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Discrimination of the closely related A and B genomes in AABB tetraploid species of *Avena*

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Abstract Fluorescent in situ (FISH) and Southern hybridization procedures were used to investigate the chromosomal distribution and genomic organization of the satellite DNA sequence As120a (specific to the A-genome chromosomes of hexaploid oats) in two tetraploid species, *Avena barbata* and *Avena vaviloviana*. These species have AB genomes. In situ hybridization of pAs120a to tetraploid oat species revealed elements of this repeated family to be distributed over both arms of 14 of the 28 chromosomes of these species. Genomes A and B were subsequently distinguished, indicating an allopolyploid origin for *A. barbata*. This was confirmed by assigning the satellited chromosomes to individual genomes, using the satellite itself and two ribosomal probes in simultaneous and sequential in situ hybridization analyses. Differences between *A. barbata* and *A. vaviloviana* genomes were also revealed by both FISH and Southern techniques using pAs120a probes. Whereas two B-genome chromosome pairs were found to be involved in intergenomic translocations in *A. vaviloviana*, FISH detected no intergenomic rearrangements in *A. barbata*. When using pAs120a as a probe, Southern hybridization also revealed differences in the hybridization patterns of the two genomes. A 1300-bp *Eco*RV fragment was present in *A. barbata* but absent in *A. vaviloviana*. This fragment was also detected in Southern analyses of A-genome diploid and hexaploid oat species.

Keywords Satellite DNA sequences · DNA hybridization · Intergenomic translocations · Allopolyploidy · *Avena barbata* · *Avena vaviloviana*

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Introduction

Four different genomes, A to D, each with a basic chromosome number of seven, are represented in the genus *Avena* L. (Poaceae) (Thomas 1992). The A and C genomes are present in some diploid species, but none have been found containing the B and D genomes. B and A occur in combination only as a tetraploid, AABB, in wild oat species, and D occurs with A and C only as AACCDD, a hexaploid form seen in wild and cultivated oats. In addition, the C genome occurs in combination with A as AACC, a tetraploid form of wild oats.

The identities of the chromosomes of each genome in the diploid and polyploid oat species was once unknown since their physical identification was impossible (Rajhathy 1963; Rajhathy and Thomas 1974). This situation changed with the discovery that oat chromosomes could be differentiated by C-banding and in situ hybridization techniques. C-banding allowed the description of the heterochromatin patterns of the constituent chromosomes of all four genomes (Fominaya et al. 1988a, b; Linares et al. 1992; Jellen et al. 1993). These studies showed C-genome chromosomes to have strong heterochromatic bands at the centromeric and interstitial regions, while A-, B-, and D-genome chromosomes were characterized by a small number of heterochromatic bands at the telomeric and interstitial regions. Based on the position of centromeres and C-banding patterns in comparative analyses of karyotypes of A-genome diploid species, different types of A-genome have been reported. These are denoted by a subscript, e.g. *Avena strigosa* AsAs and *Avena longiglumis* AlAl (Rajhathy and Thomas 1974; Fominaya et al. 1988a). Variations of these A genomes were also reported by comparing signal intensities (or their absence) after Southern hybridization of the satellite DNA sequence As120a, isolated from *A. strigosa* (As genome), to genomic DNA from *Avena* species (Linares et al. 1998). Genomic in situ hybridization (GISH) techniques using DNA from *A. strigosa* (As genome) and *Avena eriantha* (C genome) with chromosomes of hexaploid oats, allowed the detection of inter-

genomic translocations of the constituent chromosomes of the A/D and C genomes. However, it failed to distinguish between the A and D genomes (Chen and Armstrong 1994; Jellen et al. 1994; Leggett and Markhand 1995; Yang et al. 1999). These studies, together with the physical position of ribosomal loci (Linares et al. 1996), revealed that both the A and D genomes of hexaploid oats were highly homologous to the As genome. However, fluorescent in situ hybridization (FISH) techniques, using the cloned DNA sequence As120a with chromosomes of hexaploid oats, did discriminate between the closely related A and D genomes (Linares et al. 1998). This study also identified the chromosomes involved in A/C and D/C intergenomic translocations by using the cloned DNA sequences Am1 (isolated from *Avena murphyi* – AC genomes) (Solano et al. 1992) and As120a in sequential FISH experiments.

Avena barbata Pott. Ex Link., *Avena vaviloviana* (Malz.) Mordv., and *Avena abyssinica* Hochst are tetraploid species with the AB genome. They are of significant potential for use in plant breeding, having been found to be an important source of resistance to cereal cyst nematode (Leggett, 1992), crown rust (Harder et al. 1992; Ladizinsky 1992; Leggett 1992), and powdery mildew (Ladizinsky 1992; Leggett 1992). Interspecific crosses in the tribe *Avenae* have been used successfully in transferring such sources of resistance into cultivated oats (Thomas et al. 1975; Aung and Thomas 1978; Brown et al. 1986). However, few cytogenetic and molecular markers have been used to gain insight into the relationships between the A and B genomes of the tetraploid species and the A and D genomes of cultivated oats.

Earlier studies of AABB tetraploid species addressed two hypotheses on the genomic constitution and origin of these species. One considered them to have originated as allotetraploid forms from interspecific hybridization between A-genome diploid species (Rajhathy and Morrison, 1959; Fominaya et al. 1988b), the other that they arose as autotetraploid forms of *Avena hirtula* Lag – *Avena wiestii* Steudel species (As genome) (Oinuma 1952; Holden 1966; Ladizinsky 1973; Price and Kahler 1983). Recently, GISH techniques using DNA from *A. strigosa* (As genome) labelled all chromosomes of the AB tetraploids *A. barbata* (Leggett and Markhand, 1995) and *A. vaviloviana* (Katsiotis et al. 1997). In these studies, the genomic designation AAA′A′ has been suggested to describe their genomic constitution. Although the second hypothesis seems to be generally accepted, no definitive conclusion has been possible owing to the lack of conclusive cytological and molecular evidence. Our previous study, which used both C-banding and silver-stained nucleolar organizer region (NOR) techniques, suggested that the tetraploid karyotype might consist of two genomes, A and B, with the genomic formula AABB. This was based on the fact that A-genome chromosomes corresponded well with As-genome chromosomes, while B-genome chromosomes showed poorer correspondence (Fominaya et al. 1988b). To verify this primary conclusion, a molecular study with genome-specific probes was initiated in AABB tetraploid species. This paper presents the use of an A-genome specific sequence, As120a, previously isolated from *A. strigosa* (As genome) (Linares et al. 1998), to determine whether discrimination between A- and B-genome chromosomes by FISH is possible. This sequence was used in combination with either 18S-5.8S-26S (Gerlach and Bedbrook 1979) or 5S rDNA probes (Gerlach and Dyer 1980) in order to assign them to genomes. In addition, the pAs120a probe was used in Southern-hybridization experiments to differentiate the genomes of the two tetraploid species, two A-genome diploid species and three hexaploid species.

Material and methods

Plant materials and chromosome sample preparation

The names of the species employed in this study, their accession numbers and sources are listed in Table 1. Seeds were germinated on moist filter paper for 24 h at 25°C, for 36 h at 4°C, and then for 24 h at 25°C, in order to synchronize cell divisions. Seedlings were transferred to ice water for 24 h at 0°C to accumulate metaphases before fixation in ethanol-glacial acetic acid (3:1 v/v). Chromosome preparations were made as described in Fominaya et al. (1995) and used immediately for FISH.

In situ hybridization

Probe labelling and FISH were performed as described by Fominaya et al. (1995). Three DNA probes were used for FISH analyses: (1) clone pAs120a, a satellite DNA specific to the oat A genome and containing an insert of 389 bp isolated from *A. strigosa* (Linares et al. 1998), (2) clone pTa71, containing a 9-kb *Eco*RI fragment and including the 18S-5.8S-26S rDNA gene and the spacer isolated from *Triticum aestivum* (Gerlach and

Table 1 List of species studied, their accession number, genome designations (Thomas 1992) and source

Species	Genome	Accession	Source
A. strigosa *Schreb.*	AsAs	PI 258729	John Innes Centre, Norwich, UK
A. longiglumis Dur.	AlAl	BGRC0592993	Institut für Pflanzenbau und Pflanzenzüchtung Braunschweig, Germany
A. barbata Pott.ex Link	AABB	Mésico	University of California, Davis, USA
A. vaviloviana (Malz.) Mordv.	AABB	PI 412743	National Small Grain Collection, Beltsville, USA
A. byzantina C. Koch	AACCDD	cv 'Kanota'	University of Osaka, Prefecture, Japan
A. sativa L.	AACCDD	cv 'Extra Klock'	Nordic Gene Bank, Alnarp, Sweden
A. sterilis L.	AACCDD	PI 411958	National Small Grain Collection, Beltsville, USA

Bedbrook 1979), and (3) clone pTa794, including the 410-bp 5S rDNA gene and intergenic spacer isolated from *T. aestivum* (Gerlach and Dyer 1980). In simultaneous in situ hybridization experiments, the pAs120a and pTa794 clones were amplified and labelled by polymerase chain reactions (PCR) using rhodamine-4-dUTP (Amersham) or digoxigenin-11-dUTP (Roche). Alternatively, the pTa71 clone was labelled by nick translation with digoxigenin-11-dUTP or rhodamine-4-dUTP. All probes were precipitated with ethanol. For the figures, negatives were digitalized into PhotoCD format and processed with the Adobe Photoshop, using only those functions that apply equally to all pixels in the image.

Southern hybridization

Genomic DNA from seven oat species was isolated from young leaves by standard techniques (Sharp et al. 1988). This was then digested with *Eco*RV and *Hin*dIII and separated on 1% agarose gels, stained with ethidium bromide and photographed before transfer of DNA to BiodyneA membranes (Gibco BRL). Labelling, hybridization, and chemiluminescence-detection were performed according to Loarce et al. (1996). Membranes were exposed to Hyperfilm-ECL (Amersham) for 30 mn.

Genome copy numbers of As120a sequences

Genomic DNA from *A. barbata*, *A. vaviloviana*, *Avena sativa*, *Avena byzantina* and the plasmid pAs120a, were serially diluted and blotted onto a Zeta-Probe membrane (BioRad) according to the manufacturer's instructions. The pAs120a insert was labelled with digoxigenin-11-dUTP (Roche) by PCR. Hybridization, washes, and chemiluminescence detection were performed essentially as described by Loarce et al. (1996). Hybridization signals were quantified using a Millipore Bio Image. Copy numbers for the different species were calculated by comparing the signal intensities of genomic and plasmid dilutions. The genome size of the tetraploid species has been estimated at 9.5 pg/1C (Bennett and Smith 1976). The hexaploid genome size has been estimated at 11.725 pg/1C (Arumuganathan and Earle 1991).

Results

In situ hybridization analysis

The karyotype of the AABB tetraploid species was first described by Rajhathy and Morrison (1959). Fominaya et al. (1988b), using C-banding and silver nitrate staining of the NOR, studied the karyotype in greater detail. It showed two pairs of satellitized chromosomes, four pairs of metacentric chromosomes, seven pairs of submetacentric chromosomes, and one pair of subtelocentric chromosomes. To determine whether discrimination between A- and B-genome chromosomes was possible, pAs120a was used as probe in FISH analysis. The genome designation of each satellited chromosome was achieved through simultaneous FISH and re-probing the same metaphase plates with ribosomal pTa794 and pTa71 probes.

When the rhodamine-labelled pAs120a probe was hybridized with *A. barbata* metaphases, two sets of chromosomes, each with seven pairs, were clearly identified (orange in Fig. 1a). Since the As 120a tandem repeat is sequence specific to the A-genome chromosomes in diploid and hexaploid species (Linares et al. 1998), the hybridized chromosome set was consequently assigned to the A genome. The remaining seven unhybridized chromosome pairs were assigned to the B-genome chromosomes. With respect to the pAs120a hybridization pattern, the A-genome chromosomes showed a dispersed distribution over their entire lengths, except for the telomeric regions of the long arm of one metacentric chromosome pair. FISH with the pTa794 probe (green in Fig.1a) revealed eight digoxigenin hybridization sites for the 5S rDNA loci. These were located on both arms of two chromosome pairs. When FISH analysis was performed on the same metaphase plates with the pTa71 probe (green in Fig. 1b to detect 18S-5.8S-26S loci), eight hybridization sites were detected (with six strong and two weaker signals). Four strong sites were located on the two chromosome pairs carrying the 5S rDNA loci. The other two strong sites and the two weaker sites were located on two other chromosome pairs. In all cases, the loci for the NOR sequences appeared in the short arms. The number of loci seen does not agree with previous findings, which indicated the presence of two organizing chromosome pairs (Rajhathy and Thomas 1974; Fominaya et al. 1988b). The genomic identification of chromosomes carrying ribosomal (NOR and 5S) loci was achieved by taking into account their hybridization, or lack of it, with the pAs120a probe. Thus, one chromosome pair carrying both NOR and 5S rDNA loci, and one chromosome pair carrying only NOR loci, hybridized with the pAs120a probe. This indicates that these pairs belong to the A genome. In contrast, one chromosome pair carrying both the NOR and 5S rDNA loci, and the chromosome pair with the weaker NOR locus, did not hybridize with the pAs120a probe. This shows them to belong to the B genome. These results confirm the presence of two genomes in this tetraploid species. Consequently, the A-genome chromosomes could be further identified by their having two chromosome pairs hybridized by pTa71. One of them also carried sequences complementary to pTa794. One metacentric chromosome pair was identified by the absence of terminal sequences homologous to pAs120a (arrows in Fig. 2a). Three submetacentric and one subtelocentric chromosome pairs were identified from arm-ratio criteria. Similarly, the B-genome chromosomes could be identified as follows: one satellited chromosome pair by the presence of sequences complementary to both pTa71 and pTa794, one chromosome pair by the presence of a small ribosomal site, and two metacentric and three submetacentric pairs by karyotype morphology.

When the rhodamine-labelled pAs120a probe was hybridized to *A. vaviloviana* metaphases, two sets of chromosomes were also identified (orange in Fig. 1c). Seven chromosome pairs appeared highly hybridized and were identified as belonging to genome A. The remaining seven chromosome pairs showed much less-intense signals and were identified as belonging to the B genome. Differences in the hybridization patterns were observed within each chromosome set. Thus, within the A-genome chromosomes, six chromosome pairs showed a dispersed distribution of the sequences, and one metacentric chro-

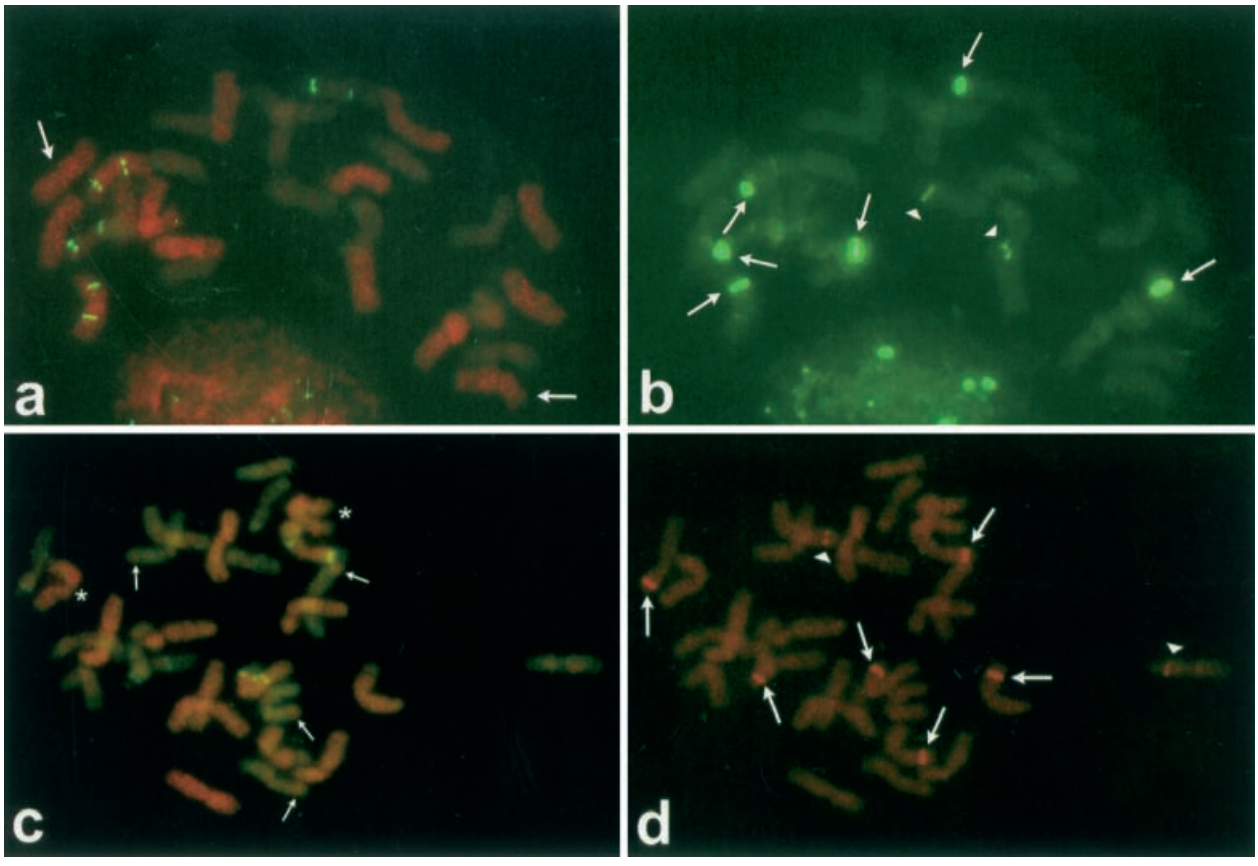

Fig. 1 FISH of metaphase plates of *A. barbata* (**a** and **b**) and *A. vaviloviana* (**c** and **d**). (**a**) Simultaneous visualization of hybridization sites of the rhodamine-labelled A-genome probe pAs120a (orange) and the digoxigenin-labelled 5S rDNA probe pTa794 (*green*). (**b**) The same cell as in **a**, shown after in situ hybridization with the digoxigenin-labelled 18S-5.8S-26S rDNA probe pTa71 (*green*). (**c**) Double FISH of the rhodamine-labelled pAs120a (*orange*) and the digoxigenin-labelled pTa794 (*green*). (**d**) The same cell as in **c**, shown after in situ hybridization with rhodamine-labelled pTa71 (*red*). In **b** and **d**, the *arrows* indicate the strong hybridization signals and the *arrowheads* indicate the weaker hybridization signals. In **c**, the *arrows* indicate the B-A translocated chromosomes

mosome pair showed unhybridized segments for the telomeric regions on both arms (asterisks in Fig. 1c). With respect to the B-genome chromosomes, two submetacentric pairs showed strong hybridization signals in the telomeric and/or interstitial regions of their long arms (arrows in Fig. 1c). FISH with pTa794 (green in Fig.1c) and pTa71 (red in Fig. 1d) probes yielded eight hybridization signals for both NOR and 5S rDNA loci similar to those for *A. barbata*. This number of loci agrees with previous findings (Katsiotis et al. 1997), indicating the presence of four nucleolar organizing chromosome pairs. These were also distributed on the A and B genomes (Fig.1c and d) in a similar fashion to that seen in *A. barbata*. Consequently, the A-genome chromosomes could be further identified as follows: two satellited chromosome pairs identified by the presence of complementary sequences to pTa71 (one also carrying sequences complementary to pTa794), one metacentric pair identified by the absence of terminal sequences homologous to pAs120a, and three submetacentric and one subtelocentric pairs identified by arm ratio. Similarly, the B-genome chromosomes could be identified as follows: one satellited pair identified by the presence of sequences complementary to both pTa71 and pTa794, one chromosome pair identified by the presence of a small ribosomal site, two submetacentric pairs identified by the presence of sequences complementary to pAs120a on telomeric and interstitial regions of the long arms, and two metacentric pairs and one submetacentric pair identified by their arm ratios.

Southern-hybridization analysis

To further analyze the differentiation of genomes between *A. barbata* and *A. vaviloviana* species, Southern blots with *Eco*RV-digested DNA from these species were hybridized with the pAs120a probe (Fig. 2a). Hybridization intensities obtained by Southern analysis were in agreement with the data obtained by in situ hybridization, indicating that the sensitivities of both techniques are similar, at least with this probe and these species. The extent of genome differentiation was further characterized by both the intensity of smear in DNA hybridizations and the distinct bands detected in the restriction digests from the two tetraploid species. *Eco*RV-digested *A. vaviloviana* DNA gave a strong smear of DNA hy-

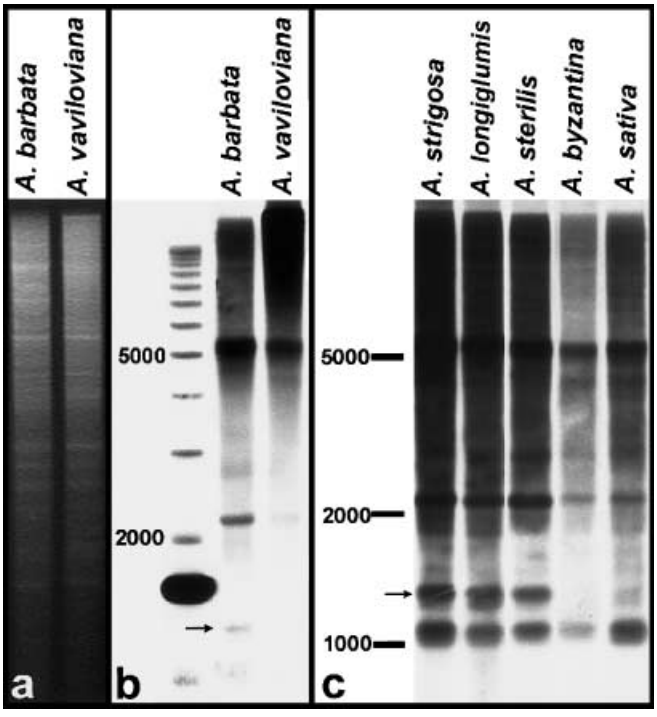

Fig. 2a–c Southern-blot analysis of the *Eco*RV-digested of diploid and polyploid oats. (**a**) EtdBr-stained 1.5% agarose gel of DNA samples. The *first lane* on the left corresponds to 1-kb Plus a molecular-weight marker (GibcoBRL). (**b**) Autoradiogram of hybridization with the pAs120a probe to *A. barbata* and *A. vaviloviana* tetraploid species shown in **a** after transfer and hybridization. (**c**) Autorradiogram of a different gel after hybridization with the pAs120a probe to diploid species *A. strigosa* and *A. longiglumis*, and hexaploid species *A. sterilis*, *A. byzantina* and *A. sativa*. *Arrows* indicate the 1300-bp hybridization fragment

bridization in which some discreet bands were found. In contrast, *Eco*RV-digested *A. barbata* DNA gave a weak smear in which pronounced bands were found. A major band of about 1300 bp was present only in *Eco*RV-digested *A. barbata* DNA. Differences in hybridization signal with pAs120a could not be attributed to differences in the quantity of DNA loaded in each gel lane, as shown by the ethidium bromide gel (Fig. 2b).

Similar genome differentiation was also visible in *Eco*RV-digested DNA from diploid and hexaploid species (Fig. 2c). In *A. strigosa*, *A. longiglumis* and *Avena sterilis*, the As120a sequence showed the hybridization pattern corresponding to *A. barbata*. In *A. byzantina* and *A. sativa*, As120a showed the profile revealed for *A. vaviloviana*. Similar DNA hybridization patterns were observed by digestion with *Hin*dIII (data not shown).

Slot-blot analysis

The number of copies of the As120a sequence in the tetraploid oat species was evaluated on the basis of hybridization of the digoxigenin-labelled pAs120a probe to slot-blots of total DNA. The *A. barbata* genome was estimated to contain 6.3×10^3 copies, whereas the *A. vaviloviana* genome contained about 7×10^3 As120a elements (data not shown). Although theses results indicate some amplification of the As120a sequence in the B genome of *A. vaviloviana*, and agree with the FISH and Southern analyses, the difference could be genotypic and requires further investigation.

Discussion

Two conflicting hypotheses to explain the origin of the AABB tetraploid species currently exist. Holden (1966) and Ladizinsky (1973), using data from meiotic chromosome pairing of a range of hybrids, proposed the AABB tetraploid species to have been derived through autotetraploidy in the *strigosa* group of diploids. Recently, this proposal has been supported by several papers describing research using GISH (Leggett and Markhand 1995; Katsiotis et al. 1997). Leggett and Markhand (1995) reported that *A. strigosa* chromatin hybridized to both A and B genomes of the *A. barbata* genome. Katsiotis et al. (1997) reported a similar observation in the *A. vaviloviana* genomes. These authors showed the uniform in situ labelling of all 28 chromosomes of *A. barbata* and *A. vaviloviana* with total genomic *A. strigosa* DNA. Similar conclusions on the structurally close relationships between the diploid *A. strigosa* and the tetraploid species were reported for in situ and Southern hybridizations using cloned DNA sequences (Katsiotis et al. 1997). Based on the absence of differences between the A and B genomes in Southern and in situ hybridizations, the above authors suggested the designation of AAA′A′ to describe the genomic constitution of these tetraploid species. In contrast, karyotypic observations support the allotetraploid origin of these species and show the presence of two different chromosome sets, only one being similar to those of *A. strigosa* (Rajhathy and Morrison 1959; Fominaya et al. 1988b). The FISH labelling-patterns of the As120a sequence observed with *A. barbata* and *A. vaviloviana* strongly confirm this hypothesis and suggest the *A. strigosa* genome to be the donor of the A genome. The origin of the B genome remains, however, uncertain. Complementary results in the present study were obtained using FISH with pTa71 and pTa794 probes. This allowed discrimination between four chromosome pairs of the AABB tetraploid species. It has been reported that the A-genome diploid species (*A. strigosa*, *Avena damascena*, *A. longiglumis*, and *Avena canariensis*) contain two chromosome pairs with NOR loci, and two chromosome pairs with 5 S loci, located on both arms of one pair of satellited chromosomes (Linares et al. 1996). In the present study, the number and position of both NOR and 5S loci observed for the A and B genomes were equal to that reported for A-genome diploid species, supporting an allopolyploid origin for these tetraploid species. Linares et al. (1998), using Southern hybridization, reported the absence of the As120a sequence in some A-genome diploid species

such as *A. damascena* (AdAd genomes) and *A. canariensis* (AcAc genomes). In the present study it has been shown that As120a sequences are present in the A-genome chromosomes and missing in B-genome chromosomes. Taken together, these data indicate that rather than *A. strigosa*, one of the above A-genome diploid species, or perhaps one not yet described which does not contain the As120a sequence, is a more-likely donor of the oat B genome.

Since the As120a sequence was able to discriminate between the A and B genomes, differences between the *A. barbata* and *A. vaviloviana* genomes were obtained by FISH analyses. In *A. vaviloviana*, pAs120a appeared to hybridize at a low level with the B-genome chromosomes, while no hybridized B-genome chromosomes were observed in *A. barbata*. These results suggest different amplifications of the As120a sequence in each tetraploid species, something also shown by the estimated copy numbers. In addition to these differences, FISH analyses showed regions of high hybridization of As120a sequences in two chromosome pairs of the B genome in *A. vaviloviana*. These regions might be explained by intergenomic translocations between chromosomes of the B and A genomes. Similar structural rearrangements have been described for the AACC tetraploid and AACCDD hexaploid species of the *Avena* genus in papers describing research using GISH (Leggett et al. 1994; Chen and Armstrong 1994; Jellen et al. 1994; Yang et al. 1999) or FISH with cloned DNA sequences (Fominaya et al. 1995; Linares et al. 1996, 1999, 2000).

Earlier cytogenetic studies identified two pairs of satellite chromosomes in the AABB tetraploid species of oat based on the presence of secondary constrictions and C-banding techniques (Rajhathy and Thomas 1974; Fominaya et al. 1988b). However, some genotypes of *A.barbata* (Ladizinsky 1974; Fominaya et al. 1988b) and *A.vaviloviana* (Rajhathy and Thomas, 1974; Fominaya et al. 1988b) have been observed to carry a third pair of satellite chromosomes. In the present study, four chromosome pairs carrying NOR sequences were detected by FISH analysis. The discrepancies observed could be due to a number of factors. Firstly, the genotype used in this study has not been previously examined and may therefore be novel, in that it carries four pairs of satellite chromosomes. Secondly, it is well known that not all conventionally stained mitotic squash preparations resolve secondary constrictions either at the NOR or elsewhere. This could be due to differential contraction of the chromosomes during pre-treatments, or to the phenomenon reported in many species where only those NORs active in the previous cell division can be visualised using silver or conventional staining techniques in subsequent mitotic divisions (Navashin 1928, 1934). Finally, as demonstrated by Brown et al. (1999), it is possible that some of the FISH signals observed using the pTa71 clone reported here, result from hybridization to the intergenic spacer sequence associated with the pTa71 clone, but which are not physically associated with NORs. Consequently, although the distribution and FISH signals of rDNA loci in tetraploid species observed in this work suggests that rRNA genes in the short arms are probably associated with NORs, future mapping of rDNA locations, using highly conserved 18S sequences, will resolve the issue.

The comparison of homologous genome-specific repetitive DNA sequences from closely related organisms not only indicates the evolution of the sequences themselves, but also highlights relationships among species (Matyášek et al. 1997; Linares et al. 1998, 1999, 2000; Taketa et al. 2000). The present examination of pAs120a hybridization patterns in diploid and polyploid *Avena* species reveals some interesting features. Though absent in the hybridization profiles of *A. vaviloviana* and cultivated oat species (indicating the lack of an *Eco*RV site), a 1300-bp hybridization band for As120a was present in *A. barbata*, the A-genome diploid, and the wild hexaploid species *A. sterilis*. This marker band reflects the differentiation of both the tetraploid and hexaploid genomes in the genotypes analyzed.

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