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Evidence for 'cross-talk' between A and B chromosomes of rye

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Spontaneous DNA insertions from supernumerary B chromosomes (Bs) into the standard A chromosome complement were detected in rye (*Secale cereale* L.), using fluorescent *in situ* hybridization (FISH) analysis with the D1100 B-specific sequence probe. The insertions were seen in individuals derived from plants possessing deleted Bs, characterized in this study by not having the B-specific sequences that are normally found at the distal part of the long arm of the standard rye B. This result supports the case for the spontaneous introgression of B-specific DNA into the A chromosome genome, and it indicates that 'cross-talking' between A and B chromosomes may occur in wild populations.

Keywords: B chromosome insertions; deleted Bs; fluorescent *in situ* hybridization with D1100 B-specific sequence

1. INTRODUCTION

Supernumerary B chromosomes (Bs) are additional and dispensable optional extras found in the genomes of hundreds of plant and animal species. They do not recombine with members of the basic A chromosome set (As), and they follow their own separate transmission and evolutionary pathways (Puertas 2002; Jones & Houben 2003). Nevertheless, the opportunity for A–B 'cross-talk' exists insofar as Bs are subject to structural mutation as are the A chromosomes; for example, the high number of structural B polymorphs in species such as *Aster ageratoides* (Matsuda 1970). There is, however, a dearth of such mutational events in the natural populations studied to date. This does not mean that translocations do not occur but that if, and when, such events do happen naturally, they have not yet been demonstrated as becoming fixed in populations, except for in the grasshopper *Eyprepocnemis plorans*. This species has been subject to extensive sampling and several independent occurrences have been observed, but the deleterious nature of the events prevents their transmission and survival (Bakkali *et al.* 2003). In plants, the only known spontaneous occurrences of A–B translocations are those reported in experimental strains of rye (Pohler & Schlegel 1990; Schlegel & Pohler 1994; Wilkes *et al.* 1995; Kubaláková *et al.* 2003), but we know little about their mode of origin or how they relate to any natural situation. This limited knowledge is a bleak scenario when we consider how relatively simple it seems to be to induce A–B translocations experimentally. Maize is the prime example, where such A–B translocations are used extensively for various forms of genetic analysis and where numerous translocation stocks are listed (Maize Genetics Cooperation Stock Centre 2004). Other species where translocations have been induced are *Pennisetum typhoides* (ethyl methanesulphonate (EMS) Pushpa 1980), *Lolium perenne* (X-irradiation; Evans & Macefield 1977), the JNK strain of rye (gamma irradiation; Puertas & Baeza 1983) and an experimental strain of rye (gamma irradiation; Hasterok *et al.* 2002), and, out of these, the last strain is the most informative.

In the study by Hasterok *et al.* (2002), fluorescent *in situ* hybridization (FISH) with the D1100 B-specific sequence probe was used to mark the distal region of the long arm of the B and to track it from an irradiated M1 sample through to the M2 progeny. The A chromosomes were marked with a subtelomeric repeat and 5S rDNA sequences, both of which are absent from the B chromosomes. A plethora of reciprocal and non-reciprocal translocations were found in eight plants from a sample of 13 M1 seedlings, and, out of these eight plants, six were selected for their high frequency of translocations, which were found in between 10% and 78% of their root-tip c-metaphase cells. In total, 174 c-metaphases with translocations were studied from the six plants concerned. Rather surprisingly, when meiotic metaphases were analysed all six plants showed regular bivalent formation with no evidence that the meiotic tissues carried these translocations: they appear to have been filtered out during the vegetative phase of growth and development. In the M2 generation derived from the six plants carrying translocations, 64 individuals were screened and found not to carry any large-scale A–B translocations. In 22 out of the 64 individuals, small fragments of the D1100 B-specific chromatin were detected at the distal tip of one or two of the A chromosomes. It seems from this study that, in terms of what can be made visible, only a tiny and particular piece of B-specific chromatin can breach the strong barrier to exchange between A and B chromosomes.

We report a novel case of the insertion of a small distal segment of the long arm of rye B chromosomes into distal regions of the A chromosomes in experimental material that has not been irradiated, that originates from the wild and in which there are some indications of a correlation between the insertions and the occurence of a spontaneous structural event in the B itself. We also briefly review the few known cases, to date, of A–B translocations.

2. MATERIAL AND METHODS

Chromosomes have been analysed using seedlings of the Japanese JNK strain of rye (*Secale cereale* L.), which occasionally carries a deleted form of the rye B. This deleted B is without the distal part of its long arm where the B-specific sequences D1100 (Sandery *et al.* 1990) and E3900 (Blunden *et al.* 1993) reside; these are markers for this region of the rye B chromosome. This deleted B is one of the structural forms of the standard rye B and has arisen at mutation frequency in a natural population.

FISH probes were used on root meristem c-metaphases, according to the schedule of Schwarzacher & Heslop-Harrison (2000), using

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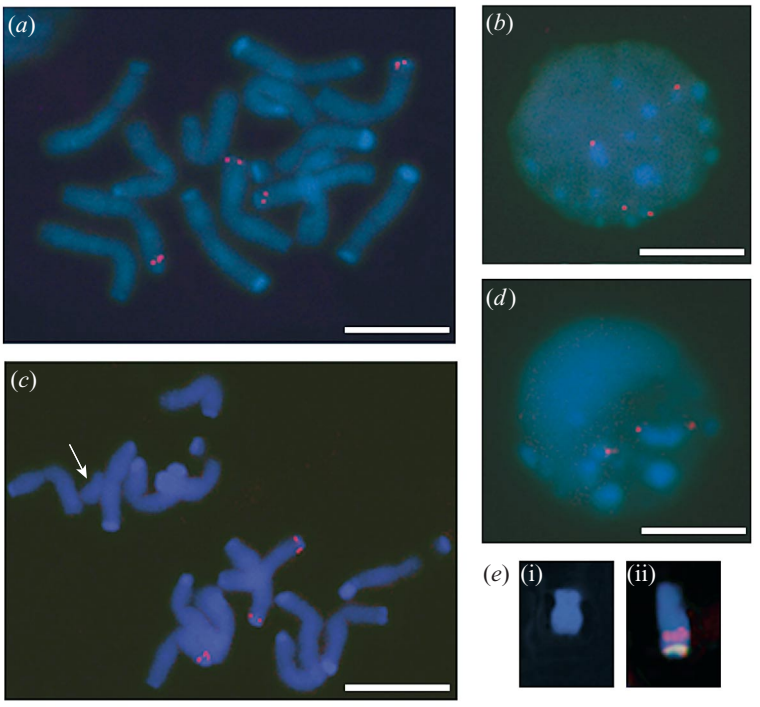

Figure 1. FISH with probes for the D1100 and E3900 B-specific sequences applied to c-metaphase and interphase root meristem nuclei of rye. (*a*) Complete c-metaphase of a plant without B chromosomes, and (*b*) its respective interphase nucleus, showing the D1100 probe signal (red) on four A chromosomes. (*c*) Complete c-metaphase of a plant with one deleted B (arrowed), showing the signal from the D1100 probe (red) on three of the A chromosomes. (*d*) Interphase nucleus from the cell with the deleted B, showing signal on three A chromosomes. (*e*) (ii) One standard form of the rye B with FISH signals from the adjacent D1100 (green) and E3900 (red) probes (the signal is yellow where the sequences colocalize), and (i) a deleted B lacking the distal end of the long arm, where we find labelling with the D1100 and E3900 probes in the standard B. Chromatin is stained blue with DAPI. Scale bars, 10 μm.

probes for both the D1100 and the E3900 sequences. The probes were labelled with either digoxigenin-11-dUTP or biotin-11-dUTP, using a nick translation kit according to the manufacturer's instructions, and cells were analysed by epifluorescence microscopy (Zeiss Axioscop 2).

3. RESULTS AND DISCUSSION

Several seedlings from the cross between a plant with a single deleted B and a plant with two deleted Bs were analysed, and all revealed signals for the D1100 sequence at subterminal locations on some of the A chromosomes. The number of D1100 signals varied from four (figure 1*a*,*b*) to three (figure 1*c*,*d*), and the number of deleted Bs also varied, from zero (figure 1*a*,*b*) to one (figure 1*c*,*d*). Confirmation that the B is the deleted form (figure 1*c*) arises from the lack of any signal for either the D1100 or the E3900 sequences. None of the A chromosomes showed any signal with the E3900 probe, indicating that the insertion event that placed the D1100 sequence on the As also separated it from the E3900 with which it is partly colocalized in the standard form of the B (figure 1*e*; see Wilkes *et al.* (1995) and Langdon *et al.* (2000) for a detailed description of the sequence organization of the B-specific region of the long arm of the standard rye B). The most significant feature of the D1100 sequence introgressions is that they apply to seedlings that originate from crosses between plants known to carry deleted Bs, and, although this material is maintained as an experimental stock, it has originated from seeds originally collected from wild populations (Kishikawa 1965). Therefore, these insertions seem to be spontaneous events, although it cannot be determined at which stage this occurred during the maintenance of the material. Nor can we tell at what stage the deleted Bs arose, since they have now been kept for a number of years by intercrossing between plants that carry them; in the wild they do not survive. It is possible that a large non-reciprocal translocation originally produced the deleted Bs and coincidentally placed a large segment of the terminal section of the long arm of the B onto some of the A chromosomes. As we have seen from the work of Hasterok *et al.* (2002) such large-scale A–B structural changes are not tolerated in the rye genome. Some restructuring of the A^B chromosomes would then be required during vegetative development to give the new A^{D1100} chromosomes, which seem to be the preferred forms allowed for 'cross-talking' between A and B chromosomes. We are biased in this respect because of the sequences we can see by 'FISH-ing'. We are making the assumption that the appearance of the D1100 sequences on the ends of the A chromosomes is associated with the process that led to the formation of the deleted Bs themselves. Whether this is in fact the case, A chromosomes still carry these tiny introgressed segments of Bs, and apparently these segments display the same form, and appear to be present at corresponding places on the A chromosomes, as that found by Hasterok *et al.* (2002). Nevertheless, we also know that many hundreds of seedlings carrying the standard form of the Bs have been subjected to FISH with the D1100 probe, in a number of laboratories, including our own under the same conditions. Similar observations have thus far, to our knowledge, not been reported, although it seems that the presence of the

deleted B is no longer needed for the As to carry the introgressed chromatin once the event has occurred (figure 1*a*,*b*). Interestingly enough, the work by Wilkes *et al.* (1995) recorded a spontaneous terminal reciprocal translocation in the experimental strain of rye, which involved the E3900 B-specific sequence and an A chromosome and which resulted in a deleted B as one of the products of this event. On this occasion the E3900 was also separated from the D1100, and it is noteworthy that this translocation involved the 1R, one of the A types reported in Hasterok *et al.* (2002). Wilkes *et al.* (1995) also discovered a number of plants in which the Bs carried some small introgressed segments of the 350–480 bp clone pAW161, which gave FISH signals at the distal end of the long arm of the standard B, although these signals were cryptic enough not to be seen in all preparations from individual plants with Bs. To complicate the story even further, Kubaláková *et al.* (2003) recently discovered A–B translocations in the 'Adams' strain of rye. A chance observation revealed an unexpected peak while flow-sorting the 'Adams' chromosomes, and cytological observations then showed the extra peak to be caused by the presence of Bs. The probe from the A chromosome *Afa* dispersed repeat was also found, for the first time, to give a prominent cluster in the middle of the long arm of the B. When used in double FISH with probes of 45S and 5S rDNA from A chromosome 1R a piece of the short arm of 1R translocated onto some of the Bs. The physical map indicates a break somewhere between the *Afa* locus and the B centromere and that this is the most common of several such translocations, the second most common being marked by just the 5S rDNA probe. Interestingly, in the present context 0.5% of sorted Bs carried a translocation with an A chromosome, but none of the chromosomes appears to have an altered morphology. The D1100 and E3900 probes were not used in this case.

The results presented here, together with those of Wilkes *et al.* (1995) and Kubaláková *et al.* (2003), highlight the possibility that cryptic 'cross-talking' between the A and the B chromosomes of rye, and indeed of other species, may happen in natural populations at a level that is invisible without the use of probes. They also lead to thoughts concerning how active the interaction may be between these two conflicting parts of genomes, what are their size limitations and could we find cases where these transgressors have arrived at fixation? We are mindful too of the patchwork nature of the A-derived sequences of some Bs (Jones & Houben 2003), which betrays their origin and ancestral relatedness to A chromosomes, a feature that could well facilitate 'cross-talking'.

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