Theor Appl Genet (2000) 100:353–360 © Springer-Verlag 2000

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Identification of C-genome chromosomes involved in intergenomic translocations in *Avena sativa* L., using cloned repetitive DNA sequences

Received: 8 April 1999 / Accepted: 30 July 1999

Abstract Four anonymous non-coding sequences were isolated from an *Avena strigosa* (A genome) genomic library and subsequently characterized. These sequences, designated As14, As121, As93 and As111, were 639, 730, 668, and 619 bp long respectively, and showed different patterns of distribution in diploid and polyploid *Avena* species. Southern hybridization showed that sequences with homology to sequences As14 and As121 were dispersed throughout the genome of diploid (A genome), tetraploid (AC genomes) and hexaploid (ACD genomes) *Avena* species but were absent in the C-genome diploid species. In contrast, sequences homologous to sequences As93 and As111 were found in diploid (A and C genomes), tetraploid (AC genomes) and hexaploid (ACD genomes) species. The chromosomal locations of the 4 sequences in hexaploid oat species were determined by fluorescent in situ hybridization and found to be distributed over the length of the 28 chromosomes (except in the telomeric regions) of the A and D genomes. Furthermore, 2 C-genome chromosome pairs with the As14 sequence, and 4 with As121, were discovered to be involved in intergenomic translocations. These chromosomes were identified as 1C, 2C, 4C and 16C by combining the As14 or As121 sequences with two ribosomal sequences and a C-genome-specific sequence as probes in fluorescence in situ hybridization. These sequences offer new tools for analyzing possible intergenomic translocations in other hexaploid oat species.

Key words Repetitive DNA sequences · Chromosomal organization · In situ hybridization · Intergenomic translocations · *Avena sativa*

Communicated by B.S. Gill

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Introduction

Repetitive DNA sequences are a major component of higher plant genomes. Some are common to many species, while others are restricted to single species or species groups (for a review, see Dvorák and Dubcovsky, 1996). They might, therefore, be used as new tools in the genome analysis of polyploid species.

The genus *Avena* is an ideal system for investigating the genomic organization and evolution of repetitive DNA sequences that come together in a common nucleus. It includes species with different degrees of ploidy (2x, 4x and 6x) and diverse genomic composition (A, B, C and D). The four hexaploid *Avena* taxa commonly recognized today, including cultivated oats (*A. sativa* and *A. byzantina*) contain the A, C and D genomes (Thomas 1992). It is now clear that chromosomal rearrangements – mainly intergenomic translocations – have played a significant role in the formation of the three constituent genomes of hexaploid oats. In recent years, conventional and molecular approaches have been used to detect and identify them.

The conventional approaches used have been based on the analysis of C-banded mitotic metaphases (Fominaya et al. 1988; Linares et al. 1992; Jellen et al. 1993a, 1993b) and multivalent configurations at meiotic metaphases of intraspecific hybrids (Jellen et al. 1993b). Molecular approaches have included the construction and comparison of genetic maps using restriction fragment length polymorphism markers (RFLPs) (Rooney et al. 1994; O'Donoughue et al. 1995; Kianian et al. 1997), and the analysis of mitotic metaphases by genomic in situ hybridization (GISH) (Chen and Armstrong, 1994; Jellen et al. 1994; Leggett and Markhand 1995). The GISH approach has provided useful information on overall differentiation among the A, C and D genomes of the hexaploid oats. When labeled genomic DNA from either A-genome or C-genome diploid species was hybridized to *A. sativa* somatic metaphases, a total of nine chromosome pairs were found to be involved in intergenomic interchanges between the A/D and C genomes. These inter-

Table 1 Oat species: genomes, accession and source

Species	Genome	Accession	Germplasm source
A. strigosa	AsAs	PI 258729	John Innes Centre, Norwich, UK
A. damascena	AdAd	CAV. 0258	Plant Research Centre, Ottawa, Canada
A. longiglumis	AlAl	BGRC 052993	Institut für Pflanzenban und Pflanzenzuechtung, Braunschweig, Germany
A. eriantha	CC	CAV. 0063	Plant Research Centre, Ottawa, Canada
A .clauda	CC	CAV. 0001	Plant Research Centre, Ottawa, Canada
A. murphyi	AACC	cc 7120	Welsh Plant Breeding Station, Aberyswyth, UK
A. maroccana	AACC	CAV. 4388	Plant Research Centre, Ottawa, Canada
A. byzantina	AACCDD	cv Kanota	University of Osaka, Prefecture, Japan
A. sativa	AACCDD	cv Previsión	National Institute of seed, Madrid, Spain
		cv. Extra Klock	Nordic Gene Bank, Alnarp, Sweden
		cv Pandora	National Institute of seed, Madrid, Spain
A. stérilis	AACCDD	PI 411958	National Small Grain Collection, Beltsville, USA

genomic translocations were detected by the presence of C chromatin in 6 A/D chromosome pairs, and A/D chromatin in 3 C-genome chromosome pairs. In addition, a common feature of both conventional and molecular approaches has been the discrimination of the C-genome chromosomes from those of the A and D genomes in hexaploid species.

The chromosomes involved in intergenomic translocations have been identified using the C-genome chromosomes as a reference. By means of C-banding (Jellen et al. 1993b, 1997; Phillips et al. 1995), RFLP mapping data (Jellen et al. 1995; Kianian et al. 1997) and in situ hybridization with either a repetitive DNA sequence specific to the C-genome chromosomes (Fominaya et al. 1995) or a combination of rDNA genes plus a C-genome-specific repetitive DNA sequence (Linares et al. 1996), a few intergenomic translocations have been identified. More recently, the isolation of a sequence able to distinguish between the closely related A and D genomes of hexaploid oats has been reported (Linares et al. 1998). In this study, the combination of rDNA genes and satellite DNA sequences specific to either the A- or C-genome chromosomes enabled the identification of one A-genome chromosome pair, plus another three D-genome pairs involved in intergenomic translocations with C-genome pairs. Such information on the chromosomal locations of new repetitive DNA sequences could provide a powerful tool for identifying translocations in the hexaploid species.

This paper reports the molecular characterization of 4 cloned repetitive DNA sequences from *A. strigosa* in oat species. These sequences were used in combination with either 18S-5.8S-26S or 5S rDNA probes (Gerlach and Bedbrook 1979, Gerlach and Dyer 1980) and a C-genome-specific sequence isolated previously (Solano et al. 1992) in order to identify those C-genome chromosomes with intergenomic translocations in cultivated oat species, using in situ hybridization.

Materials and methods

Plant materials

The plant material used in this study (Table 1) included representatives of wild diploid and tetraploid species, several cultivars of the two cultivated hexaploid species *Avena byzantina* and *A. sativa*, and the wild hexaploid species *A. sterilis*. These were kindly provided by several germplasm resource centers.

Methods

The isolation of genomic clones containing repetitive DNA sequences from a pUC19 genomic library of *A. strigosa* has been previously described (Linares et al. 1998). Of the 21 DNA clones chosen for their supposed species specificity by hybridizing with *A. strigosa*, 4 clones – pAs14, pAs121, pAs93 and pAs111 – were finally selected. These were then double digested with *Eco*RI and *Hind*III. Southern blots of the digested clones were prepared and hybridized with isolated inserts to measure levels of insert cross-hybridization. The inserts were hybridized onto membranes containing *Eco*RV and digests of genomic DNA from young leaves of the accessions summarized in Table 1 following the procedure described by Loarce et al. (1996). The hybridization probes were labeled with digoxigenin-11-dUTP by the polymerase chain reaction (PCR) according to Hoisington et al. (1994). The DNA sequences of the 4 inserts were determined for both strands using an Applied Biosystems Automated DNA Sequencer (model 377). Sequence similarity searches of the EMBL data base were performed.

Root tips were pretreated, fixed and squashed after enzyme treatment according to Maluzynska and Heslop-Harrison (1993). Fluorescent in situ hybridization (FISH) was performed essentially as described by Heslop-Harrison et al. (1991) and Linares et al. (1996). Seven DNA probes were used for FISH analyses: (1) pTa71, containing a 9-kb *Eco*RI fragment including the 18S-5.8S-26S rDNA gene and a spacer isolated from *Triticum aestivum* (Gerlach and Bedbrook 1979); (2) pTa794, including a 410-bp 5S rDNA gene and an intergeneric spacer isolated from *T. aestivum* (Gerlach and Dyer 1980); (3) pAm1, a satellite DNA sequence specific to the oat C genome containing an insert of 464 bp isolated from *Avena murphyi* (Solano et al. 1992); and (4) pAs14, pAs121, pAs93 and pAs111, isolated from *A. strigosa* (this work).

In simultaneous in situ hybridization experiments, the pTa794, pAm1, pAs14, pAs121, pAs93 and pAs111 clones were amplified and labeled by PCR with digoxigenin-11-dUTP (Boehringer Mannheim) or rhodamine-4-dUTP (Amersham). Alternatively, the pTa71 clone was labeled with digoxigenin-11-dUTP or rhodamine-4-dUTP by nick translation. All probes were precipitated with ethanol.

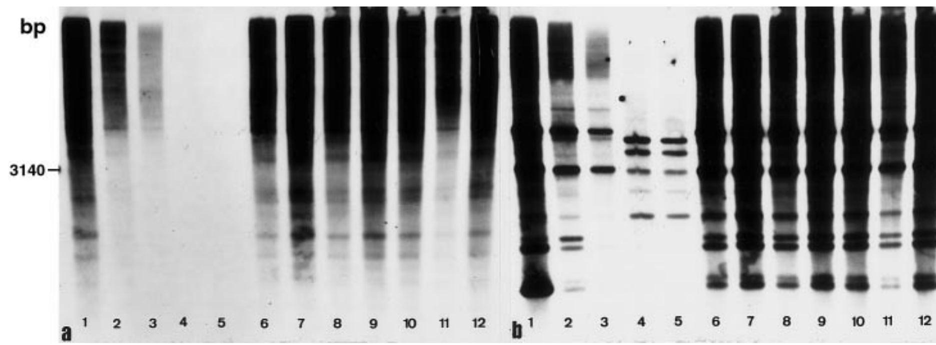

Fig. 1a, b Southern blot analysis of the *Eco*RV-digested genomic DNA of diploid and polyploid oats. The species are: *1 A. strigosa*, *2 A. longiglumis*, *3 A.damascena*, *4 A. eriantha*, *5 A. clauda*, *6 A. maroccana*, *7 A. murphyi, 8 A. sativa* cv 'Prevision', *9 A. byzantina cv ´Kanota´, 10 A. sativa* cv ´Pandora´, *11 A. sativa* cv 'Extra Klock', *12 A. sterilis*. **a** Autoradiogram after hybridization with the pAs14 probe, **b** autoradiogram showing re-hybridization of blot in **a** with pAs93 probe

Results

Four Southern blots of the digested pAs14, pAs121, pAs93 and pAs111 clones were prepared, and each was hybridized with 1 labeled insert from these clones to see if the 4 inserts were unique. The results indicate that the inserts did not cross-hybridize, suggesting that each clone contained members of different repeated sequence families.

Distribution of the cloned sequences among the *Avena* species

Hybridization of the pAs14 clone in Southern blots containing *Eco*RV-digested *Avena* species DNA revealed a strong, smeared signal. There were some prominent bands corresponding to the A-genome diploid and polyploid species, but no detectable signal for the C-genome diploid species (Fig. 1a). Hybridization of the clone pAs121 in Southern blots containing *Eco*RV-digested DNA (data not shown) of the same *Avena* species revealed high levels of hybridization for the A-genome diploid and polyploid species, but no hybridization signals for the C-genome diploid species. No interspecific variation signal for the A-genome diploid species was observed, and no interspecific variation patterns were detected. Taken together, these hybridization results suggest that sequences As14 and As121 might be specific for the A genome of the diploid and polyploid species studied. The probe hybridization patterns indicate a possible dispersed localization of these sequences in the A genome of the *Avena* species.

Hybridization of the clone pAs93 (Fig. 1b) in similar Southern blots containing *Eco*RV-digested DNA from the *Avena* species revealed a strong, smeared signal with defined hybridization bands for the A-genome diploid and polyploid species but a less intense signal with discrete bands for C-genome diploid species. This confirms the amplification of these sequences in the A genome and their presence in the C-genome diploid species. In diploid species, the sequence As93 showed two basic hybridization patterns corresponding to the A and C genomes, respectively. In polyploid species, As93 showed the profiles revealed for the A- and C-genome diploid species studied. Similar hybridization patterns were revealed after hybridization with the clone pAs111 (data not shown).

Sequencing of the cloned DNA repetitive sequences

Nucleotide sequences for the inserts of the 4 clones were determined and deposited in the European Molecular Biology Laboratory (EMBL) database. The 4 sequences were checked against the sequence database, and no significant homologies with any reported DNA sequences were detected. The 4 sequences showed no homology to each other. Comparison of each sequence with itself using the Harr plot graphic program (DNASIS, Hitachi, Tokyo) revealed that no insert contained tandem repetitive elements.

The sequence As14 (EMBL accession number AJ005499) was 639 bp long (Fig. 2a), with 59.9% of it being composed of AT nucleotide pairs. Shorter repeats were found within the element. The motif CATTTG was present in four copies. Also, two pairs of direct repeats of 9 bp were present, each one twice.

The sequence As121 (EMBL accession number AJ005500) was 730 bp long (Fig. 2b), with 48.2% consisting of AT nucleotide pairs. The motif AACCCTAG was present in three copies. A pair of direct repeats of 13 bp was separated by seven nucleotides. Also, a motif of 14 bp was present twice in the same orientation.

The sequence As93 (EMBL accession number AJ005501) was 668 bp long (Fig. 2c), with 49.3% being composed of AT nucleotide pairs. A striking feature of the sequence was the presence of six perfect repeats of 6 bp with the last two units partly overlapping. The sequence As111 (EMBL accession number AJ005502) was 619 bp long (Fig. 2d), with 48.2% being composed of AT nucleotide pairs. Two pairs of direct repeats of 10 and 12 bp were found.

Fig. 2a–d Nucleotide sequences of the fragments cloned in: **a** pAs14, which contains short CATTTG repeats (in *bold type*) and two different pairs of 9-bp direct repeats (*single* and *double underlined*); **b** pAs121, which contains AACCCTAG repeats (in *bold type*) and a 13-bp (*single underlined*) and 14-bp (*double underlined*) direct repeat, **c** pAs93, in which the locations of six 6-bp direct repeats are marked in *bold type*, **d** pAs111, in which *bold type* marks the location of a 10-bp direct repeat and *underlining* marks the location of a 12-bp direct repeat. Sequences have been deposited in the EMBL database: AJ005499 (pAs14), AJ005500 (pAs121), AJ005501 (pAs93), AJ005502 (pAs111)

a) SEQUENCE As14 639 BP; 192 A; 142 C; 191 T; 114 G.

1 GATCGTTTGG CTATCTCATT ACAATGCAGC AGAAACAACT ATAGCATGGA GTCCTTCATC 60
61 CTCAAAGTGC CACAGGCAAT CATGTTGGTT GTAGACTTTT AATCCTAATC AACACACTTC 120
121 ACTAATATCA AACAAACCTG CAACATAACA AGTTACAGCC ACAAGGGTCA ATA**CATTTG**A 180
181 ATTGTATTGG CAAGTGATAC TATGGTAGAG TAGTGGAGAC TAACATCTAT ATG**CATTTG**G 240
241 CTTGGAGGAG CTTAGGCTTG CGTTTGCTCA AAAATATATT TTCCTTCT**CA TTTG**AAAGCA 300
301 CTAAGTTTTT GTTTGGGCAA AATCACCCAA GGAACCACCA ATAGAAATTG GTGAACCTTC 360
361 ACCCTTGTTC AACCACAAGT TTTTTACTTG GAAATTGGGA TGAGGAGATT CTTGATAGCT 420
421 TCCATGTCTT GATGCTCAAA TTGTCCTTTG CCAGGGACAT GGCTAAGTAC TTAGATTTTA 480
481 CACTCTACAA AGGTGGTGCA CTTTCCCCAC AAGACTTAGT TTCACCCAAG ACAAGATTTT 540
541 TCAGAAGCCT TCCCTTAGAC CAGAATGGAC GCGCTCCAAA CCTATCATTC CCCTACCACC 600
601 AGCTATC**CAT TTG**GATAAGT TATTGCAATC TATATGATC 639

b) SEQUENCE As121 730 BP; 181 A; 197 C; 171 T; 181 G.

1 GATCTTCCTG AGCTTCCTCA ACAGTGACAT GGGTCAGACG ACCATGTCCA GCACCTTAAG 60
61 ATTTATCATT CTTCTTTGTC TGCTGATGGC CAATGGTCCG GAGAATAAGA TTCATATAAG 120
121 GAAAGTCCCG TTTCGGAGTT CGGGAATGAT TCCGATGTTT TCATCGGGAA TGTTCTAGAA 180
181 GGTTCCAAAA TGTCCGGCAC TCCGGAGGGG AAGGGGTACA TGAATGTCTC ATTGCAAGTT 240
241 GGAATGATTG CATCATGTAC TAAGTGTATT CGCTGTCCAA AATAAAGGGT TTATTCAAAT 300
301 CCTAAATTGA AAAGTCAAAC GGCTAGTTTG AAAGTCAAAC GGCAAGTTTT GTAAAGTGGA 360
361 AAAGAGGAGG AAGGAGGGAA ACTGAGTTGG GGAGGGAATC CCTCCTCCAA CCGGCGGCCC 420
421 ACCTCCAAGC CGAGTTGGGG AGGGACTCCC CTCCCTCCCC TTGGCGCGGC CGGCCCCTCC 480
481 ACCTATATAA GGAGGGGCAG GGCTCCCTCC TCTATATACA CAAGTCCCTC ACCTCA**AACC** 540
541 **CTAG**AAGCTG CAGCCCTCCA TAGCCGTCGC CACCACCTAG GGTTTTGCCT TAGCCGCCGA 600
601 TTTGGGGTCC CCGG**AACCCT AG**CCGCCGGC TCTAGCATCC CTTCCTTGGT GTAACTA**AAC** 660
661 **CCTAG**AACAC CGAGGAATCT CTCATACTCC ACCATACTCG CTCCGGCTTA AGGCGAAGCC 720
721 GTGCGGGATC 730

c) SEQUENCE As93 668 BP; 197 A; 202 C; 132 T; 137 G.

1 GATCAGCTGG CCAATGAGAA CGCAAGCGTC GCATGATGCA TCAACCAGGA AACCATCAGA 60
61 CCCCAAAGTA TCGCC**CCAAC C**CATCTCCAG GATTTGCCCC AAGAAACCAA CAGACCCCCT 120
121 ATGACCCGTC CGAACTATC**C CAACC**GTGGA AACCCTAGAC CCGGAGGAAA TCACAACAAC 180
181 CCCGGCAATT CTAGCACTTT CACCCGTGCC CCACCCAAGT TCAACA**CCAA CC**ATGCTACC 240
241 A**CCAACC**CCA ATACTGCCCC GAGGACTGGA AGCAATGCTG TGCCTGTCGC AGCCAAGGAC 300
301 AAGTCACAAG TGACCTGTTA TGAATGTGGA AATAAAGGCC ACTATTCCAA CGAGTGCCCC 360
361 AACAAGAAGA ACGCTACAGC CCCTAATACC AATGTCCCAG TCCAGCAGCA GCGTCGTGTC 420
421 CAGCCAGGGA GAAGATTTGC CCTAGGAAAT TCACTCAACC GCAATGGCCG CCTTTTTCTC 480
481 ATGCATGCTG AAGAAGCTCA GGAAGCGCCA GACGTCGTGC TGGGTATGTT TTCTGTTAAT 540
541 TTAGTACTCG CAAGAGTGTT GTTTGATTCT GGTGCATCGC ATTCATTTGT CACCGAAGAA 600
601 TTTGCATGCA CTAGTAAAAT **CCAACCAACC** AAACTGAAGC ATGTGATGAT TGGACAAATT 660
661 CCCGGATC 668

d) SEQUENCE As111 619 BP; 89 A; 156 C; 176 T; 198 G.

1 GATCTTCTCT TACGCGAACG TGCATCGTAT GGCTCCGGGC TTCTTTTCTT TTTCTTGCCA 60
61 TCAGGAGATG GGCTGCGTGA ATGACGCGGG CCTCCTCCGC CATGGTGGCT GCTAGTGGCG 120
121 ACAGAGCGGA GTGGTGGTGG GTTAGCCTTC TTGTTGTTAC CCTCCTGCGA TGCGTCACGC 180
181 TTGTCGCAGT TGCTTTTCGG ACGCGGAGGT CATGGCGCGA CCTTCTCTTG TGTCCTTGGT 240
241 TGCGCTCTCC GTCATGACGG GTCTGCTCTG TGTTGTCGCG GATGACGATG CCGCT**CTGCC** 300
301 **TTGGC**GGGGG CATATCCGTA CAACGGTGAG CCTTGGTTTC TTCGTGATGT CGGGGGGAGC 360
361 GGGGAGGTGG CGATGGGCGA AGAGGTTGGG CCTTGCCTCC ATGGTCGGAG GATATGGGGA 420
421 TAGCATCCTT CCTGTTGCGC GCATCCCTCT GTATTTGTTC TGACGAACAC GTCTGAGACC 480
481 TGACAAAACG TTGGAGGCTT GTGCTGGCGA GACTAACAAA ACGTTGGGTC AGACTCGTGC 540
541 CTCTGTGAGG TGGCCTTTTC CTATTGTTTC A**CTGCCTTGG C**CACTAGGAC ATAGACCTGG 600
601 TCCATCGCTT CAGGTGATC 619

Chromosomal distribution of cloned repeated DNA sequences in the cultivated oat genome, as revealed by FISH

To determine the chromosomal distribution of the repeated DNA sequences in the hexaploid oat genome, we used each of the 4 clones pAs14, pAs121, pAs93 and pAs111 separately as probes in FISH analysis. In each case, the chromosome identification and genome designation of each individual chromosome was achieved through both simultaneous FISH and re-probing the same metaphase plates with ribosomal (pTa71 and pTa794) and pAm1 clones (Fominaya et al. 1995; Linares et al. 1996).

Fluorescent in situ hybridization with the rhodamine-labeled pAs14 probe revealed hybridization signals on

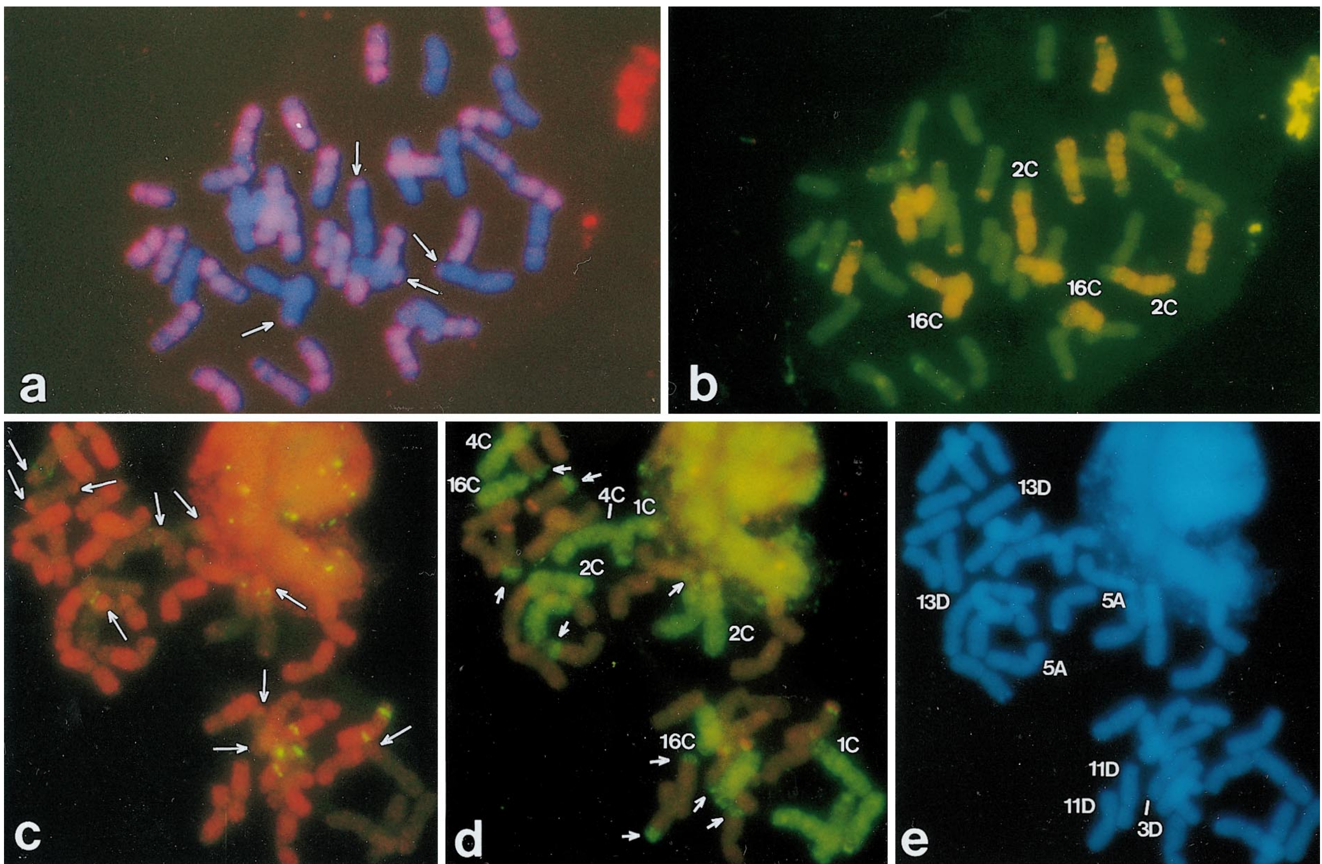

Fig. 3a–e Fluorescence in situ hybridization of metaphase plates of *A. sativa* cv 'Extra Klock'. **a** In situ hybridization of the rhodamine-labeled pAs14 (*pink*) probe, **b** the same cell as in **a** shown after in situ hybridization with rhodamine-labeled pAm1 probe (*orange*) and the digoxigenin-labeled pTa794 probe (*green*), **c** metaphase plate which is missing one 3D chromosome showing simultaneous visualization of hybridization sites of rhodamine-labeled pAs121 (*orange*) and digoxigenin-labeled pTa794 (*green*), **d** the same cell as in **c** shown after in situ hybridization with digoxigenin-labeled pAm1 (*green*) and rhodamine-labeled pTa71 (*red*), **e** the same cell as in **c** and **d** counterstained with DAPI. In **a** the *arrows* indicate the C-A/D translocations in C-genome chromosomes, which are identified in **b** with *numbers.* In **c** the *arrows* indicate the C-A/D translocations on C- genome chromosomes, which are identified in **d** with *numbers*. In **d** the arrows indicate the A-C and D-C translocations in A- and D-genome chromosomes, identified in **e** with *numbers*. Chromosomes are numbered using the generic system (Linares et al. 1996, 1998)

16 chromosome pairs in *A. sativa* cv 'Extra Klock' metaphases (pink in Fig. 3a). Two of these, plus the remaining 5 chromosome pairs, hybridized strongly over their lengths with the rhodamine-labeled pAm1 probe (orange in Fig. 3b). Taking into account the description of the pAm1 hybridization pattern (Fominaya et al. 1995), the latter chromosome group was assigned to the C genome. Consequently, the pAs14 probe identified both A- and D-genome chromosomes and two chromosome pairs belonging to the C genome. With respect to the pAs14 hybridization pattern, the A- and D-genome chromosomes showed a dispersed distribution over their entire lengths, except for the telomeric regions of the long arms. In contrast, the pAs14 probe hybridized at the telomeric regions of the long arms in two C-genome chromosome pairs identified as 2C and 16C by the presence of sequences complementary to pTa794 (green in Fig. 3b) and pAm1 (orange in Fig. 3b).

When in situ hybridization analyses of *A. sativa* cv 'Extra Klock' metaphases were performed using rhodamine-labeled pAs121 as the probe, 18 chromosome pairs showed hybridization signals (orange in Fig. 3c). Four of these, plus the remaining 3 chromosome pairs, were identified as belonging to the C genome by re-probing the same metaphase plates with the digoxigenin-labeled pAm1 probe (green in Fig. 3d). Consequently, the pAs121 probe identified both A- and D-genome chromosomes, plus 4 chromosome pairs belonging to the C-genome. Differences in the hybridization patterns were observed in chromosomes of the A and D genomes. Whereas 3 chromosome pairs were homogeneously hybridized, 5 chromosome pairs showed no hybridization in their telomeric regions. These un-hybridized segments showed complementary sequences to pAm1. Four were identified as 5A, 3D, 11D and 13D by the presence of sequences complementary to pTa794 (green in Fig. 3c) and pTa71 (red in Fig. 3d) as described by Linares et al. (1998). The fifth could not be identified since there are no chromosome markers available at present. With respect to the C-genome chromosomes, 2 chromosome pairs contained pAs121 hybridization signals located

distally on their long arms. These chromosome pairs were identified as 2C and 4C by the presence of sequences homologous to pTa794 on the long arms (Linares et al. 1996). The 1C chromosome pair showed hybridization signals close to the telomeric regions of the long arm. The 16C chromosome pair was hybridized at the telomeric regions of both arms. No hybridization signals were detected on the remaining 3 chromosome pairs of the C genome.

When in situ hybridization analyses of *A. sativa* cv 'Extra Klock' metaphases were performed using either rhodamine-labeled pAs93 or pAs111 as the probe, 14 chromosome pairs showed hybridization signals. The remaining 7 chromosome pairs were identified as belonging to the C-genome by re-probing the same metaphase plates with digoxigenin-labeled pAm1 (data not shown). Consequently, both pAs93 and pAs111 probes identified both A- and D-genome chromosomes which showed a similar hybridization pattern to the pAs14 probe. Hybridization signals with either pAs93 or pAs111 probes were dispersed over the entire chromosome lengths, except in the telomeric regions of the long arms of the A- and D-genome chromosomes. No hybridization signals were detected on C-genome chromosomes with either the pAs93 or pAs111 probes.

Discussion

This paper reports the characterization of 4 repetitive DNA sequences, pAs14, pAs121, pAs93 and pAs111, isolated from the *A. strigosa* genome. These sequences belong to 4 different repeat families, since they showed different Southern hybridization patterns and no homology to each other.

The sequences As14 and As121 were shown to be specific to the A genome. No hybridization signals were obtained with the C-genome diploid species when the clones As14 and pAs121 were used as probes in Southern hybridization experiments. This clearly indicates the presence of sequences homologous to the As14 and As121 sequences in the A genome only. In contrast, sequences homologous to both the As93 and As111 sequences were detected for the A- and C-genome diploid species when either pAs93 or pAs111 were used as probes in Southern hybridization experiments. Since the 4 sequences were isolated from the A-genome diploid species *A. strigosa,* this distinction at the DNA sequence level between both the As14 and As121 repetitive sequences from the A genome and the other repetitive sequences in the C genome may have arisen through the elimination of As14 and As121 repetitive sequences from the C genome. Alternatively, they may have suffered extensive modification during the speciation process. Similar results have been reported with 3 repetitive DNA sequences, As120, As120a and As17, also isolated from the *A. strigosa* genome (Linares et al. 1998, 1999). Homologous sequences to the fragment cloned in pAs120 are composed of a tandemly repeated DNA sequence adjacent to an unrelated sequence in both A- and C-genome diploid species. However, homologous sequences to the tandem unit subcloned in pAs120a are absent in the C-genome diploid species (Linares et al. 1998). Similarly, homologous sequences to the fragment cloned in pAs17, which has been shown to be an LTR fragment of a Ty1-*copia*-retrotransposon, are present only in the A-genome diploid species (Linares et al. 1999).

The Southern hybridization patterns and comparisons of each nucleotide sequence cloned with itself (as shown by the Harr plot graphic program), indicate that each repetitive sequence, As14, As121, As93 and As111, appears as dispersed sequences. In the case of the As14 and As111 sequences, the hybridization profiles in Southern blots containing restriction enzyme-digested oat DNA revealed a strong smeared pattern. This indicates that they are probably dispersed over the oat genome. In contrast, Southern hybridization of the As121 and As93 sequences revealed a background smear with some prominent bands, indicating the presence of numerous variant copies of a similar sequence in the genomes of the *Avena* species analyzed. Similar hybridization patterns have been described in *Leymus* (Dubcovsky et al. 1997) and *Erianthus* (Besse and McIntyre, 1998) species.

Repetitive sequences have been found to be informative in the assessment of relationships between species when using genome-specific restriction fragments in Southern hybridization experiments (Solano et al. 1992; Dubcovsky and Dvorák, 1996; Linares et al. 1998; Zhao et al. 1998). The sequences As14 and As121 appeared to be invariant in the A-genome diploid and polyploid species, except for the *Eco*RV-restriction fragment of 3747 bp detected with the As14 sequence in *A. murphyi*. Diploid species with either the A or C genome hybridized with the sequences As93 and As111, but the hybridization patterns separated the C-genome diploid species from the rest of the *Avena* species. For both sequences, polyploid species thought to bear an A genome showed DNA fragment patterns more similar to those of *A. strigosa* than to those of C-genome species. Moreover, the lack of hybridization of As93 and As111 with C-genome chromosomes in metaphases of polyploid species makes these sequences A/D-genome specific with respect to the C genome. Therefore, the 4 sequences described in this study seem to have followed evolutionary processes that have led to their selective amplification or deletion in different lineages.

Characterization of sequences in the *Avena* genus provides a clear picture of the phylogenetic relationships between the species. Absence of sequences homologous to pAs14 and pAs121 and the poor representation of sequences homologous to pAs93 and pAs111 confirm the separation of the A and C genome suggested by other authors, some of whose work includes studies with repetitive sequences (Chen and Armstrong 1994; Jellen et al. 1994; Leggett and Markhand 1995; Fominaya et al. 1995; Linares et al. 1998).

Fluorescent in situ hybridization analyses with each of the 4 *A. strigosa* clones showed high homology with

the A- and D-genome chromosomes of the *A. sativa* genome. This confirms the results of previous studies based on *A. strigosa* genomic in situ hybridization (Chen and Armstrong 1994; Jellen et al. 1994; Leggett and Markhand 1995) and on the physical mapping of 5S rDNA (Linares et al. 1996). However, the 4 clones showed different homology with individual C-genome chromosomes. Whereas sequences homologous to both As14 and As121 sequences were detected on C-genome chromosomes, no sequences homologous to either As93 or As111 were observed. These results suggest a different evolutionary pattern for the repetitive DNA sequences after the polyploidization of the *A. sativa* genome. Thus, As14 and As121 repetitive sequences were involved in intergenomic translocations between C- and A/D-genome chromosomes. In contrast, As93 and As111 repetitive sequences were restricted to the A- and D-genome chromosomes. This assumption agrees with the chromosomal location of the A-genome-specific sequence isolated from *A. strigosa*, As120a, which allowed separation of the chromosomes belonging to the A and D genomes in *A. sativa* species (Linares et al. 1998).

Genomic in situ hybridization has shown that the karyotype of *A. sativa* contains A/D-genome chromatin in the long arms of 3 C-genome chromosome pairs (Chen and Armstrong 1994; Jellen et al. 1994; Leggett and Markhand 1995). In contrast, the present study revealed As14 sequences in 2 C-genome chromosome pairs, and As121 in 4, and identified a maximum of five intergenomic translocations. Two were located on the long arms of the 2C and 4C chromosome pairs, one in the interstitial regions of the long arm of the 1C pair and the remainder on both arms of the 16C pair. The discrepancy in the number of translocations observed by genomic in situ hybridization and in situ hybridization using cloned repetitive DNA sequences may be due to low-copy numbers of the cloned repetitive DNA sequences in the oat genome combined with their low level of representation in the translocated segments. Jiang and Gill (1994) distinguished two different types of intergenomic chromosome translocations which may occur following polyploid formation. These authors classified them as either random translocations or species-specific translocations, according to whether they involved different chromosomes in different populations of the same polyploid species or specific chromosomes in every polyploid population of a species. The classification of the intergenomic translocations identified in the present study requires further investigation. These sequences may have potential as molecular markers for analyzing the presence of intergenomic translocations in other hexaploid species.

Acknowledgements The authors would like to thank the *Dirección General de Investigación Científica y Técnica* of Spain (PB92–0173 and PB95–0329) for supporting this study.

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