984, and then tracking it through several generations. The extra chromosome went through a number of rapid structural changes, including the formation of a ring chromosome, and finally stabilised as a heterochromatic isochromosome with features of a B. It showed preferential transmission, absence of any phenotypic effects, had a functional centromere and did not pair with any chromosomes of the standard complement. The sequence of events leading to the origin of this apparent B were unraveled by monitoring its life history over several generations and then characterising it by FISH with several probes. It was 'born' by the massive amplification of 5S rDNA, as a component of a nascent mini chromosome which included a centromere (Fig. 11). Telomeres were added de novo, which is known to happen, but it is not known how the nascent B undergoes preferential transmission.

Bs could also escape as small centric fragments following unequal translocation and a reduction in chromosome number to give a new species, as we speculate may have happened during the evolution of *Crepis fuliginosa* (2n = 2x = 8 +Bs) from *C. neglecta* (2n = 2x = 6) (Jones and Rees, 1982); and as proposed following an aneuploid reduction process in *Haplopappus gracilis* (2n = 2x = 4 +Bs) (Jackson, 1960).

Genomic rearrangements following interspecific hybridisation offer another opportunity for supernumeraries to arise, e.g. the derivatives *Coix gigantea* (2n = 2x = 20) hybridising naturally with *C. aquatica* (2n = 2x = 10). *C. gigantea* has four pairs of small chromosomes, about the same size as those of *C. aquatica*. In the derivatives of hybrids one or two of these small chromosomes appeared as alien extras in the genome of *C. aquatica*, giving plants with 2n = 11, and various other hybrid combinations. In plants where the additional small chromosome did not pair with the As of the *C. aquatica* genome it showed meiotic behaviour typical of a single univalent B as found in many +B species, and lacked obvious phenotypic effects. This single B could also undergo centromere misdivision to give smaller heterochromatic chromosomes. In the absence of cytological observations in these hybrid derivatives the various forms of these additional chromosomes found in population samples of *C. aquatica* would almost certainly be taken to be B chromosomes (Sapre and Deshpande, 1987).

In *Brachycome dichromosomatica* the Bs are a conglomerate of mainly tandem repeat sequences derived from different A chromosome sites, and could therefore not have originated by a single excision of an A fragment (Houben et al., 2001). It is proposed instead that B-founder sequences were 'released' from a polymorphic A chromosome region,

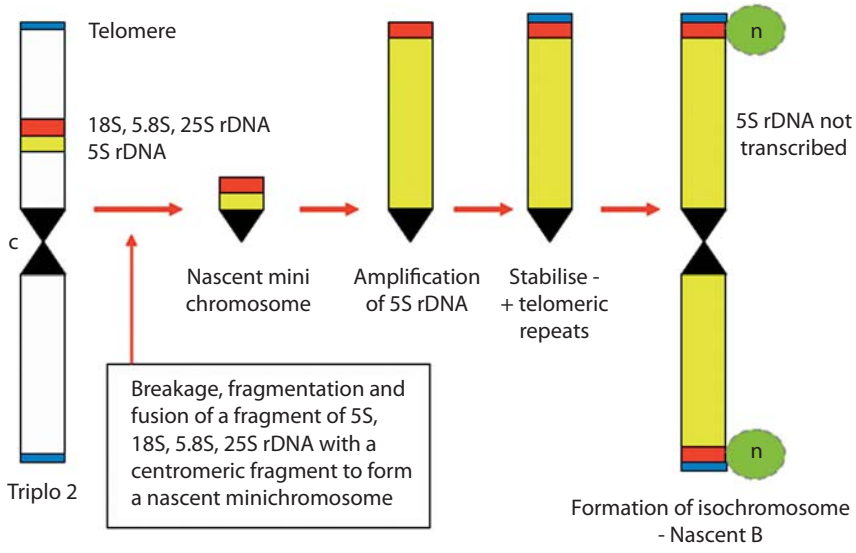

Fig. 11. Diagram representing a possible mode of origin of an apparent B chromosome from triplo 2 in *Plantago lagopus*, based on Dhar et al. (2002).

and were then stabilized by the addition of other sequences such as extrachromosomal DNA (eccDNA) and sequences necessary for their function as chromosomes (e.g. telomeric and centromeric sequences). Indeed, it should be noted that Bs contain similar types of coding and non-coding repeats as found in eccDNA of various organisms (Cohen et al., 2003) and eccDNA with similarity to tandem repeat sequences shared by A and B chromosomes has recently been identified (S. Cohen, A. Houben, D. Segal, unpublished).

The composition of the nascent B would effectively prevent meiotic pairing with any of the As, and license it to begin its own evolutionary pathway. Supernumerary A chromosome segments in *B. dichromosomatica* could also serve as potential regions to 'donate' founder sequences (Houben et al., 2001), but where the centromere would come from remains an open question, although rare de novo formation is possible (Nasuda et al., 2005). In such a dilemma we fall back on some epigenetic event to induce its activity. The Bs in maize (Cheng and Lin, 2003; Page et al., 2001) and rye (Wilkes et al., 1995) also share many sequences with the As, and a mode of origin similar to that proposed for the micro-B of *B. dichromosomatica* is one guess (Jones and Houben, 2003).

Bs thus tell us a story about how a supernumerary chromosome can arise and create new and autonomous elements as components of the genome: (i) remodel a trisomic, starting with a small centric fragment, add other repetitive sequences to give stability and isolation from recombination, and be genetically silent – which seems to be the case in all plants studied to date; (ii) arise as a small centric fragment following an unequal translocation and reduction in chromosome number; (iii) arise as a by-product of interspecific hybridisation or, (iv) excise as a small fragment, and then recruit sequences, including a centromere to enable passage through the cell cycle, and a telomere to stabilise and protect the ends of the new fragile B.

No doubt there are many such events going continuously, resulting from errors in meiosis, hybridisation, genome restructuring and other unknown processes, the products of which are aborted and never mature as B chromosomes. In any event the origin of a B is a rare event, because in species that we know well they appear to have a monophyletic origin, based on their sequence similarity across a range of cytodemes, as in *Brachycome dichromosomatica*, (Houben et al., 1999) and their virtually invariant cytological form over a broad range of geographic regions (Jones and Puertas, 1993).

Potential applications

B chromosomes have potential applications for modifying and exploring the A genomes of their host species, including crop plants (Jones, 1991). They have utility in modulating recombination, exploring the structure of the centromere and the process of nondisjunction, as well as other aspects of genome evolution as already discussed.

Roman (1947) first used translocations to study the process of nondisjunction in the pollen of maize, and since that time A-B translocations have been widely used in gene mapping in the maize A chromosome complement (Beckett, 1991; Birchler, 1991). Bs have also been widely used as a model system to investigate centromere organisation in maize, as descibed above. This is possible because when a B is univalent it often undergoes misdivision of its centromere at anaphase I of meiosis, and produces misdivision products of varying sizes (Kaszás and Birchler, 1998; Carlson, 2006).

Work in rye has shown that Bs can alter the pattern of distribution of chiasmata in the A chromosomes (Jones and Rees, 1967), and Moss (1966) had early shown a greater variability among the progenies of plants with Bs than of those without. Ayonoadu and Rees (1968) later found the same

effect in maize, and at about the same time Rhoades (1968) found genetic evidence for changes in recombination due to the Bs, and more evidence from maize followed later, as well as for other species (see Jones and Rees, 1982). Despite the potential significance of these basic studies we have yet to utilise this knowledge in crop improvement, due of the lack of any means to stabilise the transmission of a fixed number of Bs.

B chromosomes have several favourable properties for the development of artificial chromosome platforms. First, they are basically inert, and their presence in low numbers does not affect the phenotype; second, Bs do not pair with the standard A chromosomes, and third, they have accumulation mechanisms that can be used to create a dosage series for increased expression of the transgenes. There is potential to modify the B chromosome of candidate species, such as maize and rye, to construct a stably inherited plant artificial chromosome (PAC) to carry transgenes which would be outside the domain of the A chromosome genome, with all the advantages that this could entail. Birchler and colleagues have recently built the foundations for artificial chromosomes based on the B of maize (see the paper by J.A. Birchler in this volume). The Bs were transferred from the maize variety BMS to the maize line HiII-A for genetic transformation (Vega, J.M., Han, F., Peters, E.M. and J.A. Birchler, unpublished work). *Agrobacterium*-mediated transformation was first used and transgene inserts were localized among all 10 A chromosomes but no inserts were found on the Bs; thus a bias against the maize B by the *Agrobacterium*-mediated transformation was revealed (Vega et al., 2008). Yu et al. (2007) then used biolistic-mediated transformation with telomere-containing plasmids and obtained B chromosomes truncated at different positions. A GUS transgene inserted into the B-minichromosomes was expressed in all tissues of the plant that were examined, including mature kernel embryos and endosperm. The finding that the maize B chromosome can support foreign gene expression in the kernel has significant biotechnological implications (Houben and Schubert, 2007; Yu et al., 2007).

Concluding remarks

There is a huge body of literature on B chromosomes, much of it at a descriptive level in terms of occurrence in populations, structure, transmission, effects on the phenotype, and so on. This knowledge base is essential, and has value in that it provides a framework from which questions arise about the biological significance of Bs as components of their host genomes. Passengers do not get a free ride: selfishness has a cost. The 'parasite' inhabits the cells and nuclear environments of the hosts, and co-evolution is the means to the long-term future for survival of both host and 'passenger'. Current research thus leads on to studies on the genetics of transmission of Bs, and of their interaction with the A chromosomes, and the molecular basis of the ways that the Bs have found to make a selfish life of their own without destroying the host which carries them. The direction of future studies is therefore set, and a deeper understanding of these biological questions will only come about through the application of the full suite of molecular tools, and genetic analysis, we can throw at Bs to make sense of their enigmatic properties. In the end we may even find more applications for Bs themselves, as tools for experiments or having utility in the manipulations of crop plants.

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