Theor Appl Genet (2003) 107:26–35
DOI 10.1007/s00122-003-1191-6

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A genetic linkage map for hexaploid, cultivated oat (Avena sativa L.) based on an intraspecific cross 'Ogle/MAM17-5'

Received: 30 March 2002 / Accepted: 23 October 2002 / Published online: 30 April 2003

Abstract Genetic research and breeding of oat (*Avena sativa* L.) would be aided by development of a genetic linkage map for a breeding population. Such a map could be used for localization of qualitative and quantitative trait loci, marker-assisted selection and other genetic analysis in an adapted, agronomically useful background. The objectives of this research were to develop a genetic linkage map of hexaploid cultivated oat, to identify homoeologous relationships of linkage groups, and to compare homologous linkage groups between this map and the previously published hexaploid oat map from the cross 'Kanota/Ogle' (KO). A total of 510 markers, including 172 restriction fragment length polymorphisms (RFLP), 324 amplified fragment length polymorphisms (AFLP) and 14 simple sequence repeats (SSR), were assessed on a recombinant inbred population of 152 $F_{5:6}$ lines derived from the cross, 'Ogle/MAM17-5' (OM). Twenty eight linkage groups of 5 cM or longer were formed using 476 of the markers, while 34 markers remained either unlinked or in small fragments less than 5 cM. The 28 linkage groups contained from 3 to 33 markers, and varied in size from 5.2 to 123.0 cM, representing a total map length of 1,396.7 cM. Three putative homoeologous groups (OM7, OM8 and OM18; OM2 and OM23; OM13 and OM16) were identified. Comparison with the published KO map indicated that nine OM linkage groups could be determined to be homologous to linkage groups in the KO map. Further comparison of the homologous linkage groups revealed that residual differences in genomic rearrangements existed between the two hexaploid oat populations. Some linkage groups were significantly extended compared with the KO map. Since the OM mapping population is segregating for a number of agronomically important traits, this genetic map will provide a useful tool for identification of qualitative and quantitative loci for these traits.

Keywords *Avena sativa* · Genetic map · Molecular markers · Homology

Communicated by D. Hoisington

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Introduction

Oat (*Avena sativa* L.) is an important cereal crop used for both human consumption and animal feed. Cultivated oat is an allohexaploid with 21 pairs of chromosomes and a basic chromosome number of x = 7 (Rajhathy 1963). Genetic study and breeding of oat would be aided by development of a saturated genetic linkage map for an elite breeding population. Such a map could be used for localization of qualitative and quantitative trait loci, map-based gene cloning, marker-assisted selection and other genetic analyses (Tanksley et al. 1989). Linkage maps have already been developed for several important cereal crop species such as rice (Harushima et al. 1998), wheat (Messmer et al. 1999), maize (Davis et al. 1999) and barley (Heun et al. 1991; Ramsay et al. 2000). To understand and manipulate the oat genome, molecular linkage maps also have been developed for diploid (O'Donoughue et al. 1992; Kremer et al. 2001) and hexaploid (O'Donoughue et al. 1995; Portyanko et al. 2001) oat, primarily by using RFLP and AFLP markers.

The genetic map of 'Kanota/Ogle' (KO) (O'Donoughue et al. 1995) is currently the most complete hexaploid oat map. It has been used to detect quantitative trait loci (QTLs) for agronomic traits (Siripoonwiwat et al. 1996), crown rust resistance (Bush and Wise 1996) and vernalization response (Holland et al. 1997) in oat. However, the KO map was based on a wide, interspecific cross and only 71 recombinant inbred lines (O'Donoughue et al.

1995), a population which is too small to be used for accurate QTL mapping. Because a single mapping population can not be segregating for loci controlling all traits, it is necessary to derive different populations to map specific traits. Mapping populations derived from intraspecific crosses made with two breeding parents are particularly useful, because once markers are associated with QTLs or genes controlling agronomically important traits, they could be directly used in practical breeding programs.

The QTL identified for a trait could be validated through comparison of QTLs detected for different populations. Homologous and homoeologous relationships of linkage groups between the maps for the two different populations need to be identified before a comparison of QTLs is made. If one QTL for a trait is detected on a linkage group in a population, and one QTL for the same trait is identified on a homologous or homoeologous linkage group in another population, the reliability for the QTL detected would be greatly increased. Comparison of the linkage maps between two hexaploid mapping populations has been rare in oat, however. In addition, previous comparisons of the linkage maps between two mapping populations were exclusively focused on comparing linkage groups with homoeologous or unknown relationships. Limited synteny was revealed by comparing linkage maps of diploid (A) and hexaploid (A, C and D) oats (O'Donoughue et al. 1995; Kremer et al. 2001; Portyanko et al. 2001). Moreover, the three hexaploid oat genomes were defined as only 'segmentally' homoeologous (Kianian et al. 1997). Low colinearity among homoeologous linkage groups should not be surprising, however, since chromosomes within each genome have probably undergone rearrangement during the process of evolution prior to the three genomes combining at the hexaploid level. High colinearity between two homologous linkage groups for different hexaploid oat populations is expected if chromosome rearrangements are relatively fixed in hexaploid oat populations (Stebbins 1971). Relatively recent genomic rearrangements between two hexaploid oat populations could, therefore, be revealed by comparing homologous linkage groups between the two populations.

The objectives of this study were to develop a linkage map of hexaploid cultivated oat based on 152 $F_{5:6}$ recombinant inbred lines (RILs) derived from a breeding cross 'Ogle/MAM17-5' (OM), to identify homoeologous relationships based on multi-locus polymorphic markers, and to compare homologous linkage groups between this map and the previously published hexaploid oat KO map. The linkage map developed in this study is a useful supplement to, and an improvement on, other hexaploid oat maps, since some linkage groups have been significantly extended compared with the previously published hexaploid oat KO map. In addition, the OM linkage map has recently been used for QTL detection of crown rust resistance, and other important traits, in an agronomically improved genetic background (Zhu and Kaeppler 2002).

Materials and methods

Plant materials

Two hexaploid, cultivated oat (*A. sativa* L.) genotypes, 'Ogle' (CI9401) and MAM17-5, with contrasting responses to the crown rust pathogen (*Puccinia coronata*) were used as parents to produce a mapping population. MAM17-5 was selected in the spring oat breeding program at the University of Wisconsin-Madison (Moustafa et al. 1992) and has a complex pedigree. Ogle was developed in the spring oat breeding program at the University of Illinois (Brown and Jedlinski 1983). Ogle was also one of the two parents of the KO population, on which a relatively complete RFLP map was developed (O'Donoughue et al. 1995). Ogle differs from MAM17-5 in many agronomically important traits such as crown rust resistance, plant height, days to heading, barley yellow dwarf virus resistance, groat oil content, groat protein content and other quality traits. The 152 $F_{5:6}$ RILs used in this mapping experiment were derived using the single-seed descent method.

RFLP analysis

The majority of RFLP probes used in this study were selected based on the KO map, such that markers should be located approximately one every 20 cM to uniformly cover the hexaploid oat RFLP map. Additional molecular markers identified in publications as linked to crown rust and stem rust resistance genes or QTLs in oat were also included as probes. A total of 195 RFLP probes were screened; however, only 135 of them showed polymorphism between the parents and were used to characterize the RILs (Table 1). This included 17 markers putatively associated with crown rust and stem rust resistance genes or QTLs in oat (Table 2).

Clones from the BCD, CDO and WG libraries (Heun et al. 1991) were developed at Cornell University, Ithaca, N.Y., and clones from the ISU library (Rayapati et al. 1994) were developed at Iowa State University, Ames, Iowa. These clones were distributed by the Western Regional Research Center, USDA-ARS, Albany, Calif. Clones from the UMN library (Kianian et al. 1997) were kindly provided by Dr. Ronald Phillips, University of Minnesota, St. Paul, Minn. Clones from the OG (Goffreda et al. 1992) and RZ (Causse et al. 1994) libraries were generously provided by Dr. Mark Sorrells and Dr. Susan McCouch, respectively, Cornell University, Ithaca, N.Y. (Table 1).

Leaf tissue samples of the parents, Ogle and MAM17-5, and of the 152 RILs (15 plants per line) were collected from greenhouse-grown plants at Zadoks Growth Stage 31 (Zadoks et al. 1974). Samples were lyophilized, and ground through a 1-mm mesh with a cyclone sample mill (UDY Co., Fort Collins, Colo.). A modified hexadecyl trimethylammonium bromide (CTAB) procedure (Saghai-Maroof et al. 1984) was used for DNA extractions. Restriction digestions were made of extracted genomic DNA with one of the four enzymes *Dra*I, *Eco*RI, *Eco*RV, and *Hin*dIII (Promega, Madison, Wis.) using standard restriction digestion procedures recommended by the manufacturer. Twenty microgram samples of restricted DNA were loaded onto 0.8% agarose gels, electrophoresed for approximately 16 h at 35 V, and transferred onto either an Immobilon-S (Millipore Co., Bedford, Mass.) or Hybond-N^+ (Amersham Pharmacia, UK) nylon membrane with 5 × SSC. Probes were labeled with [^{32}P] by random priming (Feinberg and Vogelstein 1983). Hybridizations were done in capped glass tubes in a roller oven overnight at 65 °C. After hybridization, membranes were first rinsed 2 × 10 min at 65 °C with 2 × SSC and 0.1% SDS in the tubes. The membranes were then washed for 2 × 20 min at 65 °C with 0.2 × SSC and 0.1% SDS in plastic tubs. BioMax films (Eastman Kodak Co., Rochester, N.Y.) were exposed at –80 °C for 2–15 days.

Table 1 Molecular markers used for the Ogle/MAM17-5 map

Designation	Source	No. clones or primer pairs	No. loci	No. polymorphic loci/clone or primer pair
RFLP				
BCD	Barley leaf cDNA	30	39	1.3
CDO	Oat leaf cDNA	69	87	1.3
ISU	Oat root cDNA	18	25	1.4
OG	Oat genomic	2	3	1.5
RZ	Rice leaf cDNA	1	1	1
UMN	Oat endosperm cDNA	13	14	1.1
WG	Wheat genomic	2	3	1.5
AFLP				
em		21	324	15.4
SSR				
AM	Oat microsatellite	4	8	2
hvm	Barley microsatellite	1	2	2
Wisc	Oat microsatellite	2	4	2
Total			510	

Table 2 Molecular markers putatively associated with crown rust and stem rust resistance genes or QTLs in oat

Molecular markers	Linked genes or QTLs	Source
ISU707, CDO1467, UMN162, CDO309, CDO942, CDO1509, CDO1196, UMN23, UMN498	*Prq*	Chen et al. 2000
UMN145	*Pc 91*	Rooney et al. 1994
OG176	*Pc 92*	Rooney et al. 1994
OG41	*R264B*	Bush et al. 1994
ISU1719, ISU1774	*R203*	Bush et al. 1994
ISU 2192	Pca	Rayapati et al. 1994; Wise et al. 1996
CDO1385	*Pg 9/Pc X*	O'Donoughue et al. 1996
BCD1562	Pg 13	O'Donoughue et al. 1996

AFLP analysis

AFLP analysis was performed according to the protocol provided by the manufacturer (Gibco-BRL Life Technology, Inc., Gaithersburg, Md.) with minor modifications. The primers and their sequences are listed in Table 3. Three modifications were made to the protocol: (1) 1/4 of the reaction size suggested by the protocol was used in step 1 (digestion) through the last step (selective amplification); selective amplifications were performed in only 5-μl reactions with the PTC-100 thermal controller (MJ Research, Inc. Watertown, Mass.); (2) DNA of parent lines and RILs was digested for 3 h instead of 2 h in order to allow complete digestion of genomic DNA; and (3) No dilution was made to the ligation mixture, and only a 1:20 dilution instead of 1:50 was made to the pre-amplified PCR products since the genome size of hexaploid oat is almost twice as large as what the AFLP System is optimized for. The samples were size-fractionated through a 7% denaturing polyacrylamide sequencing gel at 85 W for 2 h. Gels were dried and exposed to BioMax films (Eastman Kodak Co., Rochester, N.Y.) at room temperature for about 3 days. All AFLP reactions were performed three times to confirm that the results were repeatable. Polymorphic bands were numbered consecutively from the bottom of the films using the primer abbreviations in Table 3.

Table 3 Sequences of AFLP primers used for selective amplifications

Primer[a]	Selective base[b]	Primer	Selective base
e1	AAC	m1	CAA
e2	AAG	m2	CAC
e3	ACA	m3	CAG
e4	ACC	m4	CAT
e5	ACG	m5	CTA
e6	ACT	m6	CTC
e7	AGC	m7	CTG
e8	AGG	m8	CTT

[a] Primers e and m indicate *Eco*RI and *Mse*I primers. *Eco*RI primer core sequence: 5′-GACTGCGTACCAATTC-3′, and *Mse*I primer core sequence: 5′-GATGAGTCCTGAGTAA-3′

[b] Selective bases added to the 3′ end of the core sequences in the final selective amplification. The first nucleotide A or C in the selective bases were used in the pre-selective amplification

SSR analysis

Microsatellite or SSR analysis was performed according to Chin et al. (1996), except that the separation and detection of the amplified products were done on polyacrylamide sequencing gels. The AM primer sequences (Li et al. 2000) were graciously provided by Dr. Graham Scoles of the University of Saskatchewan, Saskatoon, SK, Canada. The hvm primer sequences were listed in Liu et al. (1996). The Wisc primers were developed at the University of Wisconsin. The Wisc48 primer sequences are 5′-CAATGG GCATT GAGAG ATTAA G-3′ and 5′-TATGG CTGGT GGAGT TGTTT TG-3′. The Wisc115 primer sequences are 5′-TCATG TCTGT GGGGC CAGG-3′ and 5′-CAGGC GCACT TTGGAT CTATT T-3′.

Map construction

Chi-square tests were performed on each locus for goodness-of-fit to the expected 1:1 segregation ratio, ignoring heterozygous genotypes. Markers that deviated significantly ($p < 0.01$) from the expected segregation ratio were checked for influence on linkage grouping and map order. Skewed loci were first excluded from map construction, and then added into the map construction. If no ambiguous linkage groups and map order were produced, the distorted markers were kept in the map.

Linkage analysis and map construction were performed by using MAPMAKER/EXP 3.0b (Lander et al. 1987). Initially, linkage groups were obtained using two-point analysis with a log-likelihood of odds (LOD) score of 8.0 and a maximum recombination level of 0.30. This step was implemented by using the "group" command. These are the same grouping criteria used to construct the KO map (O'Donoughue et al. 1995). The criteria were utilized in this study to standardize data for comparison of results from the current study with the KO map in previous research. LOD scores of 10.0 and 6.0 were also tested, but resulted in either too many small fragments or too few, reasonable-sized linkage groups, which deviated too much from the 21 pairs of chromosomes for hexaploid oat (Rajhathy 1963). Next, linked markers within each linkage group were ordered using multi-point analysis with the "compare" command. For large linkage groups, a subset of the most informative markers was first chosen using the "suggest subset" command to construct a framework order. The best intervals for the rest of the linked markers within the linkage group were then determined using multi-point analysis with the "try" command. Finally, the map distances were calculated using the "map" command. The map distances were converted to centi-Morgans using the Kosambi function (Kosambi 1944).

Map comparisons

The OM and KO mapping populations have one parent, Ogle, in common, and the majority of RFLP markers for this study were chosen in an attempt to place one marker every 20 cM, based on the KO map. Therefore, homologous loci could be identified between the two maps. Homologous loci were identified by comparing fragment sizes of bands from 'Ogle', 'Kanota' and MAM17-5 produced by the same restriction enzyme-probe combination in the two studies and mapped in both the KO and the OM map (Fig. 1). The sizes of bands mapped in the KO map were obtained from autoradiograms for the KO mapping parents, which are available in the GrainGenes database (http://wheat.pw.usda.gov /ggpages / kxo_autorads.html). Homologous linkage groups were putatively defined as two linkage groups sharing at least one homologous locus. Only putative homologous linkage groups between the two maps were extensively compared. The percent of genome examined was calculated as the proportion of linkage-group segments in the OM map flanked by marker loci in common with the KO map (Van Deynye et al. 1995). The percent of the genome conserved was calculated as the length of the segments in the OM map that could be considered colinear between the two maps divided by the length of the total segments examined. Colinearity refers to at least three markers that cross-hybridize to two linkage groups and retain a linear order on both linkage groups. In this paper, homoeologous linkage groups were putatively defined as two linkage groups having regions defined by at least two markers in common.

Results

Detection of polymorphism

The two parents, Ogle and MAM17-5, of the RI mapping population were screened with 195 RFLP clones probed to genomic DNA digested with four restriction enzymes.

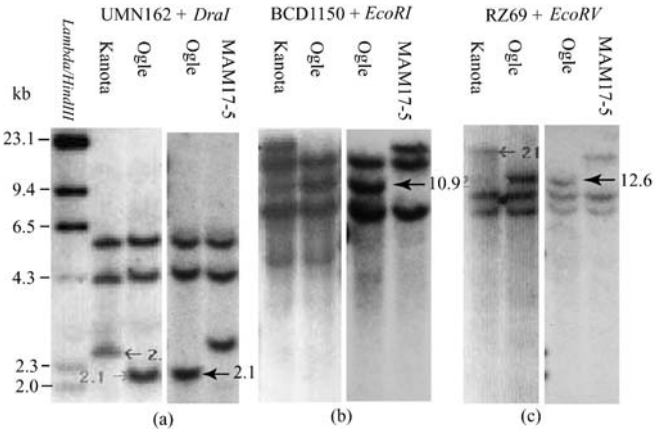

Fig. 1 Examples of RFLP markers detecting homologous loci between the 'Ogle'/MAM17-5 (OM) and 'Kanota'/'Ogle' (KO) maps. A band of 2.1 kb (**a**), 10.9 kb (**b**) and 12.6 kb (**c**) in Ogle was identified by UMN162, BCD1150 and RZ69, respectively. These loci were mapped in both maps. Autoradiograms for the KO mapping parents were downloaded from the GrainGenes database (http://wheat.pw.usda.gov/ggpages/kxo_autorads.html)

Of the clones screened, 69.2% (135) detected RFLPs between genomes of the two parents when digested with at least one of the four enzymes. The 135 clones used for characterizing the population of RILs had an average of 1.3 polymorphic bands per clone, producing a total of 172 RFLP marker loci (Table 1).

Sixty four combinations of AFLP primers (Table 3) were used to fingerprint genomic DNA of the two parents, Ogle and MAM17-5. The total number of amplified fragments, as well as the number of polymorphisms, varied among the AFLP primer pairs. On average, each primer pair produced about 100 bands. The 21 pairs selected for mapping had an average of 15.4 polymorphic bands per primer pair, producing a total of 324 AFLP loci (Table 1).

Molecular linkage map

The final map consisted of 476 marker loci that formed 28 linkage groups of 5 cM or longer. Thirty four markers remained either linked in small fragments less than 5 cM or unlinked (Fig. 2). The linkage groups contained from 3 to 33 markers, with a mean of 17, and varied in size from 5.2 to 123.0 cM, with an average of 49.9 cM per linkage group. The 'Ogle'/MAM17-5 map represented 1,396.7 cM. There were 137 markers (28.8%) co-segregating with other mapped loci in pairs or groups, which had no recombinants between them. The average distance between the mapped loci was 4.1 cM.

Table 4 Comparisons between the maps of 'Ogle'/MAM17-5 (OM) and 'Kanota'/'Ogle' (KO) based on putatively homologous regions

LG[a]	OM map		LG	KO map		Cons[c] (%)	No. of loci shared
	Region compared	Exam.[b] (%)		Region compared	Exam. (%)		
OM1	cdo795-bcd327	12.4	KO22	bcd327-cdo795	17.8	100.0	4
OM2	bcd1150-bcd1261a	61.9	KO3	bcd1150-bcd1261b	16.8	100.0	5
OM2	cdo393b-umn28	39.8	KO5	cdo393b-umn28	34.8	100.0	4
OM3	rz69-cdo1464	20.6	KO11	cdo1464-rz69	75.2	100.0	4
OM6	cdo1158-cdo539	36.5	KO2	cdo1158-cdo539	64.8	100.0	3
OM7	cdo122-bcd1186	44.8	KO28	cdo122-bcd1186	98.3	100.0	6
OM11	cdo772-cdo527	44.0	KO33	cdo527-cdo772	87.0	100.0	3
OM23	cdo309d-cdo309c	100.0	KO6	isu1774-cdo309b	13.9	47.0	8
Mean		45.0			51.1	93.4	

[a] Linkage group of the OM or KO map
[b] The percent of genome examined
[c] The percent of genome conserved

Comparison between the maps of 'Ogle'/MAM17-5 and 'Kanota'/'Ogle', and putative homoeology

Since the majority of RFLP probes were polymorphic for digestion with different restriction enzymes between the two studies, only 13 homologous loci were identified (Fig. 2). Nine linkage groups were revealed by the homologous loci to be partly, if not completely, the same (homologous) as in the KO map. Each pair of homologous linkage groups shared not only the homologous loci, but up to seven more loci as well (Table 4), which also could be homologous loci. For example, homologous linkage groups OM1 and KO22 shared two homologous loci, CDO1196 and BCD327, which segregated for identical size-restriction fragments from Ogle, plus two more loci, CDO795 and UMN575, which were detected by the same probes in each population, but polymorphic with different restriction enzymes between the two studies; and thus their homology could not be confirmed. The linkage group homologies were: OM1 = KO22, OM3 = KO11, OM6 = KO2, OM7 = KO28, OM11 = KO33, OM23 = KO6, OM25 = KO8 and OM26 = KO30. One linkage group, OM2, was found to correspond to two linkage groups, KO3 and KO5 (Fig. 2). The homologous locus, BCD1150, on one end of OM2 was mapped on KO3, and the homologous loci, CDO1173 and WG282, on the other end of OM2 were mapped on KO5. The BCD1150 end of OM2 showed loose linkage to the other end containing markers CDO393B, BCD1261A and BCD1562. Three marker loci CDO393, BCD1261 and BCD1562 were also linked to BCD1150 on KO3, however (O'Donoughue et al. 1995). This suggested that the two ends of OM2 were truly linked. However, further research is needed to verify the linkage.

The order of markers (3 to 6) shared by the homologous linkage groups was, on average, 93.4% conserved. A mismatch was observed in the comparison of OM23 and KO6, which was identified by further comparing the homologous linkage groups between the two maps (Table 4). The marker locus CDO309D on one end of OM23 apparently is a duplicate of CDO309C (Fig. 2). Moreover, only 45.0% of the length of homologous linkage groups in the OM and 51.1% in the KO map were covered by the shared markers and could be examined for reliable comparison (Table 4). Our results indicate that residual differences in genomic rearrangements between two hexaploid oat populations still exist, which is in agreement with cytogenetic findings that intervarietal chromosome interchanges exist in cultivated oat (Singh and Kolb 1991).

Linkage groups could be putatively declared to be homoeologous if they had at least two markers in common, or the homoeology could be defined transitively. For instance, OM7 and OM18 did not share any polymorphic marker loci, but each of them shared two marker loci with OM8. OM7, OM8 and OM18 could, therefore, be considered as one homoeologous group. Based on this line of reasoning, set OM7, OM8 and OM18, set OM2 and OM23, and set OM13 and OM16 could be defined as three different putative homoeologous groups (Fig. 2). Linkage groups KO3, KO5 and KO6, corresponding to OM2 and OM23, were also identified as a putative homoeologous group (Portyanko et al. 2001), an observation consistent with our designation of OM2 and OM23 as a homoeologous group. In addition to nine OM linkage groups showing homology to the KO linkage groups, 14 OM linkage groups were identified as homoeologous to 1–5 linkage groups from the KO map. Finally, the remaining five linkage groups were clearly not comparable with groups in the KO map (Fig. 2). Since the homoeologous relationship among chromosomes is not yet clear, and linkage groups have not been assigned to chromosomes, the assignments of linkage groups to different homoeologous groups must remain tentative.

Segregation ratios of molecular markers

Chi-square tests were performed on all 510 markers characterized. Four hundred and sixty four markers fit the expected 1:1 segregation ratio ($p > 0.05$), ignoring residual heterozygosity. Thirty markers deviated at a 0.01 level of significance and 16 at the 0.05 level. Of the 46 (9.0%) with skewed segregation markers, 28 (60.7%)

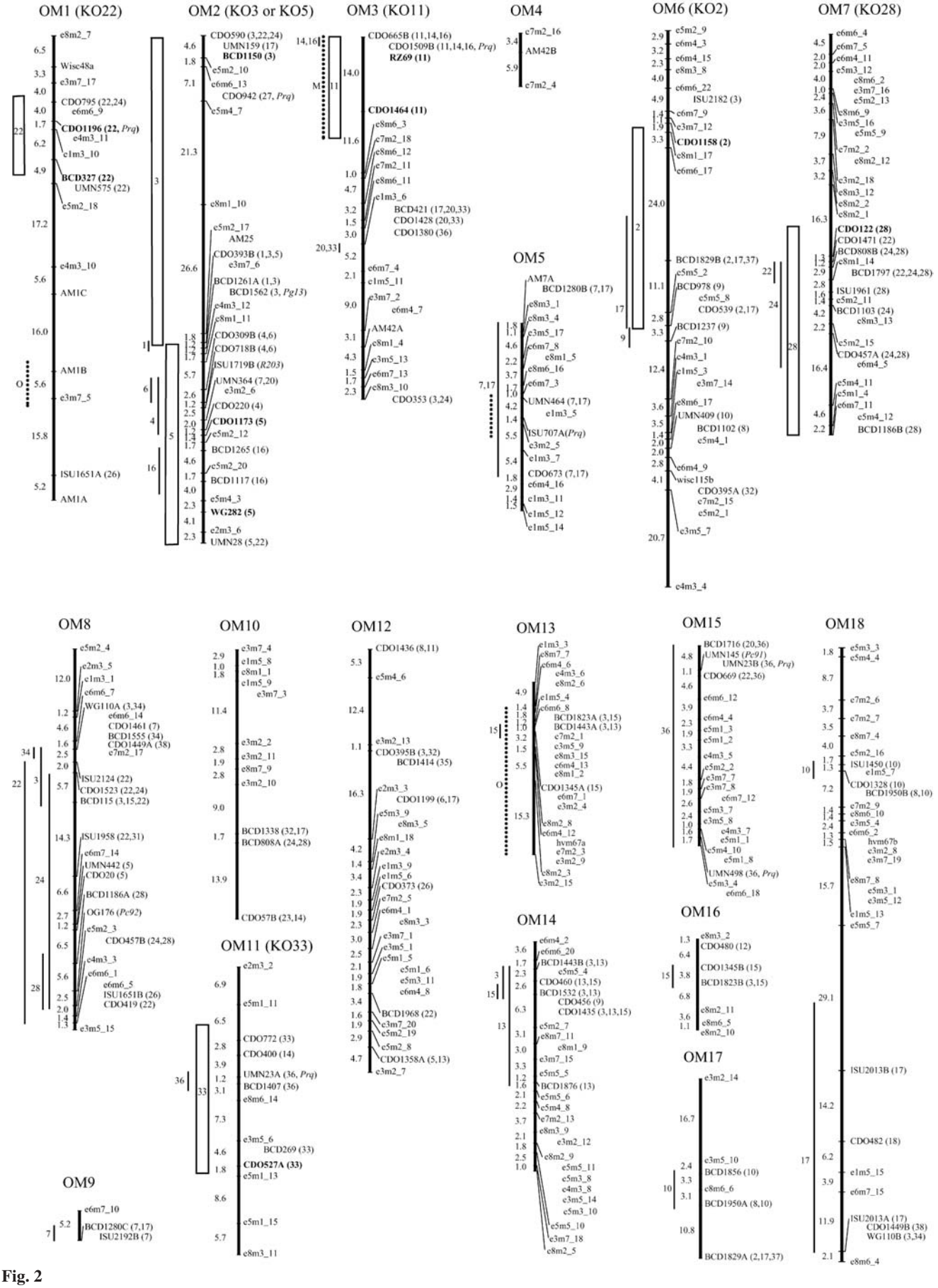
OM1 (KO22)
OM2 (KO3 or KO5)
OM3 (KO11)
OM4
OM5
OM6 (KO2)
OM7 (KO28)
OM8
OM9
OM10
OM11 (KO33)
OM12
OM13
OM14
OM15
OM16
OM17
OM18

Fig. 2

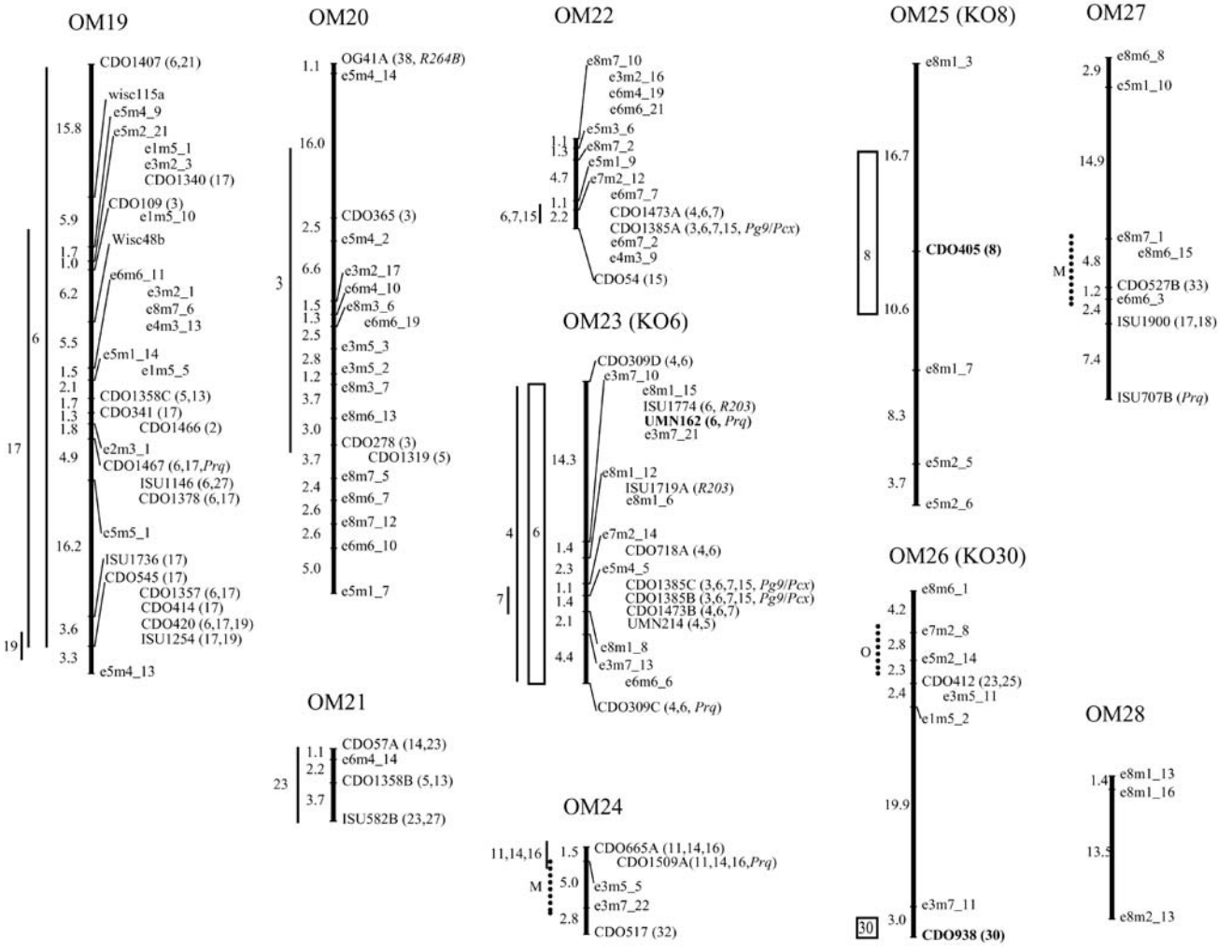

Fig. 2 Linkage map of hexaploid cultivated oat (*A. sativa* L.) based on 152 recombinant inbred lines from the cross 'Ogle/MAM17-5' (*OM*). Map distances are given in centi-Morgans (Kosambi). *Numbers in parentheses* following each marker indicate linkage groups where the marker is mapped in the hexaploid oat 'Kanota'/'Ogle' (KO) map or putatively associated rust resistance genes/QTL in oat. Markers in *bold* correspond to homologous loci mapped in the KO map. The *names in parentheses* following the names of OM linkage groups indicate the corresponding homologous linkage group in the KO map. *Boxes* to the left of linkage groups show regions homologous to the corresponding KO linkage groups indicated within the *boxes*. *Solid lines* to the left of linkage groups illustrate segments homoeologous to the indicated KO linkage groups. *Dotted lines* indicate the region with markers significantly ($p < 0.05$) skewed towards the parents, *O* (Ogle) and *M* (MAM17-5). If a homologous, homeologous or skewed locus flanks an interval with a locus that has unknown relationship with the KO map or is not skewed, half of the interval will be considered as a homologous, homoeologous or skewed region

were skewed towards the female parent, Ogle, and 18 (39.3%) were skewed towards the male parent, MAM17-5. The majority of skewed markers formed extended regions within which all skewed markers showed an excess of the same parental type. Seven linkage groups showed regions of skewed segregation ratios. Segments exhibiting distortion on linkage groups OM1, 13 and 26 had an excess of female Ogle alleles, and those on OM3, 5, 24 and 27 skewed towards male MAM17-5 alleles (Fig. 2).

Relative distribution of RFLP and AFLP markers

In general, RFLP and AFLP markers showed very similar distributions. However, one linkage group, OM28, was identified that contained only AFLP markers. Some linkage groups were greatly extended by AFLP markers. For example, the region of OM7 covered by RFLP markers was exactly the same as the linkage group KO28 (O'Donoughue et al. 1995), but OM7 was extended to twice that size using AFLP markers (Fig. 2). These results suggest that the addition of PCR-based markers such as AFLPs, which are not limited to coding regions, would enhance the development of molecular linkage maps

through the increase of map density and the extension of linkage groups.

Discussion

Deviation of marker segregation

Nine percent of the molecular markers tested displayed significant distortion from the expected segregation ratio. Similar results were reported in other mapping studies of oat (O'Donoughue et al. 1992, 1995; Yu and Wise 2000; Portyanko et al. 2001) and other crops (Castiglioni et al. 1999; Messmer et al. 1999). One possible cause of skewed segregation is inadvertent selection. This was largely avoided, however, since the 152 RILs used in this study were developed from the randomly harvested 152 F_2 plants using a single-seed descent method and care was taken to preserve all lineages. The segment spanned by cdo1464 and rz69 on OM3 was skewed towards MAM17-5, whereas the homologous segment on KO11 was skewed towards Ogle (O'Donoughue et al. 1995). It is interesting that both MAM17-5 and Ogle were used as males in the two mapping populations, respectively. The deviation of markers from expected segregation ratios seemed related to passage through male or female gametes rather than depending on a specific genotype. Genetic control of segregation disturbance has been reported in rice and *Aegilops tauschii* (Xu et al. 1997; Faris et al. 1998). The apparent preferential transmission of one parental genotype in each of the areas of the genome suggests that these areas may carry genes that affect gamete viability.

Additionally, disposal of skewed markers should be done with caution when a molecular linkage map is constructed. For example, adding 22 skewed loci to the diploid oat map combined linkage groups A and C into AC, and B and C into BC (Portyanko et al. 2001). The updated diploid map exhibited improved colinearity with another well-developed diploid map (O'Donoughue et al. 1992) relative to the original map with the skewed markers discarded (Kremer et al. 2001).

Comparative mapping and structural rearrangements

Theoretically, a probe representing a DNA sequence is normally present as a single copy per genome if an allohexaploid has no duplications or deficiencies for a DNA sequence complementary to the RFLP probe. The probe should detect from one to three bands when there are no restriction sites within the target sequence. Two linkage groups containing the same markers in different hexaploid oat maps can not be designated as belonging to the same (homologous) linkage group unless the autoradiograms for a marker in the two maps are compared. It is necessary to show that the same sequence is detected. Therefore, comparison of one linkage map with another in hexaploid oat must be done with caution.

One linkage group, OM2, was found to correspond to two linkage groups, KO3 and KO5, in the KO map by comparing homologous loci (Fig. 2). Reasons for the mismatch in linkage groups could be the following. First, the KO map developed by O'Donoughue et al. (1995) consisted of 38 linkage groups, 17 more than expected on the basis of the haploid chromosome number of oat. The OM map in this study consisted of 28 linkage groups, which were seven more than expected. Therefore, it was expected that more than one linkage group in the KO map would be represented by one linkage group in the OM map, or more than one linkage group in the OM map would be represented by one linkage group in the KO map. Second, it has been shown that intervarietal chromosome structural rearrangements exist in cultivated oat (Singh and Kolb 1991). It was also noted that parents of the KO mapping population, 'Kanota' and 'Ogle', differed for at least three intergenomic translocations (Jellen et al. 1994). 'Ogle' (*Avena sativa*) has the reciprocal 7C-17 translocation whereas 'Kanota' (*Avena byzantina*) does not. In general, genotypes in *A. sativa* have the 7C-17 translocation (Jellen and Beard 2000). Thus, MAM17-5 may also have it. The OM mapping population was, therefore, probably homozygous in the 7C-17 translocation whereas the F_1 of the KO mapping population was heterozygous in the translocation. This difference could introduce complications in mapping the linkage groups related to the chromosomes 7C and 17. Moreover, linkage group KO3 represented a portion of chromosome 7C as determined by aneuploid analysis (Kianian et al. 1997). A single linkage group in another hexaploid map was identified as homologous to two linkage groups, KO3 and KO1 (Portyanko et al. 2001). Once again, KO3 was found to be involved in an ambiguous linkage grouping. This result was in agreement with the finding that chromosome 7C, including a portion of KO3 (Kianian et al. 1997) (homologous to OM2), was often involved in chromosome rearrangements in hexaploid oat (*A. sativa*).

Map size and recombination

Cultivated oat is an allohexaploid with 21 pairs of chromosomes and a basic chromosome number of $x = 7$ (Rajhathy 1963). The number of linkage groups identified in this research indicated that the OM map is not yet complete. Compared with the estimated complete map size of 2,932 cM for hexaploid oat (O'Donoughue et al. 1995), the OM map size, 1,396.7 cM, covered about 50% of the hexaploid genome. Compared with the 1,482 cM and the 38 linkage-group KO map originally published by O'Donoughue et al. (1995), the current OM map was more uniform, with 21 linkage groups longer than 25 cM, possibly corresponding to 21 haploid chromosomes.

The map developed in this study was significantly shorter than the updated 2,351 cM KO map, in which AFLP markers had been added (Jin et al. 2000), and the 2,049 cM 'Ogle'/TAM O-301 map (Portyanko et al.

2001). To some extent, map size can be affected by mapping strategy. Although these maps were constructed using the same program, linkage groups were formed in this study by using a very stringent LOD score of 8.0 for the OM map, whereas in the 'Ogle'/TAM O-301 map a LOD score of only 3.0 was used. The latter strategy could result in some spurious linkage groups and produce a larger map size. Reduced recombination frequencies, leading to reduced map distances, have been observed for more distantly related crosses than those that are closely related in maize (Doebley and Stec 1991), rice (McCouch et al. 1988) and potato (Gebhardt et al. 1991). About 29% (137) of the markers in this map co-segregated with other markers, which is much more than 13% in the KO map and 6% in the 'Ogle'/TAM O-301 map. Many RFLP markers turned out to be closely linked in this map, although they were selected to be spaced at 20-cM intervals based on the KO map. 'Ogle' and MAM17-5 belong to the same species *A. sativa*, but they may be divergent in some areas of the genome since MAM17-5 has two diploid and two tetraploid progenitors. Initially MAM17-5 was developed for crown rust resistance. Sharma and Forsberg (1974) found that crown rust resistance was associated with the presence of an alien chromosome in a monosomic alien substitution line (20" + 1' + 1A'), derived from a cross between tetraploid CI7232 and hexaploid cv 'Clarion'. However, the alien chromosome did not pair with any *sativa* chromosomes, and was not transmitted through the pollen, resulting in instability of rust resistance. To overcome the low pairing affinity between the alien chromosomes and the *A. sativa* chromosomes, Sharma and Forsberg (1977) irradiated seeds from the alien substitution lines with thermal neutrons to produce a stable line, DCS1789, one of the MAM17-5 progenitors. A similar strategy was used to transfer resistance genes from *Avena strigosa* line CI3436 to oat N560 lines and N770 lines (Forsberg 1990). One of the N770 lines was used as another progenitor of MAM17-5. The irradiation employed during the development of MAM17-5 was believed to introduce fragments of the alien chromosomes carrying resistance genes into *sativa* chromosomes (Forsberg 1990), and it could also have caused other unknown variation in the genome. Finally, the cross 'Ogle'/MAM17-5 may not be ideal for developing a complete linkage map since the cross is intraspecific, and there may be no polymorphism in some areas of the genome between 'Ogle' and MAM17-5.

Despite these possibilities, the OM map contributes significantly to our understanding and study of the complex genome of cultivated oat. Overall, the OM map is very useful to supplement other hexaploid oat genetic maps, since some linkage groups, such as OM7, have significantly extended homologous linkage groups, such as KO28, of either the original KO map (O'Donoughue et al. 1995) or the updated KO map (Jin et al. 2000). Assignment of putative homology and homoeology among or between linkage groups from this study will greatly assist further genetic mapping in oat. Detection of residual differences in genomic rearrangements between two hexaploid oat populations in this research suggests that a consensus map should be confirmed with genetic maps from different mapping populations. Finally the OM map will be invaluable for QTL detection of agronomically important traits since the mapping population is derived from a breeding cross and consists of twice as many lines as the original KO population.

Acknowledgements We thank Dr. S.M. Kaeppler for his useful suggestions concerning this research, and Dr. T.C. Osborn for his helpful review of the manuscript. We would also thank Dr. E.B. Buyukunal-Bal for her contribution of preliminary SSR work to the study. This research was supported by Hatch Grant No. WIS03920 and a Plant Breeding and Genetics Fellowship from Pioneer Hi-Bred International, Inc.

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