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Identification of homoeologous chromosomes in hexaploid oat (*A. byzantina* cv Kanota) using monosomics and RFLP analysis

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Abstract The use of RFLP markers, together with a partial set of monosomics available in *Avena byzantina* cv Kanota, has enabled us to identify putative homoeologous chromosome sets in hexaploid *Avena* species (2n = 6x = 42, AACCDD). We first identified probes producing distinct three-band patterns on Southern blots that possibly reflect orthologous loci of the three genomes present in the hexaploid. Using monosomic analysis, 51 different restriction fragments that hybridized to 26 probes were localized to 12 different chromosomes for which monosomic stocks were available. These DNA restriction fragments were localized to specific monosomics using image analysis to quantify band intensity relative to other bands in the same lane. From these data, we have tentatively identified two complete homoeologous sets of three chromosomes each and two partial sets of two of the three chromosomes. The results indicate that RFLP dosage analysis is useful in the characterization of homoeologous chromosomes in hexaploid oat where nullisomics for many of the chromosomes are not available.

Key words *Avena byzantina* · *A. sativa* · RFLP · Homoeology · C-banding · Aneuploids · RFLP mapping

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Introduction

Attempts to identify the homoeologous chromosome sets in polyploid oat (*Avena* spp.) have been largely unsuccessful. Hexaploid cultivated oats (*A. sativa* L. and *A. byzantina* C. Koch) have a cytogenetic structure and genome size comparable to bread wheat (*Triticum aestivum* L.), yet the sets of homoeologous chromosomes in bread wheat have been known for nearly 40 years. This lag in identifying homoeologous chromosomal relationships in oat is due to the inability to develop comprehensive sets of cytogenetic stocks and the lack of suitable genetic markers.

Cytogenetic stocks of various types have been used to identify homoeologous chromosomes in wheat and wheat-related species (*Triticeae*). Sears (1966) used nullisomic-tetrasomic aneuploid stocks to determine if extra copies of a specific chromosome would compensate for the absence of another. When a tetrasomic chromosome could phenotypically complement a nullisomic, the nullisomic and tetrasomic chromosomes were deemed to be homoeologous. With this method, Sears classified all seven homoeologous groups of chromosomes in hexaploid wheat. Rajhathy and Thomas (1974) demonstrated that nullisomic-tetrasomic compensation could occur in *A. sativa*, but the chromosomes involved in the aneuploids could not be identified due to similarities in morphology within the oat chromosomal complement (Rajhathy 1963). Ansari and Thomas (1983) used a set of aneuploids in the oat cultivar 'Sun II' to show that Sun II chromosomes VI and XIII would compensate for each other in appropriate nullisomic-tetrasomic combinations. Their results indicated that these chromosomes were homoeologous; our results confirm this interpretation. However, an incomplete aneuploid series in oat, and the inability to produce the

needed aneuploid combinations, have hindered further research in this area.

An alternative method of identifying homoeologous chromosome sets is the use of genetic markers. Isozyme markers were valuable in the cytogenetic confirmation of homoeologous sets in hexaploid wheat (Hart 1987). More recently, RFLP technology has facilitated extensive genetic studies of homoeology. Liu et al. (1992) used RFLP maps to characterize several intergenomic translocations in hexaploid wheat. In hexaploid *Avena*, the use of classical genetic markers is limited by their small numbers and the lack of genetic linkage information. McGinnis (1966) proposed that three independent chlorophyll-synthesis genes were on chromosomes constituting a homoeologous group although none of the mutants were ever located to a chromosome. MacKenzie et al. (1970) determined that genes for seedling resistance to stem rust were located in three linkage groups. Based on these results they speculated that these linkage groups may constitute a homoeologous set although no chromosomal associations were made.

The combination of aneuploidy and RFLP techniques in the hexaploid cereals provides a powerful means of localizing genes to chromosomes and identifying the homoeologous chromosome sets (McIntosh 1987). The initial assignment of chromosomes to a homoeologous set can be confirmed as RFLP linkage maps are completed for the crop. These linkage maps will also be useful for identifying translocations that have occurred in the hexaploid species.

In theory, the use of aneuploids to localize RFLP markers to chromosomes, and chromosomes to homoeologous sets, is simple. If an allohexaploid has no duplications or deficiencies for a DNA sequence complementary to the RFLP probe, then a probe representing a DNA sequence normally present in a single copy per genome should detect from one to three bands when there are no restriction sites within the target sequence. If there are no chromosomal interchanges present, these sequences will usually map to a homoeologous chromosome set, although probes mapping to non-homoeologous loci have been identified in wheat (Anderson et al. 1992). If a complete set of nullisomics were probed with such a sequence producing three bands with euploid stocks, the three nullisomics each missing a different band would reflect a homoeologous set. Sharp et al. (1989) used compensating nullisomic-tetrasomic and ditelocentric stocks to identify a set of 14 RFLP probes that mark each chromosome arm in the hexaploid wheat genome.

The use of monosomics to identify homoeologous sets of chromosomes is similar to the procedure used for nullisomics. Gale et al. (1988) described the potential usefulness of monosomes for localizing probes to chromosomes. In the monosomic condition a band will not disappear, but theoretically will have only one-half the intensity of the two disomic bands in the same lane of the gel. Since determining the absence versus presence of a band is easier than scoring reduced intensity, it is more desirable to use nullisomics than monosomics if a choice is available. However, a complete set of nullisomics in the hexaploid oat is not available, and attempts to produce such a set have been unsuccessful. Morikawa (1985) reported the production of a complete set of 21 monosomic lines in *A. byzantina* cv Kanota. More recently, Jellen et al. (1993a) determined that only 12 of the 21 potential monosomics are actually present in this series. Nevertheless, the partial set of monosomics is useful to localize sequences to chromosomes and to identify homoeologous chromosomes. This information simplifies hexaploid oat RFLP mapping efforts.

The objectives of the present study were: (1) to develop a method of quantifying signal intensity to determine the chromosomal location of an RFLP marker by dosage analysis in a monosomic, (2) to localize RFLP sequences to chromosomes, (3) to identify homoeologous sets of chromosomes in 'Kanota', and (4) to correlate the homoeologous sets of markers with their physical (C-banded) chromosome.

Materials and methods

Aneuploid sources

A set of 21 monosomics in 'Kanota' was supplied by Dr. T. Morikawa from the University of Osaka Prefecture, Saikai, Osaka, Japan (Morikawa 1985). Recent analysis of the Kanota monosomic series using RFLP and C-banding showed that the monosomics series is not complete; only 12 of the 21 possible monosomics are actually present (Jellen et al. 1993a). From the 21 monosomic stocks, nullisomics were derived from three of the lines, specifically monosomics K8, K19, and K21. In addition, each monosomic line was crossed to *A. sativa* cv 'Ogle'. The complete set of monosomics, the three nullisomics, and the F_1 progeny of the monosomic × Ogle crosses were grown in growth chambers under a 20 °C day/15 °C night with 12-h days for the first 4 weeks and 16-h days thereafter. Microsporocytes were collected from individual plants to confirm monosomy. Monosomics were identified either by cytogenetic analysis of diakinesis or the presence of a high frequency of micronuclei in the microspore quartet stage.

RFLP analysis

For RFLP analysis, plants from all sources were grown in a greenhouse or growth chamber. From each line, leaves and stems from 4- to 5- week-old seedlings were harvested, freeze-dried, mechanically ground, and stored at − 20 °C until used for DNA extraction. DNA was extracted using a modified version of the technique described by Saghai-Maroof (1984). Restriction enzyme digestions were made using one of the following enzymes: *Eco*RI, *Eco*RV, *Dra*I, *Bam*HI, or *Bgl*II. Restricted DNA was electrophoresed for 24 h at 30 V in an 0.8% agarose gel. Southern blotting was completed using 10 × SSC onto Immobilon-N membrane. Probes were radioactively labelled using random-priming (Feinberg and Vogelstein 1984) and hybridizations were done in capped plastic tubes in a roller oven at 65 °C. Post-hybridization washes were as follows: 2 × SSC, 1% SDS for 30 min and 0.1 × SSC, 0.1% SDS twice for 30 min. Autoradiographs were exposed to Kodak XAR X-ray film for 1–4 days in exposure containers with intensifying screens at − 70 °C.

The probes for RFLP analysis were primarily from three different sources. Single-copy oat *Pst*I genomic probes (denoted with an OG prefix) and cDNA probes (denoted with a CDO prefix) from an etiolated-leaf cDNA library were provided by Dr. M. E. Sorrells of Cornell University. An oat endosperm cDNA library, characterized at the University of Minnesota, was the third source of RFLP probes (denoted with a UMN prefix).

Probes to test on DNA from monosomics were selected based on hybridization patterns to screening blots containing restricted DNA from the cultivars Kanota and Ogle. The probes selected for analysis produced three clear and distinct bands in Kanota with little or no background hybridization. If there was a polymorphism between Kanota and Ogle, the F_1 monosomic progeny from a cross of each Kanota monosomic × Ogle were screened with the probe to detect the chromosomal location of the sequence by absence of the polymorphic Kanota band in monosomic F_1 progeny (Helentjaris et al. 1986).

Image analysis and densitometry

To detect the bands where signal intensity was reduced, band intensity was measured using Image 1.43, a software program developed for image enhancement and manipulation techniques on the Macintosh computer. Image 1.43 is available through the National Institutes of Health from the developer, W. Rasband. This program assigns a gray value from 0 (white) to 255 (black) to each pixel composing the image. Short-term exposure autoradiograms were optically scanned into the Macintosh IIx computer using a 300 dpi Apple Scanner with the AppleScan software. Images of blots were saved as PICT files and opened in Image 1.43. A densitometry estimate for each band is based on the average intensity of all pixels within the delineated band. To account for DNA loading variation, three relative intensity ratings (RIR) were created for each band with each probe (Table 1). These ratings were obtained by sequentially using each band as a standard (assigned an RIR of 1.0) and dividing the densitometer estimate of each of the other two bands by the densitometer estimate of the standard band (Tables 1–2). Thus, the

Table 1 Generation of relative intensity ratings (RIRs) to localize DNA fragments to which probe OG 41 hybridizes to selected monosomic chromosomes. (A) = densitometric readings for probe OG 41 hybridized to DNA from ten monosomics. (B), (C), and (D) = the standardization and RIRs for bands 1, 2, and 3, respectively. Chromosomal location is indicated when the RIRs of the two tester bands were consistently larger than the average value of the standard band. Bold, italicized numbers indicate chromosomal location

Item	Kanota monosomic number										Average
	K1	K3	K6	K7	K8	K14	K16	K17	K19	K20	
(A)[a]											
Band 1	172	162	121	78	82	186	167	118	172	117	
Band 2	200	181	169	130	110	231	211	111	194	156	
Band 3	120	154	136	110	86	196	206	141	186	148	
(B)											
Band 1 (standard)	1.0	1.0	1.0	***1.0***	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Band 2	1.2	1.1	1.4	***1.7***	1.4	1.3	1.2	1.1	1.1	1.3	1.3
Band 3	0.7	0.9	1.1	***1.4***	1.1	1.1	1.2	1.2	1.1	1.3	1.1
(C)											
Band 1	0.9	0.9	0.7	0.6	0.7	0.8	0.8	***1.1***	0.9	0.8	0.8
Band 2 (standard)	1.0	1.0	1.0	1.0	1.0	1.0	1.0	***1.0***	1.0	1.0	1.0
Band 3	0.6	0.9	0.8	0.8	0.8	0.9	1.0	***1.3***	1.0	0.9	0.9
(D)											
Band 1	***1.5***	1.0	0.9	0.7	0.7	0.9	0.8	0.8	0.9	0.8	0.9
Band 2	***1.7***	1.2	1.2	1.2	1.3	1.2	1.0	0.8	1.0	1.1	1.2
Band 3 (standard)	***1.0***	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

[a] The RFLP pattern was created using the restriction enzyme *Eco*RV

Table 2 Relative intensity ratings (RIRs) calculated from the densitometry data presented in Fig. 1A for probe UMN 249 hybridized to *Dra*I-restricted genomic DNA. Readings are presented from two monosomic plants from each line to demonstrate consistency of results. (A), (B), and (C) = the standardization and RIRs for bands 1, 2, and 3, respectively. Chromosomal location is indicated by the bold, italicized numbers. Band 1 was localized to K6 (data not shown)

Item	Kanota monosomic number										Average
	K9	K9	K10	K10	K11	K11	K12	K12	K13	K13	
(A)											
Band 1	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Band 2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	1.2	0.8	0.8	1.1
Band 3	0.9	0.9	1.0	1.0	1.2	1.3	1.3	1.3	1.2	1.3	1.1
(B)											
Band 1	0.8	0.8	0.9	0.9	0.8	0.8	0.8	0.8	***1.3***	***1.2***	0.9
Band 2	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	***1.0***	***1.0***	1.0
Band 3	0.8	0.7	0.9	0.9	1.0	1.1	1.0	1.1	***1.6***	***1.6***	1.1
(C)											
Band 1	***1.1***	***1.1***	1.0	1.0	0.8	0.8	0.8	0.8	0.8	0.8	0.9
Band 2	***1.3***	***1.4***	1.1	1.1	1.0	0.9	1.0	0.9	0.6	0.6	1.0
Band 3	***1.0***	***1.0***	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0

two alternative bands were used as internal standards for the band being measured. RIR results were then compared across all monosomics. To assign a sequence to a chromosome, the RIR of the other two bands used as standards had to be higher than the RIR average across the bands for the other monosomics. Each test was repeated on two different monosomic plants from each Kanota aneuploid line (Table 2), and a chromosomal location is reported only when the results were consistent across both tests.

C-banding and chromosomal analysis

Root tips were collected from at least ten seedlings of each of the 21 Kanota monosomic lines. Chromosome preparation methods and the C-bandling protocol using Wright's stain were as described in Jellen et al. (1993b).

Results and discussion

Densitometry methods

The densitometric measurement procedure described in Materials and methods proved to be a useful technique for quantifying signal intensity differences on blots involving DNA from oat monosomics. Analysis with radioanalytical scanners was also attempted; this method was accurate, but time constraints limited its practical usefulness. Probes that hybridized to three sequences of different lengths and produced little or no background hybridization were the most compatible with the system used. If the probe produced more than three bands, the extra bands could be due to internal restriction sites or duplicate copies of the sequence within one of the three genomes. Conversely, if a probe produced fewer than three distinct bands, the most likely causes were restriction-site homology in the homoeologues or loss of the complementary sequence from one or two of the homoeologues. Because the actual reason for the variable number of bands was not always obvious, only probes that produced three bands were included in this study. Even then, caution must be used in interpreting three-band patterns as representing three homoeologous chromosomes because the patterns could result either from absence of an homologous sequence in one genome while the probe hybridizes to two sequences in one genome and a third sequence in a second genome or else be due to hybridization to sequences in non-homoeologous chromosomes.

In some cases, independent confirmation of monosomic assignments was possible. When a sequence was localized to a chromosome where the nullisomic was available, the probe was screened against the nullisomic. Assignments were confirmed when the same fragment that produced a band of reduced intensity with the monosomic was absent in the nullisomic. A second method of confirmation was the use of monosomic polymorphism tests (Helentjaris et al. 1986). If a band produced by a probe was polymorphic between Kanota and Ogle, then the assignment of a band to a monosome was possible. The monosomic F_1 progeny of the appropriate crosses were screened and an absence of the Kanota band for the same monosomic confirmed the original assignment (Fig. 1b). Even in cases where the homoeologous sets of chromosomes could not be identified, the method was useful in localizing sequences to syntenic groups. Of 51 chromosome assignments made by monosomic analysis, 13 were confirmed using nullisomics and seven were confirmed using monosomic polymorphisms.

In the present study, probes were derived from three sources consisting of two cDNA libraries and an oat genomic library. From these sources, approximately 150 probes were screened to identify suitable candidates that met the requirements for identifying homoeologous chromosome sets. In total, 26 probes were used to localize sequences to homoeologous chromosome sets in the Kanota monosomic series. In localizing sequences to chromosomes, all three libraries supplied useful probes to the mapping effort.

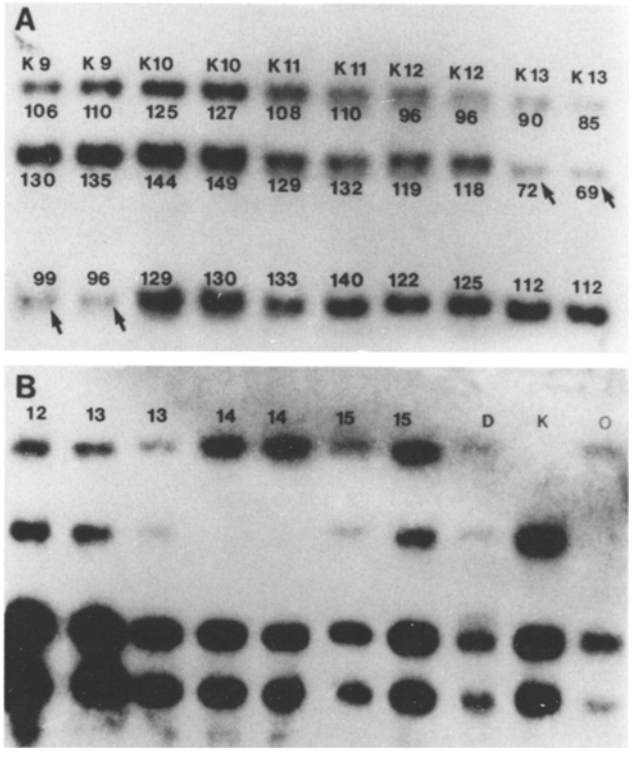

Fig. 1A–B Autoradiogram of probe UMN 249 hybridized to Kanota monosomic series DNA restricted with *Dra*I. Two monosomic plants from each monosomic line are presented to demonstrate consistency of results. Monosomics *K9 through K13* are included. Next to each band is the densitometric intensity of the band as measured by direct scanning of the autoradiograms. *Arrows* indicate the restriction fragment whose chromosomal location has been determined. Relative intensity ratings for these data are in Table 2. **B** Autoradiogram of probe UMN 114 hybridized to DNA of monosomic F_1 progeny from the crosses of Kanota (*K12 through K15*) monosomic series with Ogle. When hybridized to Kanota (*K*) and Ogle (*O*) DNA restricted with *Dra*I, UMN 114 is polymorphic for a 9.0-kb fragment in Kanota. This fragment is absent in K14 × Ogle F_1 monosomic progeny. (*12–15* = F_1 monosomics of Kanota monosomic × Ogle, *D* = F_1 plant of disomic Kanota × Ogle)

Identification of homoeologous sets of chromosomes

A total of 51 different restriction fragments hybridizing to 26 probes were localized to the 12 different and unique monosomic chromosomes (Table 3, Fig. 2). RFLP markers were assigned to all available aneuploids but, because the monosomic series was incomplete, not all sequences identified by RFLP probes could be assigned to chromosomes. As stated previously, each of these probes produced a hybridization pattern composed of

Table 3 Syntenic sequences recognized by RFLP probes hybridized to the 12 unique monosomics in the hexaploid *A. byzantina* cv Kanota

Homoeologous set	Monosomic chromosome	RFLP markers to monosome	Fragment size (kb)	Restriction enzyme
1	1C[a] (K1, K2)	OG 41	5.0	*Eco*RV
		pzc 3[b]	4.0	*Eco*RI
		CDO 77	3.5	*Eco*RI
		CDO 99	16.0	*Eco*RI
		UMN 34	12.0	*Hin*dIII
		UMN 28	4.4	*Eco*RI
1	14 (K7, K13)	OG 41[d]	20.0	*Eco*RV
		pzc 3[b]	5.0	*Eco*RI
		CDO 77	7.0	*Eco*RI
		CDO 99	8.0	*Eco*RI
		UMN 34	8.0	*Hin*dIII
		UMN 28	2.0	*Eco*RI
		UMN 249	5.0	*Dra*I
		pOGL 1[c]	3.0	*Bgl*II
1	17 (K11, K17)	OG 41[d]	9.0	*Eco*RV
		pzc 3[b]	8.0	*Eco*RI
		CDO 77	8.5	*Eco*RI
		CDO 99	5.0	*Eco*RI
		UMN 34	6.0	*Hin*dIII
		UMN 28	3.0	*Eco*RI
		pOGL 1[c]	6.7	*Bgl*II
2	2C (K3, K4)	UMN 41	9.0	*Eco*RI
		UMN 44	9.5	*Eco*RV
		UMN 71	17.0	*Eco*RI
		CDO 348	20.0	*Eco*RV
2	15 (K5, K10, K15, K20)	UMN 41	7.0	*Eco*RI
		UMN 44	9.5	*Eco*RV
		UML 71	20.0	*Eco*RI
2	11 (K19)	UMN 41[e]	4.0	*Eco*RI
		UMN 44[e]	8.0	*Eco*RV
		UMN 71[e]	12.0	*Eco*RI
		OG 49[e]	8.0	*Hin*dIII
		CDO 348[e]	8.0	*Eco*RV
		UMN 114[e]	5.0	*Dra*I
3	16 (K18)	OG 19[d]	5.0	*Eco*RI
		UMN 145	3.0	*Eco*RI
		CDO 113	12.0	*Dra*I
3	18 (K21)	OG 19[e]	7.5	*Eco*RI
		UMN 145[e]	6.0	*Eco*RI
4	8 (K6)	OG 110	9.5	*Eco*RV
		OG 176	2.0	*Eco*RV
		UMN 249	6.7	*Dra*I
4	13 (K9, K16)	OG 110	20.0	*Eco*RV
		OG 176[d]	4.4	*Eco*RV
		UMN 249[d]	2.3	*Dra*I
		pOGL 1[c]	9.4	*Bgl*II
Unknown	3C (Sun II-XI)	UMN 145[e]	12.0	*Eco*RI
Unknown	5C (K8)	CDO 59[e]	5.1	*Eco*RV
		CDO 370[e]	4.1	*Eco*RI
		UMN 51[e]	10.0	*Dra*I
Unknown	19 (K12, K14)	UMN 114[d]	13.0	*Dra*I
		OG 49[d]	20.0	*Eco*RV

[a] Monosomic chromosome designation is based on Jellen et al. (1993a). The monosomic lines missing the respective chromosome are given in the parentheses
[b] pzc3 is a cDNA clone assigned to chromosome in wheat by Jellen (1992)
[c] pOGL is a full-length cDNA clone of (1–3, 1–4)-beta-glucanase (Yun 1992)
[d,e] Represent a monosomic chromosomal assignment that was confirmed using monosomic F_1-polymorphisms (d) or nullisomics (e)

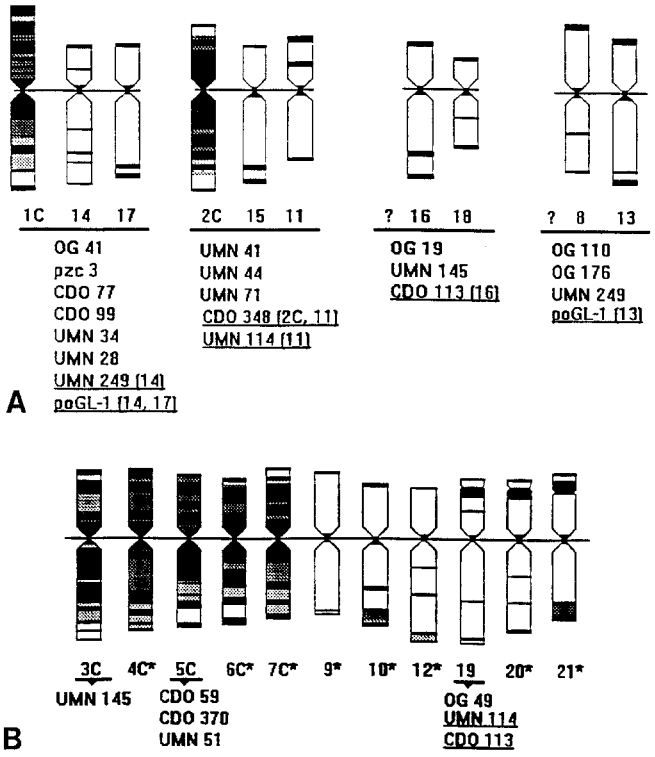

Fig. 2A–B Sets of homoeologous chromosomes identified in the current study and their karyotype based on C-banding. The numerical designation below each chromosome identifies the chromosome based on karyotype (Jellen et al. 1993a) and the listed RFLP probes indicating homoeology. **B** C-banded karyotypes of chromosomes that were not assigned to homoeologous sets. RFLP probes listed below chromosomes 3C, 5C and 19 detected fragments in these chromosomes. The remaining chromosomes are not available as aneuploids in the current monosomic series. Fragments (or sequences) detected by *underlined* RFLP probes did not conform to the predicted pattern of homoeology and the defined chromosomal location of these fragments is in *parentheses*

three bands. We assumed that the three bands represented different alleles, each located on a different but homoeologous chromosome. Our results were consistent with our assumption in that six probes hybridized to restriction fragments on the putative homoeologues 1C, 14, 17, and at least two probes hybridized to fragments for each of the other putative homoeologous sets (Table 3, Fig. 2).

Based on these results, these 26 probes tentatively identified two complete sets of homoeologous chromosomes and 2/3 of two additional homoeologous sets. The homoeologous sets of chromosomes are as follows, using the generic hexaploid oat numbering system described by Jellen et al. (1993a): 1C, 14, 17; 2C, 11, 15; 16, 18; and 8, 13. Chromosome 3C is monosomic in the Sun II aneuploid series and one RFLP marker identified by probe UMN 145 was localized to this chromosome, indicating that at least part of 3C may be homoeologous to chromosomes 16 and 18 (Table 3, Fig. 2). However, only one RFLP marker has produced these results and confirmation with additional probes is necessary prior to assignment of the chromosome to a homoeologous set.

C-banding of mitotic root-tip cells provides a second form of testing the correspondence of homoeologous chromosomes. Jellen et al. (1993a) reported that each chromosome in the cultivar Kanota can be identified using C-banding. Previous reports (Fominaya et al. 1988; Jellen et al. 1993a) have indicated that the C genome is darker staining than either the A or D genome. Since a homoeologous set of chromosomes is composed of a single chromosome from the A, C, and D genomes, a single dark-staining chromosome would be expected within each of the homoeologous sets of chromosomes. Both of the putatively complete homoeologous sets have a single dark-staining chromosome; however, the dark-staining chromosome in the remaining two sets is unidentified because the monosomics for those chromosomes are not available in the Kanota monosomic series (Jellen et al. 1993a).

The sets of homoeologous chromosomes identified here are in agreement with the limited data collected previously pertaining to homoeologous chromosome sets in oat. Ansari and Thomas (1983) reported successful nullisomic-tetrasomic compensation of Sun II nullisomic VI with Sun II tetrasomic XIII. RFLP analysis has shown that Sun II nullisomic VI and Sun II nullisomic XIII are missing the respective chromosomes 16 and 18 (W. L. Rooney and S. F. Kianian, unpublished data). These two chromosomes were also determined to be homoeologues in our study (Fig. 2).

The data indicate that exceptions to the homoeologous sets occur (Table 3, Fig. 2). Almost 30% of the probes gave hybridization patterns that did not agree with the homoeologous sets of chromosomes recognized by the remaining 70% of the probes. These exceptions make the identification of homoeologous chromosomes useful but not definitive. Exceptions may be due to a combination of two factors. First, probes which map to non-homoeologous loci have been identified in wheat (Anderson et al. 1992; Devos et al. 1992). It is possible that they occur in hexaploid oat as well. Second, intergenomic interchanges that have occurred during the evolution of the allohexaploid oat, or in crosses during cultivar development, may have disrupted sequence colinearity of homoeologues.

Many, but not all, low-copy number DNA probes can be expected to identify homoeologous chromosome sets. In developing an RFLP-based chromosomal arm map for wheat, Anderson et al. (1992) analyzed 210 clones, mainly oat and barley cDNAs plus a few wheat genomic clones, that were chosen as low copy (six or fewer bands) in wheat with preference to clones yielding three bands of approximately equal hybridization intensity. Of the 210 probes, 163 hybridized only to homoeologous chromosomes. Of the 47 probes hybridizing to fragments on chromosomes of more than one homoeologous group, seven detected previously-hypothesized translocations. In the majority of the remaining 40, three or more major fragments were assigned to chromosomes in one homoeologous group and only minor fragments were assigned to the other groups.

Evidence that translocations have played an important role in genome differentiation in *Avena* has been extensively documented (Ladizinsky 1970; Rajhathy and Thomas 1974). In meiotic pairing studies of hexaploid *A. sativa* cultivars, Singh and Kolb (1991) found interchange heterozygosity in many of the intraspecific crosses. Evidence for chromosomal rearrangement and structural differentiation among oat cultivars has been confirmed using C-banding (Jellen et al. 1993b). Jellen et al. (1993a) detected interchanges in two Kanota monosomics using C-banding. In addition, evidence that at least seven chromosomes in Ogle carry intergenomic translocations between A/D- and C-genome chromosomes has been obtained using genomic in-situ hybridization (Jellen et al. 1994).

If interchanges are common, homoeologous chromosomes may not be as easy to identify in oat as they are in hexaploid wheat. "Homoeology" in hexaploid oat may actually involve homoeologous chromosomal regions localized over several chromosomes, so that the original "homoeologous" chromosomes will be identified only by RFLP molecular markers linked to the centromeres. Intergenomic interchanges between sets of homoeologous chromosomes have been described in hexaploid wheat. Detailed RFLP maps were extremely useful in identifying intergenomic translocations among chromosomes 4A, 5A, and 7B in *T. aestivum* (Liu et al. 1992). Comparisons of oat and wheat RFLP maps may help clarify syntenic-group homoeology in oat, as well as evolutionary relationships between the Triticeae and Avenae. For example, we have found that pzc 3 and OG 41 hybridize to opposite arms of homoeologous Group-1 chromosomes of wheat using ditelosomics, and that two opposite-arm Group-1 wheat probes hybridize to the 1C/14/17 group of Kanota (unpublished results). Thus, a distantly-homoeologous relationship should exist between 1C, 14, and 17 of oat and Group-1 chromosomes of the Triticeae. The exact nature of the interchanges and their complicating effect on "homoeologous" chromosome assignments should be clear once an RFLP map that includes the location of multiple loci per probe is available in hexaploid oat.

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