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ORIGINAL PAPER

A new chromosome nomenclature system for oat (*Avena sativa* L. and *A. byzantina* C. Koch) based on FISH analysis of monosomic lines

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Abstract Fluorescent in situ hybridization (FISH) with multiple probes was used to analyze mitotic and meiotic chromosome spreads of *Avena sativa* cv 'Sun II' monosomic lines, and of *A. byzantina* cv 'Kanota' monosomic lines from spontaneous haploids. The probes used were *A. strigosa* pAs120a (a repetitive sequence abundant in A-genome chromatin), *A. murphyi* pAm1 (a repetitive sequence abundant in C-genome chromatin), *A. strigosa* pITS (internal transcribed spacer of rDNA) and the wheat rDNA probes pTa71 (nucleolus organizer region or NOR) and pTa794 (5S). Simultaneous and sequential FISH employing pairs of these probes allowed the identification and genome assignation of all chromosomes. FISH mapping using mitotic and meiotic metaphases facilitated the genomic and chromosomal identification of the monosome in each line. Of the 17 'Sun II' lines analyzed, 13 distinct monosomic lines were found, corresponding to four monosomes of the A-genome, five of the C-genome and four of the D-genome. In addition, 12 distinct monosomic lines were detected among the 20 'Kanota' lines examined, corresponding to six monosomes of the A-genome, three of the C-genome and three of the D-genome. The results show that 19 chromosomes out of 21 of the complement are represented by monosomes between the two genetic backgrounds. The identity of the remaining chromosomes can be deduced either from one intergenomic translocation detected on both 'Sun II' and 'Kanota' lines, or from the single reciprocal, intergenomic translocation detected among the 'Sun II' lines. These results permit a new system to be proposed for numbering the 21 chromosome pairs of the hexaploid oat complement. Accordingly, the A-genome contains chromosomes 8A, 11A, 13A, 15A, 16A, 17A and 19A; the C-genome contains chromosomes 1C, 2C, 3C, 4C, 5C, 6C and 7C; and the D-genome consists of chromosomes 9D, 10D, 12D, 14D, 18D, 20D and 21D. Moreover, the FISH patterns of 16 chromosomes in 'Sun II' and 15 in 'Kanota' suggest that these chromosomes could be involved in intergenomic translocations. By comparing the identities of individually translocated chromosomes in the two hexaploid species with those of other hexaploids, we detected different types of intergenomic translocations.

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Introduction

In allopolyploid plant breeding and evolutionary studies, there is a need to determine the origins of the constituent subgenomes and determine other genomic relationships, for example, homoeology. For economically important plants, this information can be used to facilitate the introduction and expression of alien genes that increase the plant's value. The evolution of the genome of common or white oat (*Avena sativa* L.) and its Mediterranean counterpart red oat (*A. byzantina* C. Koch, both $2n = 6x = 42$, AACCDD

subgenomes) was presumably a complex process involving divergence at the diploid level from a common diploid ancestor, then convergence through amphidiploidization, followed by divergence at the tetraploid level and then further divergence after the addition of the third subgenome at the hexaploid level (Thomas 1992). Oat is an important cereal crop raised both for human consumption and animal feed. Cultivated hexaploid oat (*A. sativa* L. and *A. byzantina* C. Koch) is classified as a secondary crop, i.e., derived from weeds of the primary cereal domesticates of the Near East, *Avena sterilis* L., the oldest hexaploid oat, has been proposed as the putative progenitor of all other cultivated and wild hexaploid oat species (Coffman 1946).

The earliest efforts to identify individual chromosomes of hexaploid oats involved meiotic chromosome pairing analysis and somatic karyotyping (Thomas 1992). Later, C-banding, a technique that preferentially stains heterochromatin, was used to distinguish the C-genome chromosomes from those of the A and D genomes (Linares et al. 1992; Jellen et al. 1993a, b, 1997). The latter of these authors also demonstrated the existence of C-banding polymorphisms among the hexaploid species they studied. Genomic in situ hybridization (GISH) using fluorescent labels has proven to be a very useful tool for allocating chromosomes to genomes in allopolyploid species. However, GISH has so far been unable to separate the A- and D-genome chromosomes in hexaploid oats, although discrimination between the A/D- and C-genome chromosomes has been demonstrated by several authors (Jellen et al. 1994; Chen and Armstrong 1994; Leggett and Markhand 1995; Yang et al. 1999; Hayasaki et al. 2000).

Fluorescent in situ hybridization (FISH) using probes from different families of satellite sequences leads to the detection of chromosome- and genome-specific patterns, and consequently allows for the recognition of pairs of chromosomes. For example, FISH involving the satellite sequence pAs120a isolated from *A. strigosa* has been used to distinguish the A- and D-genome chromosomes (Linares et al. 1998). Moreover, pAs120a in combination with ribosomal genes probes and the sequence pAm1, a satellite specific to the C-genome chromosomes (Fominaya et al. 1995), has been successfully used to characterize chromosome complements when single pairs could not be recognized on the basis of their morphology—as is the case with *A. sativa* cv 'Sun II' (Irigoyen et al. 2002).

C-banding and GISH have also shown that intergenomic translocations are present in hexaploid oats. Such studies have shown small C-genome segments to be translocated onto the A- or D-genome chromosomes (abbreviated as A/D–C translocations) and vice versa (abbreviated as C–A/D) for A- or D-segments translocated onto C-genome chromosomes (Jellen et al. 1994; Chen and Armstrong 1994; Leggett and Markhand 1995; Yang et al. 1999; Hayasaki et al. 2000). The chromosomes involved in some translocations have been identified using combinations of satellite sequences as probes in sequential FISH experiments (Linares et al. 2000; Irigoyen et al. 2002). These studies have allowed distinctions to be made between specific intergenomic translocations as follows: C-genome segments translocated onto either A-genome chromosomes (A–C translocation) or D-genome chromosomes (D–C translocation); A-genome segments translocated onto either C-genome chromosomes (abbreviated as C–A translocation) or D-genome chromosomes (D–A translocation); and D-genome segments translocated onto either A-genome chromosomes (A–D translocation) or C-genome chromosomes (C–D translocation).

Monosomic lines have proven useful in the assignment of oat linkage groups to specific chromosomes (Rooney et al. 1994; Jellen et al. 1997; Fox et al. 2001). Thus, efforts have been made to develop monosomic lines of cultivated oats; these have been isolated mainly from two sources. Twenty monosomic lines (K1–K20) and one nullisomic (K21) were isolated from *A. byzantina* cv 'Kanota' (Morikawa 1985), and 18 monosomic lines (SI–SXVIII) were isolated from *A. sativa* cv 'Sun II' spontaneous haploids (Hacker and Riley 1965). Additional monosomic lines have been isolated from 'Sun II' haploid plants obtained from oat × maize hybridizations (Rines and Dahleen 1990; Davis and Rines 1991). Haploids were allowed to self-pollinate and were partially fertile, presumably due to unreduced gametes. A few attempts have been made to determine the monosome or nullisome in each line. In the 'Kanota' monosomic series, the identification of individual mitotic or meiotic chromosomes in the 21 monosomic lines, and their allocation to specific genomes, was reported using classical karyotyping (Morikawa 1985) and Giemsa C-banding (Linares et al. 1992). Wright C-banding combined with RFLP marker data was used to show that the 'Kanota' monosomic series represented 12 of the possible 21 chromosomes (Jellen et al. 1993a, b; Rooney et al. 1994). In the 'Sun II' monosomic series, 10 different monosomic chromosomes were identified in the SI–SXVIII set using RFLPs (Mendu et al. 1993), GISH (Leggett and Markhand 1995) and C-banding (Jellen et al. 1997). Among 27 'Sun II' monosomic lines obtained from intergeneric hybridization-derived haploids, six new lines were identified by C-banding (Jellen et al. 1997). Both C-banding (Jellen et al. 1993a, b, 1997) and GISH (Leggett and Markhand 1995) have been used to identify seven pairs of C-genome chromosomes; however, the remaining 14 pairs of chromosomes could not be reliably assigned to the A or D genomes. In contrast, the genomic allocation and the morphological identification of the aneuploid chromosome in each 'Sun II' monosomic line was made by FISH mapping using a combination of ribosomal sequences and

satellite sequences specific to either the A-, D- or C-genome chromosomes (Irigoyen et al. 2002).

It is noteworthy that the authors of all the above studies employed different nomenclature systems for each individual chromosome pair. This complicated the comparison of results from different research groups that have been working to identify monosomic chromosomes or those involved in intergenomic translocations.

The aims of the present study were to (1) develop a FISH map of the chromosomes of 'Sun II' and 'Kanota', (2) to use these to identify the missing chromosome in monosomic lines of 'Sun II' and in monosomics of 'Kanota' and (3) to develop a uniform nomenclature system to describe each individual chromosome.

Materials and methods

Seeds of 17 'Sun II' monosomic lines were kindly supplied by Dr. H.W. Rines of the University of Minnesota (St. Paul, MN). This set of lines had been isolated either from screening large populations of 'Sun II' for spontaneous aneuploids or from haploids generated by the 'Sun II' oat × maize crosses (Jellen et al. 1997). The 'Sun II' parent used in making the oat–maize hybrids lacked the 3C (10C)-14 interchange and, consequently, oat–maize derived haploids also lack it (H. Rines, personal communication). Thus, the S-monosomic-lines without this reciprocal translocation are new monosomics derived from oat × maize crosses. A set of 20 monosomic lines of *A. byzantina* C. Koch cv 'Kanota' was kindly supplied by Dr. T. Morikawa of the University of Osaka Prefecture (Osaka). Both 'Kanota' and 'Sun II' monosomic lines were screened for monosomy ($2n = 41$) by standard Feulgen method. Root tips and anthers of monosomic plants were used in cytological preparations for FISH. Root tips and anthers were pretreated, fixed and squashed after enzyme treatment according to Fominaya et al. (1995) and Irigoyen et al. (2002) respectively. FISH with multiple probes was performed as described by Linares et al. (1996). Two probes, each labeled with a different fluorochrome were routinely combined in the same hybridization mixture (30 µL/slide). After washing in 4× SSC containing 0.2% v/v Tween-20, re-hybridization with two additional probes was carried out under the same conditions. No appreciable alteration was detected in chromosome morphology after the second probing. Slides were examined using a Zeiss Axiophot epifluorescence microscope. Separate images were captured for each filter using a cooled CCD camera (Nikon).

Five DNA probes were used for FISH analyses: (1) pAs120a, a satellite DNA sequence specific to the oat A-genome and containing an insert of 114 bp isolated from *A. strigosa* (Linares et al. 1998, (2) pAm1, a satellite DNA sequence specific to the oat C-genome containing an insert of 464 bp isolated from *A. murphyi* (Solano et al. 1992), (3) two ribosomal probes, either pITS, containing the gene 5.8S and both intergeneric spacers ITS1 and ITS2 isolated from *A. strigosa* (this work) or pTa71, containing the 18S-5.8S-28S rDNA gene isolated from *Triticum aestivum* (Gerlach and Bedbrook 1979) and (4) pTa794, including a 410 bp 5S rDNA gene and intergeneric spacer isolated from *T. aestivum* (Gerlach and Dyer 1980). In simultaneous in situ hybridization experiments, the pAs120a, pAm1, pITS and pTa794 clones were amplified and labeled by PCR with digoxigenin-11-dUTP (Boehringer Mannheim) or biotin-16-dUTP (Roche). The pTa71 clone was labeled with digoxigenin-11-dUTP or biotin-16-dUTP by nick translation. All labeled probes were precipitated with absolute ethanol and resuspended in sterile water.

Results

Each chromosome pair of the 'Sun II' and 'Kanota' monosomic lines (Fig. 1) was assigned to a specific genome by sequential FISH performed using pAs120a and pAm1 as probes. Positive hybridization with pAs120a identified chromosomes of the A-genome; positive hybridization with pAm1 identified the C-genome chromosomes; negative hybridization with both probes identified the D-genome chromosomes. It has been shown that the pAs120a- and pAm1-homologous sequences detected by these probes are distributed homogeneously along all the chromosomes in *A. strigosa* (Linares et al. 1998) and *A. pilosa* (Fominaya et al. 1995), respectively. The chromosomal identities based on size, arm ratios and FISH pattern follow the nomenclature used in previous karyotyping analyses (Linares et al. 1998; Irigoyen et al. 2002).

FISH mapping of 'Sun II' monosomic lines

Based on the results of FISH analyses of *A. sativa* cv 'Sun II' reported by Irigoyen et al. (2002), a FISH karyotype of the S-mono-8 monosomic line was generated to establish the hybridization patterns of the chromosomes belonging to the A, C and D subgenomes (Fig. 1a, b). Probing chromosomes of this line with labeled pAs120a and labeled pAm1 led to the clear identification of three sets of 14 chromosomes each. The chromosome set that hybridized with the pAs120a insert was identified as the A-genome (red in Fig. 1a), the chromosome set that hybridized with the pAm1 insert was identified as the C-genome (green in Fig. 1b), and the remaining chromosomes—unhybridized with either probe—were identified as the D-genome.

The A-genome chromosomes could be further characterized. One satellited chromosome pair was identified as 12A

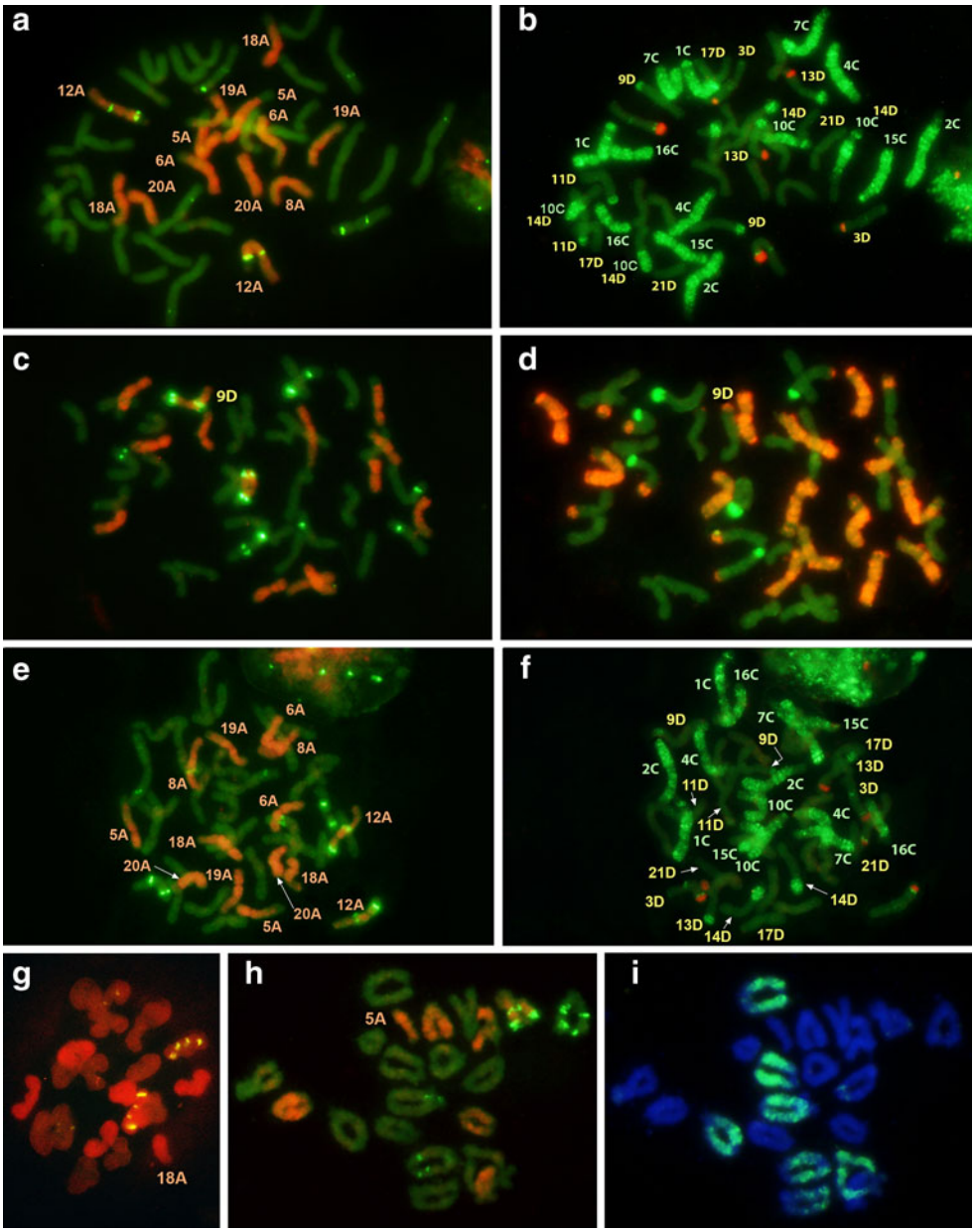

Fig. 1 Fluorescent in situ hybridization (FISH) of mitotic metaphase plates of monosomic lines S-mono-8 17/7/2010, S-mono-10 (**c**, **d**), and euploid Kanota (**e**, **f**) and meiotic plates of monosomic lines Kanota-10 (**g**) and Kanota-11 (**h**, **i**). The aneuploid chromosome is identified as 8A in line S-mono-8 (**a**, **b**); 9D in line S-mono-10 (**c**, **d**); 18A in line Kanota-10 (**g**); and 5A in line Kanota-11 (**h**, **i**). **a** Double FISH of biotin-labeled pAs120a (*red*) and digoxigenin-labeled pTa794 (*green*) probes. *Numbers* indicate the A-genome chromosomes. **b** The same cell as in **a** shown after simultaneous FISH with the digoxigenin-labeled pAm1 (*green*) and biotin-labeled pITS (*red*) probes. *Numbers* indicate the C- and D-genome chromosomes. Intergenomic translocation 10C-14D is also labeled. **c** Double FISH of biotin-labeled pAs120a (*red*) and digoxigenin-labeled pTa794 (*green*) probes. *Numbers* indicate the aneuploid chromosome. **d** The same cell as in **c** shown after simultaneous FISH with biotin-labeled pAm1 (*red*) and digoxigenin-labeled pITS (*green*) probes. **e** Double FISH of biotin-labeled pAs120a (*red*) and digoxigenin-labeled pTa794 (*green*) probes. *Numbers* indicate the A-genome chromosomes. **f** The same cell as in **e** shown after simultaneous FISH with the digoxigenin-labeled pAm1 (*green*) and biotin-labeled pITS (*red*) probes. *Numbers* indicate the C- and D-genome chromosomes. **g** Double FISH of biotin-labeled pAs120a (*red*) and digoxigenin-labeled pTa794 (*yellow*) probes. *Number* indicates the aneuploid chromosome. **h** FISH of biotin-labeled pAs120a (*red*) probe. *Number* indicates the aneuploid chromosome. **i** The same cell as in **h** shown after FISH with the digoxigenin-labeled pAm1 (*green*) and counterstained with DAPI

based on the presence of sequences complementary to pITS and pTa794. The presence/absence of terminal sequences homologous to pAs120a or pAm1 and karyotype morphology enabled the remaining A-genome chromosomes to be tentatively identified (Fig. 1a). One chromosome pair, 8A, was labeled over its entire length on both sides of the

centromere. Another pair, 5A, showed a lack of sequences homologous to pAs120a in the telomeric region of its long arm. Using labeled pAm1 as a probe (green in Fig. 1b), however, this chromosome pair was shown to carry a telomeric segment of the C-genome, denoting it as an A–C intergenomic translocation. The four remaining chromosome pairs (6A, 18A, 19A and 20A) showed a lack of sequences homologous to pAs120a and pAm1 on the telomeric region of their long arms, denoting the possible existence of A–D intergenomic translocations. However, it could not be ruled out that these chromosome segments carry low densities of A or C specific satellite probe sequences.

Two pairs of C-genome chromosomes (green in Fig. 1b), 1C and 15C, were identified by the presence of sequences homologous to pAm1 over their entire lengths, except in the interstitial regions of their long arms. Four chromosome pairs (2C, 4C, 7C and 16C) were identified by the presence of sequences homologous to pAm1 over the entire length of the short arms, and by segments of variable length on the long arms. The 10C pair, labeled $10C^{14D}$ in Fig. 1b was identified by the presence of sequences homologous to pAm1 only on the long arms. All these chromosome pairs showed a lack of sequences homologous to pAs120a, suggesting that they carry C–D intergenomic translocations. Chromosome pairs 2C and 4C also harbored 5S rDNA (green in Fig. 1a) at the distal end of their long arms.

With regard to the D-genome chromosomes (dark green in Fig. 1b), two pairs, 3D and 13D, were satellited; they were also identified by the presence of sequences homologous to pAm1 in the telomeric region of their long arms. Moreover, the 3D chromosome pair contained 5S sequences on both chromosome arms in a patterns similar to that seen in 12A—strong morphological evidence that these two chromosomes are homoeologous. Two chromosome pairs, 9D and 11D, were identified by the presence on their long arms of segments of variable length containing sequences homologous to pAm1. The 14D pair, labeled $14D^{10C}$ in Fig. 1b was identified by the presence of sequences homologous to pAm1 over the entire length of its long arms. These results indicate that these five chromosome pairs carry D–C intergenomic translocations. The remaining two pairs, 17D and 21D, showed no sequences homologous to pAm1.

This FISH mapping was used to assign either the individual meiotic univalent or the aneuploid somatic chromosome to each of the 17 S-monosomic lines. Figure 1a–d illustrates the FISH experiments performed. Table 1 summarizes the in situ hybridization data recorded.

Table 1 Presence (+) or absence (−) of fluorescence in situ FISH experiments using the repetitive sequences pAs120a, pAm1, pITS and pTa794 as probes to examine the aneuploid chromosome of each S-monosomic-lines

S-monosomic-lines	Hybridization with				Genome allocation	Intergenomic translocation	Aneuploid chromosome (Linares et al. 1998)	Reciprocal translocation $10C^{14D}$	
	pAs120a	pAm1	pITS	pTa794				Absence	Presence
S-mono-1C	−	+	−	−	C	C–D (i, l)	15C	+	−
S-mono-2C (−1)	−	+	−	+	C	C–D (t, l)	4C	+	−
S-mono-4C	−	+	−	−	C	C–D (t, l)	7C	+	−
S-mono-5C (AVA 228)	−	+	−	−	C	C–D (i, l)	1C	−	H_t
S-mono-6C (AVA 126)	−	+	−	−	C	C–D (i, l)	15C	−	H_t
S-mono-7C (AVA 157)	−	+	−	−	C	C–D (t, l)	16C	+	−
S-mono-8 (AVA 352)	+	−	−	−	A	A–D (t)	8A	−	*H*
S-mono-9 (AVA 559)	−	−	−	−	D	–	17D	+	−
S-mono-10 (AVA 116)	−	$+_{(t,l)}$	−	−	D	D–C (t, l)	9D	+	−
S-mono-11 (AVA 580-1)	+	−	−	−	A	–	6A	+	−
S-mono-12 (AVA 120)	−	$+_{(t,l)}$	−	−	D	D–C (t, l)	9D	+	−
S-mono-15 (AVA 576)	+	−	−	−	A	A–D (t, l)	8A	−	*H*
S-mono-16 (AVA 72-2)	+	$+_{(t,l)}$	−	−	A	A–C (t, l)	5A	−	*H*
S-mono-18 (AVA 578-2)	−	−	−	−	D	–	21D	−	*H*
S-mono-19 (XII b-3)	+	−	+	+	A	–	12A	−	H_t
S-mono-20 (AVA 572)	+	−	+	+	A	–	12A	−	H_t
S-mono-21 (AVA 569)	−	$+_{(t,l)}$	−	−	D	D–C (t, l)	13D	−	*H*

i, *t* and *l* the presence of FISH signals in interstitial or telomeric regions of the long arm. Also reported for each S-monosomic line is the presence in homozygosity (*H*), or heterozygosity (H_t), or the absence (+) of the 10C–14D reciprocal translocation, the genome allocation, the absence (−) of the intergenomic translocation or its identification, and the identification of the aneuploid chromosome

Hybridization of chromosome preparations of S-monosomic-lines with labeled pAs120a revealed six lines (S-mono-8; Fig. 1a, b), S-mono-11, S-mono-15, S-mono-16, S-mono-19 and S-mono-20) to be aneuploids for A-genome chromosomes (Table 1). S-mono-16 also hybridized with labeled pAm1 in the telomeric region of the long arm, indicating the existence of an A–C intergenomic translocation (Table 1). The monosomic chromosomes of S-mono-19 and S-mono-20 lines also showed hybridization signals with labeled pITS in interstitial regions of the short arm, and with labeled pTa794 in interstitial regions of both arms.

Hybridization with pAm1 revealed six lines (monosomic lines 1C, 2C, 4C, 5C, 6C and 7C) to be aneuploid for C-genome chromosomes. Monosomic chromosomes in lines 2C, 4C and 7C showed no hybridization signals with either labeled pAm1 or pAs120a in the telomeric region of the long arms, indicating the possible existence of C–D intergenomic translocations. Monosomic chromosomes in lines 1C, 5C and 6C showed no hybridization signals with labeled pAm1 or pAs120a in the interstitial regions of the long arms, suggesting they carry C–D intergenomic translocations. The remaining five lines (9, 10, 12, 18 and 21) were aneuploid for D-genome chromosomes (Table 1). Monosomic chromosomes in lines 10 (Fig. 1c, d) and 12 showed hybridization signals with labeled pAm1 in the telomeric regions of the long arms, indicating the existence of D–C intergenomic translocations. Monosomic chromosome in line 21 showed hybridization signals with labeled pITS and pAm1.

FISH mapping of the aneuploid chromosome of each of the S-monosomic lines was then compared with the pattern of the A-, C- and D-genome chromosomes previously characterized to determine their identities (Table 1). As these were assigned it became obvious that monosomic lines 8 and 15 were aneuploid for the same chromosome (chromosome 8 of the A-genome); monosomic lines 19 and 20 were aneuploid for chromosome 12 of the A-genome; monosomic lines 1C and 6C were aneuploid for chromosome 15 of the C-genome; and monosomic lines 10 and 12 were aneuploid for chromosome 9 of the D-genome. However, monosomic line 1C was different than monosomic line 6C in that the intergenomic translocation between chromosome pairs 10C and 14D detected in S-monolines was absent.

FISH mapping of *A. byzantina* Koch cv. 'Kanota' and its monosomic lines

Probing the chromosomes of the euploid *A. byzantina* Koch cv. 'Kanota' with labeled pAs120a (red in Fig. 1e) marked the entire length of chromosome pairs 6A, 8A and 12A. Chromosome pair 12A also carried pTa794 (for 5S rDNA, green in Fig. 1e) and pITS (red in Fig. 1f) hybridization signals on both sets of arms or the short arms respectively. The remaining pairs (5A, 18A, 19A and 20A) were labeled over their entire length, except in the telomeric regions of their long arms. These chromosomes remained unlabeled at the telomeric regions with probe pAm1 (green in Fig. 1f), suggesting the possible existence of A–D intergenomic translocations.

Probing the chromosomes with pAm1 (green in Fig. 1f) labeled seven chromosome pairs on both sides of the centromere. Two pairs, 1C and 15C, were labeled over their entire length except in the interstitial regions of the long arms. Chromosomes 2C, 4C, 7C and 10C were labeled over the entire length of their short arms, as were segments of variable length of the long arms. Chromosome pairs 2C and 4C also carried 5S rDNA (green in Fig. 1e) at the distal end of their long arms. The 16C pair was labeled over its entire length, except in the telomeric regions of both arms. Since the pAm1 non-hybridized segments were not labeled by the pAs120a probe they might carry C–D intergenomic translocations.

Probing the chromosomes with both pAs120a (red in Fig. 1e) and pAm1 (green in Fig. 1f) showed seven chromosome pairs that appeared unlabeled in the pericentromeric regions, indicating they corresponded to the D-genome (Fig. 1f). The pITS probe (red in Fig. 1f) labeled the distal end of the short arm of the chromosome pairs 3D and 13D. Chromosome pair 3D is characteristic in that it also has two 5S rDNA loci (green in Fig. 1e) distributed in a manner similar to that seen in chromosome pair 12A: with one locus adjacent to the proximal side of the NOR and the other toward the proximal end of the long arm. The pAm1 probe (green in Fig. 1f) labeled the distal ends of the long arms of the chromosome pairs 9D, 11D, 13D and 14D, indicating they carry D–C intergenomic translocations. Chromosome pairs 17D and 21D remained unlabeled with all the probes employed.

FISH mapping was used to try to identify either the individual meiotic univalent or the aneuploid somatic chromosome in each of the 20 'Kanota' monosomic lines. Figure 1g–i illustrates the FISH experiments performed to physically map the chromosomes. In situ hybridization data are summarized in Table 2. Probing with labeled pAs120a revealed 12 lines [monosomic lines 5, 6, 9, 10 (Fig. 1g), 11 (Fig. 1h–i), 13, 15, 16, 17, 18, 19 and 20] to be aneuploid for A-genome chromosomes (Table 2; Fig. 1g–i). Probe pAm1 revealed four monosomic lines (1, 2, 4 and 8) to be aneuploid for C-genome chromosomes. The remaining four lines (3, 7, 14 and 21) were aneuploid for D-genome chromosomes (Table 2).

The FISH maps of the aneuploid chromosomes of each of the 'Kanota' monosomic lines was then compared with the FISH map of disomic 'Kanota' to determine their identities (Table 2). In a manner similar to the 'Sun II' lines, several 'Kanota' lines were duplicate monosomics for the

Table 2 Presence (+) or absence (−) of fluorescence in situ FISH experiments using the repetitive DNA sequences pAs120a, pAm1, pITS or pTa71 and pTa794 as probes to examine the aneuploid chromosome of each K-monosomic-line

K-monosomic-lines	Hybridization with				Genome allocation	Intergenomic translocation	Aneuploid chromosome
	pAs120a	pAm1	pITS/pTa71	pTa794			
K1	−	+	−	+	C	C–D (t, l)	2C
K2	−	+	−	+	C	C–D (t, l)	2C
K3	−	−	+	+	D	–	3D
K4	−	+	−	+	C	C–D (t, l)	4C
K5	+	−	−	−	A	A–D (t, l)	18A
K6	+	−	−	−	A	–	8A
K7	−	−	−	−	D	–	17D
K8	−	+	−	−	C	C–D (i, l)	1C
K9	+	−	−	−	A	–	20A
K10	+	−	−	−	A	A–D (t, l)	18A
K11	+	−	−	−	A	A–D (t, l)	5A
K13	+	−	−	−	A	–	20A
K14	−	−	−	−	D	–	17D
K15	+	−	−	−	A	A–D (t, l)	18A
K16	+	−	−	−	A	–	20A
K17	+	−	−	−	A	–	6A
K18	+	−	−	−	A	A–D (t, l)	19A
K19	+	−	−	−	A	–	6A
K20	+	−	−	−	A	A–D (t, l)	18A
K21	−	−	−	−	D	–	21D

pITS and pTa 71 were indistinctly used in FISH analyses

i, *t* and *l* the presence of FISH signals in the interstitial or telomeric regions of the long arm. Also reported is the genome allocation, the absence (−) of the intergenomic translocation or its identification, and the identification of the aneuploid chromosome in each of the K-monosomic lines

same chromosome. Thus, monosomic lines 5, 10, 15 and 20 were aneuploid for the same chromosome (chromosome 18 of the A-genome); monosomic lines 17 and 19 were aneuploid for chromosome 6 of the A-genome; monosomic lines 9, 13 and 16 were aneuploid for chromosome 20 of the A-genome; monosomic lines 1 and 2 were aneuploid for chromosome 2 of the C-genome; and monosomic lines 7 and 14 were aneuploid for chromosome 17 of the D-genome.

Comparison of *A. sativa* 'Sun II' and *A. byzantina* 'Kanota' FISH patterns

Several individual chromosomes of the two species studied showed clear differences in terms of the presence and nature of the intergenomic translocations detected. With the probes used in this work, it was normally only possible to identify the genomic origin of the translocated chromatin; only infrequently was a chromosomal source identified. Further, the sizes of the translocated regions were not quantitatively evaluated. Thus, the identification of an intergenomic translocation common to *A. sativa* 'Sun II' and *A. byzantina* 'Kanota' is taken with caution.

With respect to the A-genome chromosomes (Tables 1, 2), one A–C intergenomic translocation involving chromosome 5A was identified in the S-monosomic lines; this same chromosome was also possibly involved in one intergenomic translocation with D-genome chromatin in 'Kanota'. Moreover, an A–D intergenomic translocation involving the 6A chromosome was found only in the S-monosomic lines. The other three A–D translocations involving chromosomes 18A, 19A and 20A were shared by the two species.

Most of the translocations detected in the C-genome chromosomes appear to be common to both species (Tables 1, 2). Although chromosome 16C of *A. byzantina* showed D-genome chromatin in the terminal portion of both arms, this type of chromatin was only present in the long arm of chromosome 16C in *A. sativa*.

The 3D chromosome showed a different FISH pattern in the two aneuploid series. It did not appear to bear any translocation in 'Kanota', but it showed a D–C translocation in 'Sun II' and its derived lines. Moreover, a large reciprocal translocation involving chromosomes 10C and 14D was found in some 'Sun II' monosomic lines and one involving

16C and 14D was found in 'Kanota' lines. While the breakpoint of the translocation lies within the centromeric region in the 14D chromosome of 'Sun II', in 'Kanota' it carries C-genome chromatin on its long arm.

Discussion

Monosomic series are a valuable source of individual chromosomes that can be used to localize molecular markers on chromosomes, and to identify homoeologous chromosome sets. However, the direct exploitation of these aneuploids requires detailed knowledge of the cytogenetic structure of monosomic series and such knowledge can be gained using a molecular cytogenetic approach as demonstrated here. In the present study molecular probes coupled with FISH analyses were successfully used to determine the cytogenetic identities of the 'Sun II' monosomic lines derived from *A. sativa*, and those of 'Kanota' monosomic lines derived from *A. byzantina*. The paucity of useful FISH markers for analyzing oat chromosomes did not hamper a clear picture of each single chromosome of the *A. sativa* and *A. byzantina* chromosome complements being obtained. The discussion of the present results focuses on the following points: (1) the detection and significance of the intergenomic translocations found; and (2) the proposal of a new nomenclature system for individual oat chromosomes.

Detection of intergenomic translocations

Rajhathy and Thomas (1974) indicate that chromosome rearrangements such as translocations probably represent a major genomic divergence mechanism in allopolyploid oat evolution. In hexaploid oat species, evidence to support this has come from in situ hybridization experiments using genomic DNA (Chen and Armstrong, 1994; Jellen et al. 1994; Leggett and Markhand 1995; Yang et al. 1999; Hayasaki et al. 2000; Ueno and Morikawa 2007), C-genome-specific sequences (Fominaya et al. 1995) and repetitive DNA sequences (Linares et al. 1996, 1998, 2000, 2001). The present results show that most of the chromosomes of the two cultivars studied have segments translocated from chromosomes of other genomes.

The presence of intergenomic chromosome translocations in nature is particularly interesting in the context of polyploid evolution since they represent new genomic arrangements that are possible only once a polyploid appears. Three hypotheses may be invoked to explain the different types of intergenomic translocations seen in the *A. sativa* cv 'Sun II' and *A. byzantina* cv 'Kanota' hexaploid species. First, some `common translocations' should be present in homologous chromosomes among all the closely related hexaploid oat species: *A. byzantina, A. fatua, A. sativa* and *A. sterilis*. In the present work, 12 intergenomic translocations were detected in *A. sativa* and *A. byzantina* when using pAs120a and pAm1 as probes in FISH experiments: three A–D (chromosomes 18A, 19A and 20A), six C–D (chromosomes 1C, 2C, 4C, 7C, 15C and 16C) and three D–C (chromosomes 9D, 11D and 13D) translocations. It might, therefore, be postulated that these translocations were present in the ancestor species from which *A. sativa* and *A. byzantina* were domesticated—most likely weedy *A. sterilis*. Although several intergenomic translocations have been detected in hexaploid species by GISH (Chen and Armstrong 1994; Jellen et al. 1994; Leggett and Markhand 1995; Yang et al. 1999) and FISH (Fominaya et al. 1995; Linares et al. 1996, 1998, 2000, 2001), the identity of chromosomes with translocated segments can be unclear because the chromosome nomenclature used is different in these different reports. The use of a more robust naming system for the identification of the oat chromosomes, as proposed in the present work, would help address this problem.

Second, 'species-specific' translocations should also be present. These would involve certain chromosomes and should be exclusive to *A. byzantina* or, alternatively, *A. sativa* to be deemed species-specific. These rearrangements would have been specific to the different populations of *A. sterilis* in which the "domestication mutations" presumably occurred to give rise to *A. byzantina* and *A. sativa*. One example of this might be the 7C-17 (16C-5A in the present study) translocation described by Jellen and Beard (2000), which was predominantly found in *A. sativa*, but not in *A. byzantina*. This rearrangement was also present in 70–80% *A. sterilis* populations (Zhou et al. 1999) and in essentially all populations of *A. fatua* (E. Jellen, personal communication).

A third group of translocations, those that are `cultivar-specific′, would also be expected. This is especially true in autogamous *Avena* species. True breeding, translocation-fixed genotypes would not be expected to have a negative effect on population fitness since the appearance of semi-sterile translocation heterozygotes would be a rare phenomenon.

There are at least two possible explanations for the presence of abundant translocations in allopolyploid genera like *Avena.* Gill (1991) proposed the 'nucleocytoplasmic interaction (NCI) hypothesis' by which a newly formed polyploid must pass through a 'bottleneck' of sterility resulting from the adverse interaction between the male nuclear genome and the nuclear and cytoplasmic genomes of the female parent. Certain bottleneck (species-specific) chromosomal changes must occur in the nuclear genome to restore fertility and nucleocytoplasmic compatibility.

A second mechanism leading to "species-specific" types of translocations in oat might involve homoeologous, or potentially even random, chromosome associations and exchanges during polyhaploid meioses—such as occur either in spontaneous haploids or in the non-doubled amphihaploid in the initial generation of the novel allopolyploid and such non-homologous chromatin exchanges—including reciprocal translocations—would then be subject to fixation within relatively few generations via genetic drift. This phenomenon has been observed indirectly, via cytological characterization of oat monosomics derived from haploids, as the appearance of unique intergenomic rearrangements. For example, Jellen et al. (1993b) identified a unique 7C-14 (16C-14D in the present study) reciprocal translocation in five of the 'Kanota' monosomic lines and a 3C-14 reciprocal translocation (10C-14D in the present study) found in 15 of the original lines of 'Sun II' monosomics (Jellen et al. 1997; Irigoyen et al. 2002). We have also recently identified a unique A–D reciprocal translocation in a new set of 'Sun II' monosomics from haploids derived by oat × maize hybridization (A. Fominaya, E. Jellen and H. Rines, personal communication).

By physically mapping two highly repetitive DNA sequences, two intergenomic translocations, on chromosomes 5A and 3D, were found only in monosomic genotypes of *A. sativa* (Linares et al. 1998, 2000; Irigoyen et al. 2002). This observation is particularly interesting for 5A, which corresponds to 17 in the nomenclature system of Jellen et al. (1993a) and was previously shown by C-banding to carry the 7C translocation segment (mentioned above) in 77 out of 79 (97%) *A. sativa* accessions (Jellen and Beard 2000).

Duplicate monosomic lines and a proposed chromosome nomenclature

It has been suggested on the basis of C-banding (Jellen et al. 1993b; Rooney et al. 1994; Jellen et al. 1997) and RFLP analysis (Rooney et al. 1994; Fox et al. 2001) that some monosomic lines of the 'Sun II' series and some lines of the 'Kanota' series may be duplicates. The results

Table 3 Comparison of the monosomic lines derived from both the 'Sun II'/maize haploids and 'Kanota' and the chromosome missing in each

Genome	Aneuploid chromosome (Linares et al. 1998)	S-monosomic-lines	Kanota-monosomic lines	Sun II monosomic lines (Irigoyen et al. 2002)
A	5A	S-monosomic-16	K11	SVI
	6A	S-monosomic-11	K17; K19	SI
	8A	S-monosomic-8	K6	SXV
		S-monosomic-15		
	12A	S-monosomic-19	–	SXII
		S-monosomic-20		
	18A	–	K5; K10; K15; K20	SXVI; SXVII; SXVIII
	19A	–	K18	–
	20A	–	K9; K13; K16	–
C	1C	S-monosomic-5C	K8	–
	2C	–	K1; K2	–
	4C	S-monosomic-2C	K4	–
	7C	S-monosomic-4C	–	SIV
	10C	S-monosomic- $3C^{14}$	–	SXI
	15C	S-monosomic-1C	–	SVII; SXIV
		S-monosomic-6C		
	16C	S-monosomic-7C	–	-
D	3D	–	K3	–
	13D	S-monosomic-21	–	SVIII; SIX; SX
	9D	S-monosomic-10 S-monosomic-12	–	SV
	11D	–	–	–
	14D	–	–	–
	17D	S-monosomic-9	K7; K14	–
	21D	S-monosomic-18	K21	SII; SIII; SXIII

Roman numerals refer to previously characterized 'Sun II' aneuploids (Irigoyen et al. 2002). Chromosomes are numbered according to Linares et al. (1998)

described in this paper confirm this hypothesis. The S-monosomic lines involve only 13 distinct lines (Table 1), while the 'Kanota' monosomic series analyzed has only 12 distinct lines (Table 2). Among the S-monosomic lines, four were of the A-genome (S-monosomic-8/S-monosomic-15, S-monosomic-11, S-monosomic-16 and S-monosomic-19/S-monosomic-20); five of the C-genome (S-monosomic-1C/S-monosomic-6C, S-monosomic-2C, S-monosomic-4C, S-monosomic-5C and S-monosomic-7C); and four of the D-genome (S-monosomic-9, S-monosomic-10/S-monosomic-12, S-monosomic-18 and S-monosomic-21). Among the monosomic lines of the 'Kanota' series, six lines were of the A-genome (K5/K10/K15/K20, K6, K9/K13/K16, K11, K17/K19 and K18); three of the C-genome (K1/K2, K4 and K21); and three of the D-genome (K3, K7/K14 and K21). However, some authors who have studied these lines report discrepancies in the identification of duplicate monosomic lines. Thus, S-monosomic-8 and S-monosomic-15 were originally described as containing different monosomes; the same was true for S-monosomic-1C and S-monosomic-6C (Jellen et al. 1997). Similarly, all the 21 chromosomes originally were reported to be represented as monosomics in the 'Kanota' monosomic series (Linares et al. 1992), as deduced by C-banding analysis. There are at least three possible explanations for these discrepancies. The first involves the different origin of the monosomic lines analyzed. The duplicated monosomic lines identified in the present paper correspond to new monosomic lines produced in the 'Sun II' genetic background using derivatives of haploids generated by oat × maize crossing techniques. The second involves the different technique used in the identification of the monosomes. The C-banding technique used in the studies mentioned above allows identification of most of the individual chromosomes of the A and D genomes, but does not permit assignment of chromosomes to A versus D. The use of A- and C-genome-specific repetitive DNA sequences in the present work clearly distinguishes chromosomes and allows the monosomic lines to be assigned the A, C or D genomes. The third hypothesis explaining confounded chromosome identifications within the two series involves monosomic (univalent) shifts (Khush 1973).

In this report, the identification of the aneuploid chromosome of each monosomic line in both the S-monosomic and 'Kanota' series was undertaken using the same four repetitive DNA sequences as probes in FISH. This approach has also been followed in the identification of the aneuploid chromosome in another set of 11 'Sun II' monosomic lines (Irigoyen et al. 2002). A comparison of the three monosomic series can therefore be made on the basis of their respective shared, or unique, monosomes; consequently there appear to 19 chromosome pairs are represented by monosomes between the 2 genetic backgrounds (Table 3).

Table 4 Proposed chromosome nomenclature for numbering the hexaploid complement, taking into account the chromosome designations used in the present study and the C-banding-based designations of Jellen et al. (1997)

Genome	Chromosome (Linares et al.1998)	Chromosome (Jellen et al. 1997)
A	5A	17
	6A	11
	8A	8
	12A	19
	18A	15
	19A	16
	20A	13
C	1C	5C
	2C	1C
	4C	2C
	7C	4C
	10C	3C
	15C	6C
	16C	7C
D	3D	20
	13D	21
	9D	12
	11D	10
	14D	14
	17D	9
	21D	18

Although an intact chromosome 14D is not represented in either of the two monosomic series, its identity can be deduced from the reciprocal intergenomic translocation 10C-14D. Also, the identity of the chromosome 11D can be deduced from the presence of a C-segment translocated. Molecular markers should be used to confirm the cytological work and will likely identify additional smaller intergenomic translocations.

The FISH analyses of monosomic series of two hexaploid species of *Avena* genetic backgrounds reported in this paper allow a new nomenclature system to be proposed for numbering the 21 chromosome pairs of the hexaploid complement (Table 4; Fig. 2). This system is based on the chromosome number deduced in C-banding analyses from karyotype of *A. sativa* cv 'Sun II' (Jellen et al. 1997), along with genome allocation deduced from FISH analyses using satellite sequences specific to either the A or C-genome chromosomes of *A. sativa* cvs 'Prevision' (Linares et al. 1998) and 'Sun II' (Irigoyen et al. 2002). Consequently, the A-genome contains chromosomes 8A, 11A, 13A, 15A, 16A, 17A and 19A. The C-genome contains chromosomes 1C, 2C, 3C, 4C, 5C, 6C and 7C. Finally, the D-genome contains chromosomes 9D, 10D, 12D, 14D, 18D, 20D and 21D.

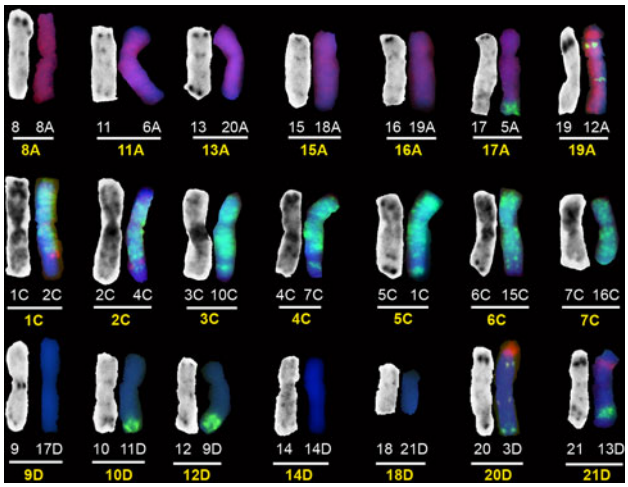

Fig. 2 Karyotype of hexaploid oat (*A. sativa* cv 'Sun II') with C-banded and the FISH-probed chromosomes. *Each panel* of chromosomes is numbered according to Jellen et al. (1997) for C-banding patterns (*left*), Linares et al. (1998) for FISH patterns (*right*), and the uniform nomenclature system proposed in the present paper (*under the line*). The seven A-genome chromosomes show the FISH pattern of biotin-labeled pAs120a (*red*). Chromosome 19A presents hybridization signals with both biotin-labeled pITS (*red*) and digoxigenin-labeled pTA794 (*green*) probes. Chromosome 17A shows hybridization signal with digoxigenin-labeled pAm1 (*green*). The seven C-genome chromosomes show the FISH pattern of digoxigenin-labeled pAm1 (*green*). Chromosomes 1C and 2C show hybridization signals with biotin-labeled pTa794 (*red*). Four D-genome chromosomes, namely, 10D, 12D, 20D and 21D show hybridization signals with digoxigenin-labeled pAm1 probe (*green*). Moreover, chromosome 20D shows hybridization signals with both biotin-labeled pITS (*red*) and digoxigenin-labeled pTa794 (*green*). Chromosome 21D shows hybridization signal with biotin-labeled pITS (*red*) probe

This new, uniform nomenclature system should be useful in oat cytogenetics, facilitating the identification of homeologous relationships among the three chromosome sets in hexaploid oat.

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