A linkage map of hexaploid oat based on grass anchor DNA clones and its relationship to other oat maps

V.A. Portyanko, D.L. Hoffman, M. Lee, and J.B. Holland

Abstract: A cultivated oat linkage map was developed using a recombinant inbred population of 136 $F_{6:7}$ lines from the cross 'Ogle' × 'TAM O-301'. A total of 441 marker loci, including 355 restriction fragment length polymorphism (RFLP) markers, 40 amplified fragment length polymorphisms (AFLPs), 22 random amplified polymorphic DNAs (RAPDs), 7 sequence-tagged sites (STSs), 1 simple sequence repeat (SSR), 12 isozyme loci, and 4 discrete morphological traits, was mapped. Fifteen loci remained unlinked, and 426 loci produced 34 linkage groups (with 2–43 loci each) spanning 2049 cM of the oat genome (from 4.2 to 174.0 cM per group). Comparisons with other *Avena* maps revealed 35 genome regions syntenic between hexaploid maps and 16–34 regions conserved between diploid and hexaploid maps. Those portions of hexaploid oat maps that could be compared were completely conserved. Considerable conservation of diploid genome regions on the hexaploid map also was observed (89–95%); however, at the whole-chromosome level, colinearity was much lower. Comparisons among linkage groups, both within and among *Avena* mapping populations, revealed several putative homoeologous linkage group sets as well as some linkage groups composed of segments from different homoeologous groups. The relationships between many *Avena* linkage groups remain uncertain, however, due to incomplete coverage by comparative markers and to complications introduced by genomic duplications and rearrangements.

Key words: *Avena*, linkage map, comparative mapping, homoeology.

Résumé : Une carte génétique de l'avoine cultivée a été produite à l'aide d'une population de 136 lignées recombinantes fixées ($F_{6:7}$) issues du croisement 'Ogle' × 'TAM O-301'. Un ensemble de 441 marqueurs, dont 355 polymorphismes de la longueur du fragment de restriction (RFLP), 40 polymorphismes de la longueur du fragment amplifié (AFLPs), 22 AND polymorphes amplifiés au hazard (RAPDs), 7 marqueurs spécifiques de site (STSs), 1 microsatellite, 12 locus isoenzymatiques et 4 marqueurs morphologiques, ont été placés sur la carte. Quinze locus n'ont pu être placés, tandis que les 426 autres locus ont été groupés en 34 linkats (ayant de 2 à 43 locus) couvrant 2049 cM du génome de l'avoine (à raison de 4,2 à 174,0 cM par linkat). Des comparaisons réalisées avec d'autres cartes génétiques du genre *Avena* ont révélé 35 régions montrant une synténie chez les cartes d'avoines hexaploïdes et 16 à 34 régions conservées entre l'avoine diploïde et hexaploïde. Ces portions du génome pouvant être comparées se sont montrées complètement conservées. Un degré de conservation considérable (89 % à 95 %) des régions génomiques diploïdes a également été observé sur la carte des hexaploïdes, tandis qu'au niveau de l'ensemble des chromosomes, la co-linéarité était beaucoup plus faible. Des comparaisons entre linkats, tant parmi qu'entre les populations de cartographie du genre *Avena*, ont permis de révéler plusieurs groupes de liaison potentiellement homéologues de même que certains linkats composés de segments provenant de différents groupes homéologues. Les relations entre plusieurs linkats du genre *Avena* demeurent incertaines cependant en raison de la couverture incomplète des marqueurs permettant les comparaisons et en raison des complications découlant des duplications et réarrangements génomiques.

Mots clés : *Avena*, carte de liaison génétique, cartographie comparée, homéologie.

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Introduction

Cultivated oat, *Avena sativa* L., is a self-pollinating, disomic, hexaploid ($2n = 6x = 42$) species. The genome of hexaploid *Avena* species consists of three basic subgenomes, referred to as the A, C, and D genomes, each of which contains seven pairs of chromosomes (Rajhathy and Thomas 1974). At the cytogenetic level, this type of genome organization superficially resembles that of the model allopolyploid crop, bread wheat, *Triticum aestivum* L. (McFadden and Sears 1946). Diploid, tetraploid, and hexaploid *Triticum* species exist, and there are close relationships between the A, B, and D genome chromosomes of bread wheat and those of the *Triticum* A, B, and D diploid species relatives, respectively (Singh 1993). Furthermore, the three subgenomes of *T. aestivum* clearly represent homoeologous chromosome sets that can compensate for each other (Sears 1966). Genetic mapping studies have indicated that the relative order of DNA fragments is highly conserved among members of each of the seven homoeologous chromosome groups of the Triticeae (Chao et al. 1989; Marino et al. 1996; Nelson et al. 1995*a*, 1995*b*, 1995*c*; Van Deynze et al. 1995*a*). Like wheat, cultivated oat has a large genome ($1C = 11\ 315$ Mb; Arumuganathan and Earle 1991); diploid, tetraploid, and hexaploid oat species exist; and there seem to be gross cytogenetic similarities between the A and C subgenomes of the hexaploid oat, and the genomes of the A and C diploid *Avena* species, respectively (Leggett and Thomas 1995). Unlike wheat, however, oat lacks a complete set of either nullisomic or nullitetrasomic lines, which has hindered the localization of DNA marker loci to specific chromosomes. Furthermore, the three hexaploid oat subgenomes are only segmentally homoeologous (Kianian et al. 1997). Comparisons between restriction fragment length polymorphism (RFLP) linkage maps of diploid oat and hexaploid oat also revealed only limited synteny (Kremer et al. 2001; O'Donoughue et al. 1995). Surprisingly, however, considerable synteny of genome regions or segments has been reported between a diploid oat linkage map and maps of related grass genera. Alignment of this diploid oat map, based on a comparative "anchor" DNA probe set, to genome maps of Triticeae, rice (*Oryza sativa* L.), and maize (*Zea mays* L.) revealed significant conservation (71–84%) between the genomes of diploid oat and these other cereal species (Van Deynze et al. 1995*a*). This result is consistent with data demonstrating a high level of synteny across grass species as a whole (Bennetzen and Freeling 1993; Devos and Gale 1997; Van Deynze et al. 1995*b*). Based on the putative synteny of most grass genomes, it is surprising that observed map conservation within *Avena* is not greater. Understanding this phenomenon will require comparisons of detailed genome maps of hexaploid and diploid oat and other cereal species.

Previously published DNA marker linkage maps of hexaploid oat are of limited utility for addressing this issue. The first published linkage map of hexaploid oat was based primarily on oat cDNA clones that did not allow comparisons with the published maps of other grass species, because these clones had not been mapped in other species (O'Donoughue et al. 1995). More recently, amplified fragment length polymorphisms (AFLPs) have been used to de-velop oat linkage maps, but these cannot be compared across species (Jin et al. 2000). Therefore, development of a hexaploid oat map based on DNA probes that have been mapped in other *Avena* species and in other grass species should help to clarify the genome relationships within the *Avena* genus and between *Avena* and other grasses.

The objective of this study was to develop a linkage map of cultivated hexaploid oat and to compare the linkage relationships among DNA marker loci in As-genome diploid and ACD-genome hexaploid *Avena* populations. Alignment of maps was facilitated by the use of probes selected from the Cornell University "Grass Anchor Set" of comparative DNA clones (Van Deynze et al. 1998); wheat DNA probes from the John Innes Center (Norwich, U.K.), which also have been widely used for genetic mapping in the Gramineae (Devos and Gale 1997); and probes previously mapped on the 'Kanota' × 'Ogle' cultivated hexaploid oat molecular map (O'Donoughue et al. 1995).

Materials and methods

Mapping population

A population of 136 F_6-derived F_7 recombinant inbred lines (RILs) was developed by single-seed descent without selection from 136 F_2 plants randomly chosen from the cross between oat cultivars Ogle (*A. sativa*, ssp. *sativa* L.) and TAM O-301 (*A. sativa*, ssp. *byzantina* C. Koch.). 'Ogle' was also one of the parents of the hexaploid oat molecular map developed by O'Donoughue et al. (1995). At each generation, panicles were bagged to prevent cross-pollination. The F_2, F_3, and F_5 generations were grown in the greenhouse or growth chamber, and the F_4 and F_6 generations were grown in the field at Aberdeen, Ida.

RFLP analysis

Total genomic DNA was isolated from lyophilized leaf and stem tissue pooled from seven to eight random plants of each RIL or parental variety. DNA isolation and RFLP detection were performed according to Veldboom et al. (1994) as modified by Kremer et al. (2001). DNA samples (20 μg/sample) were digested with each of six restriction enzymes (*Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Sst*I) and DNA fragments were separated by electrophoresis on 1% agarose gels, followed by transfer to Hybond Nfp (Amersham Corp., Arlington Heights, Ill.) nylon membranes. After hybridization, membranes were washed 4×20 min at 65°C with 0.5× SSC (0.15 M NaCl and 0.015 M sodium citrate), 0.1% SDS and exposed to X-ray films at –80°C for 1–22 days. To identify polymorphic DNA probes, parental varieties were screened first. Putatively codominant markers were preferentially selected over dominant markers.

Molecular markers

Most of the molecular markers used were DNA clones that produced 355 restriction fragment length polymorphism (RFLP) loci (Table 1). An additional 86 marker loci were mapped, and these included amplified fragment length polymorphisms (AFLPs), randomly amplified polymorphic DNAs (RAPDs), sequence-tagged sites (STSs), a simple sequence repeat (SSR), isozymes, and discrete morphological traits.

Comparative DNA probes

In order to compare the linkage groups of the cultivated oat map with those of other species, we used primarily DNA clones mapped in other grasses (Table 1). The CDO probes represent oat leaf cDNA (Heun et al. 1991). ISU probes originating from oat root tis-

Table 1. Markers used for 'Ogle' × 'TAM O-301' linkage map.

Marker designation	Marker or clone type	No. of markers	No. of polymorphic loci	Average no. of polymorphic loci per marker
RFLP markers				
BCD	Barley cDNA	29	48	1.6
CDO	Oat cDNA	65	106	1.6
RZ	Rice cDNA	25	45	1.8
OG	Oat genomic	2	5	2.5
WG	Wheat genomic	1	3	3.0
PSR	Wheat cDNA or wheat genomic	28	35	1.2
KSU	*T. tauschii* genomic	4	6	1.5
ISU	Maize cDNA	21	45	2.1
OISU	Oat cDNA	16	27	1.7
UMN	Oat cDNA	8	18	2.2
Adh2	Maize cDNA	1	3	3.0
Disease resistance gene analogs				
Srgh	Sorghum genomic	4	6	1.5
PIC	Maize genomic	2	4	2.0
pLrk, pLw	Wheat genomic	2	4	2.0
PCR-based primer sets				
E	AFLP	2	40	20.0
AA-AD	RAPD	11	22	2.0
KV1.9	STS	1	3	3.0
Rast1_4	SSR	1	1	1.0
Amy, Glav	Intron	3	4	1.3
Other markers				
	Isozyme	9	12	1.3
	Trait	4	4	1.0

sue cDNA (Rayapati et al. 1994) are referred to here as OISU clones to distinguish them from the maize ISU clones also used on the same map. UMN clones from the University of Minnesota represent oat cDNAs (Kianian et al. 1997). Of the heterologous probes used, BCD probes represent barley (*Hordeum vulgare* L.) leaf cDNAs (Heun et al. 1991); RZ probes represent rice leaf cDNAs (Causse et al. 1994); PSR probes are both cDNA and genomic clones from wheat (Gale et al. 1995; M.D. Gale, personal communication; Sharp et al. 1989); KSU probes are *Triticum tauschii* genomic DNA (Gill et al. 1991); and ISU probes are maize cDNA clones (Pereira et al. 1994). Genomic clones of oat, OG (Goffreda et al. 1992), and wheat, WG (Heun et al. 1991), were developed at Cornell University. A probe for maize gene *Adh2* (pZmL 184.1) was provided by Dr. E.H. Coe of the University of Missouri–Columbia. Several putative disease resistance gene probes or disease resistance gene analog clones from other cereal crops were also used. PIC20 is a clone of the *Rp1-D* gene of maize (Collins et al. 1998, 1999), which confers resistance to common rust (*Puccinia sorghi* Schwein.). PIC21 is a disease resistance gene analog clone that maps near the maize *Rp3* gene, which also confers resistance to common rust (Collins et al. 1998). The Lrk10 clone is a resistance gene analog from wheat that co-segregates with the *Lr10* gene, which confers resistance to leaf rust (*Puccinia recondita* Roberg et Desmaz) (Feuillet et al. 1997). Lw25 is a clone representing the polymerase chain reaction (PCR) product of conserved regions of the nucleotide binding site (NBS) of a gene with an NBS and leucine-rich motif sequence from wheat (C. Feuillet, personal communication). Disease resistance gene analog probes sRGH1, sRGH8, sRGH25, and sRGH32 from sorghum (*Sorghum bicolor* (L.) Moench.) were supplied by Dr. Jeff Benetzen of Purdue University.

AFLPs and RAPDs

The protocol for AFLP analysis followed that of Hoffman et al. (2000) with two exceptions. First, oat DNA was isolated from plants at the three-leaf stage using the chloroform extraction procedure of Doyle and Doyle (1987). Secondly, instead of labeling *Eco*RI primers with [^{33}P]dATP, an infrared dye was attached to *Eco*RI primers that were custom-made by the Li-Cor Corp. (Lincoln, Nebr.). The final amplification products were run in a 7% w/v Long Ranger® polyacrylamide gel inside a LiCor 4200L automated DNA sequencer. Primer pairs were selected based on the number of polymorphisms and strength of amplification observed in a previous screening experiment. Polymorphisms were scored visually off of a digitally produced image generated by the Base ImagIR® program. AFLP loci were named according to the convention developed by Vuylsteke et al. (1999), where "t" refers to a fragment from 'TAM O-301', and "o" refers to a fragment from 'Ogle'.

The procedure used for RAPD analysis followed that detailed in Hoffman and Bregitzer (1996). Primers were selected based on the number of readable polymorphisms from a previous screening analysis.

STSs

The KV1.9 locus was a sequence-tagged site identified by polymerase chain reaction (PCR) amplification using primers designed by Tragoonrung et al. (1992) based on barley Beta-1-Hordein sequences (GenBank accession X03103; Forde et al. 1985). The primer sequences for this locus were KV1: 5′-CCACCATGA-AGACCTTCCTC-3′, and KV9: 5′-TCGCAGGATCCTGTA-CAACG-3′. The Glav3 loci were detected by designing a PCR

Fig. 1. A linkage map of hexaploid oat constructed using 136 $F_{6:7}$ recombinant inbred lines from the cross 'Ogle' × 'TAM O-301'. Numbers to the left of linkage group intervals refer to map distances of the intervals (in cM). Numbers at the bottom of the linkage groups refer to cumulative lengths of each linkage group. Marker loci with tick marks were assigned as framework loci at LOD > 2.0. Marker loci shown in italics without tick marks were assigned to intervals only (at LOD < 2.0). Probes that produced banding patterns identical to clones included in the framework map are shown in parentheses with the = sign. Probes sharing all except one DNA fragment with framework markers are shown in parentheses with the ~ sign. Loci demonstrating segregation distortion due to excess of one homozygous parental class at $P = 0.05$ or $P = 0.01$, are marked with * and **, respectively. Loci with an excess of heterozygotes at $P = 0.05$ or $P = 0.01$ are marked with † or ††, respectively. Numbers in square brackets indicate linkage groups where a probe is mapped in the 'Kanota' × 'Ogle' molecular map (numbers) and in the *A. atlantica* × *A. hirtula* map (letters).
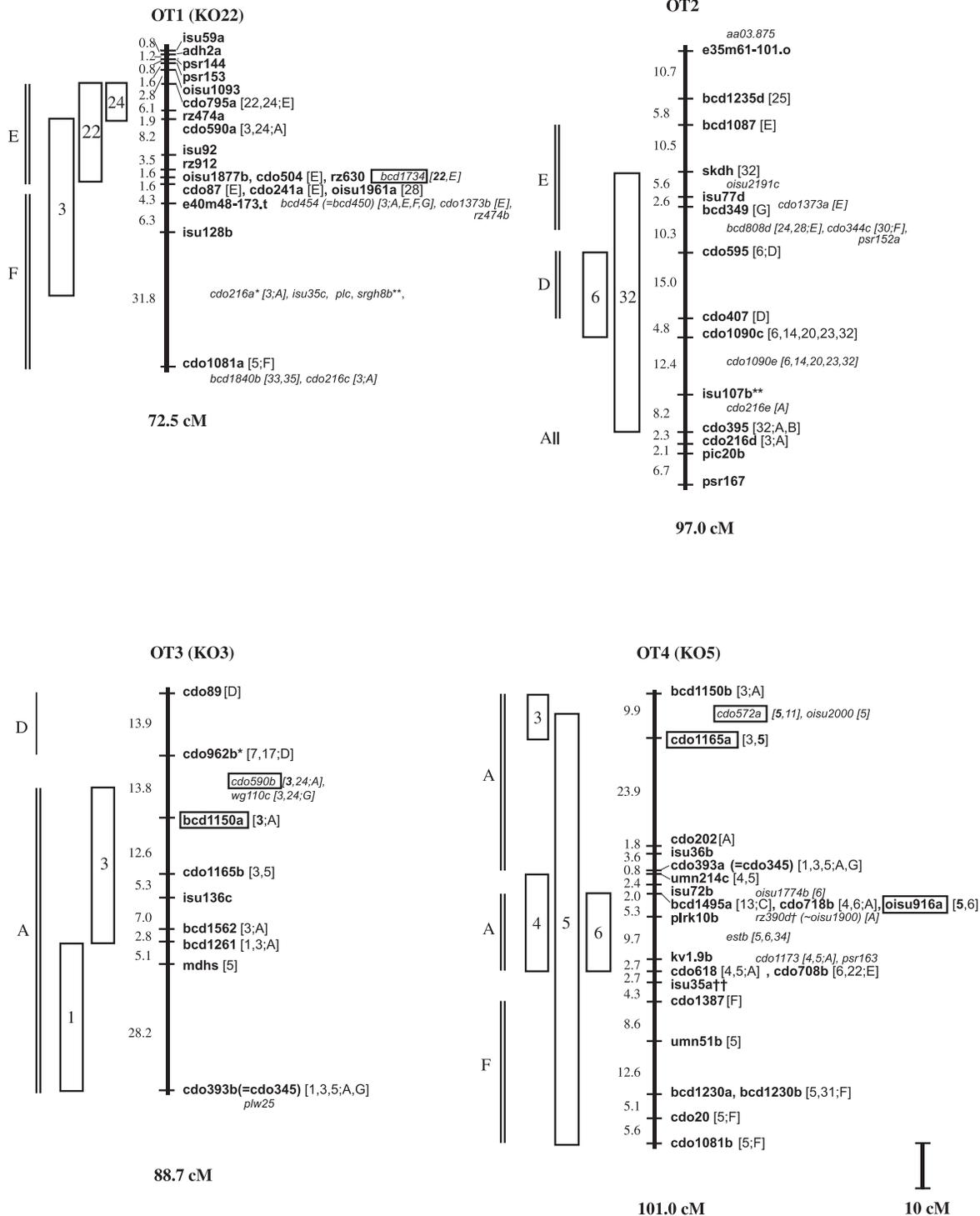
Loci with names in boxes correspond to homologous (allelic) loci mapped in the 'Kanota' × 'Ogle' map, and numbers in bold print in brackets following such loci refer to the linkage group containing the homologous locus on the 'Kanota' × 'Ogle' map. The corresponding homologous linkage group in the 'Kanota' × 'Ogle' map is listed in parentheses following the name of the 'Ogle' × 'TAM O-301' linkage group. Boxes to the left of linkage groups show regions conserved relative to the corresponding 'Kanota' × 'Ogle' linkage groups indicated within boxes. Double solid lines to the left of linkage groups demonstrate segments conserved with the indicated *A. atlantica* × *A. hirtula* linkage groups. Single solid lines show areas putatively rearranged between the 'Ogle' × 'TAM O-301' and *A. atlantica* × *A. hirtula* maps. Figure 1 continued on following pages.
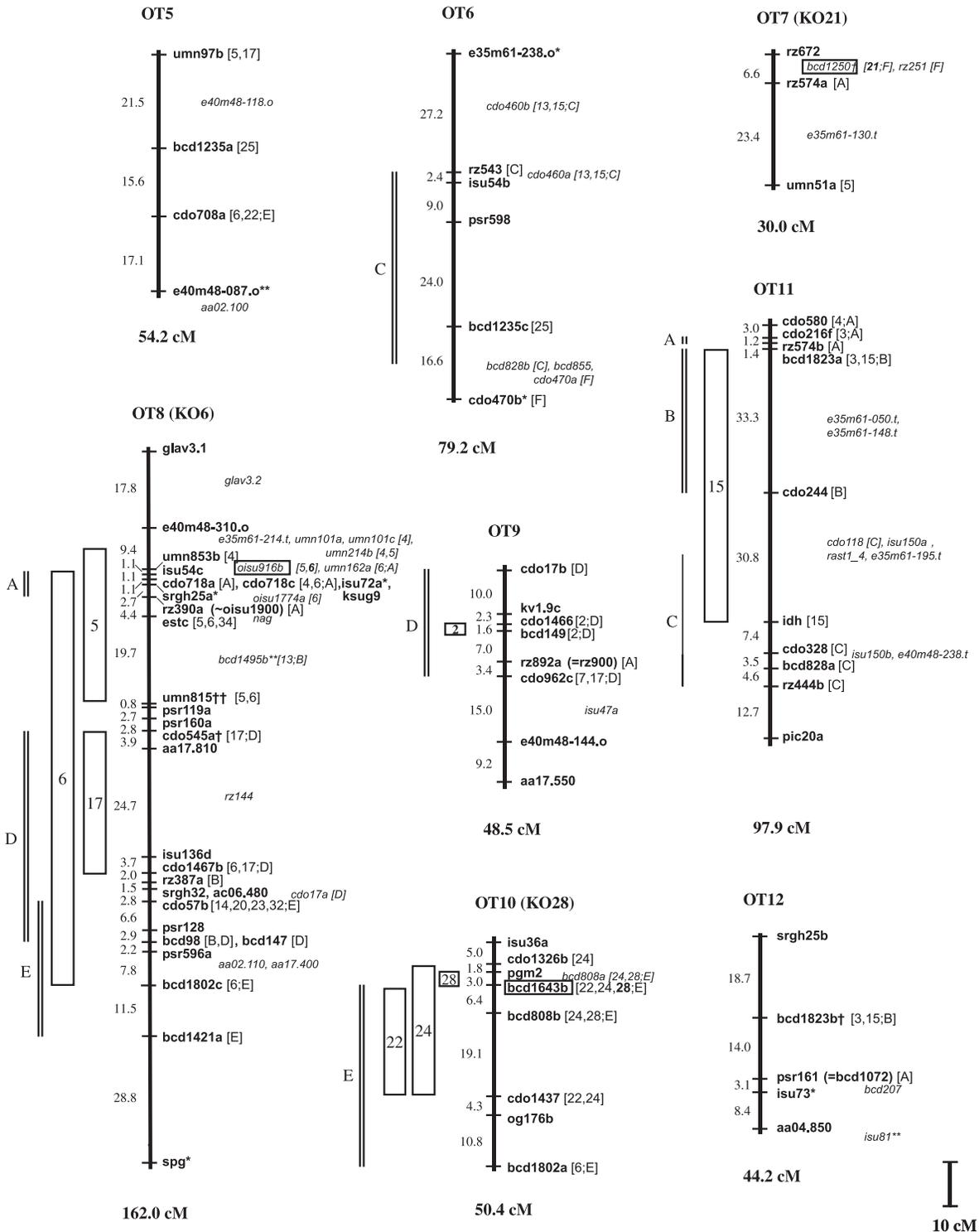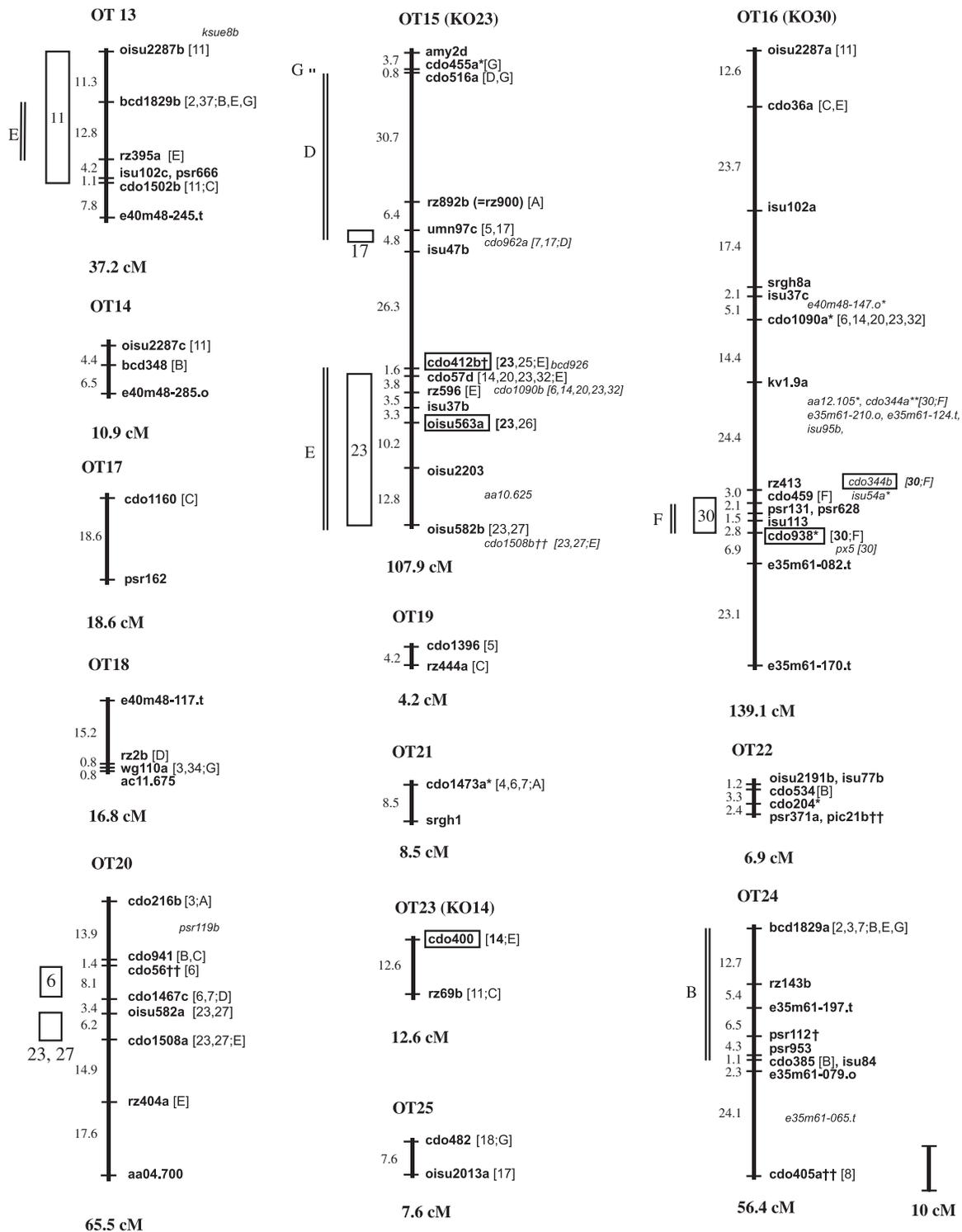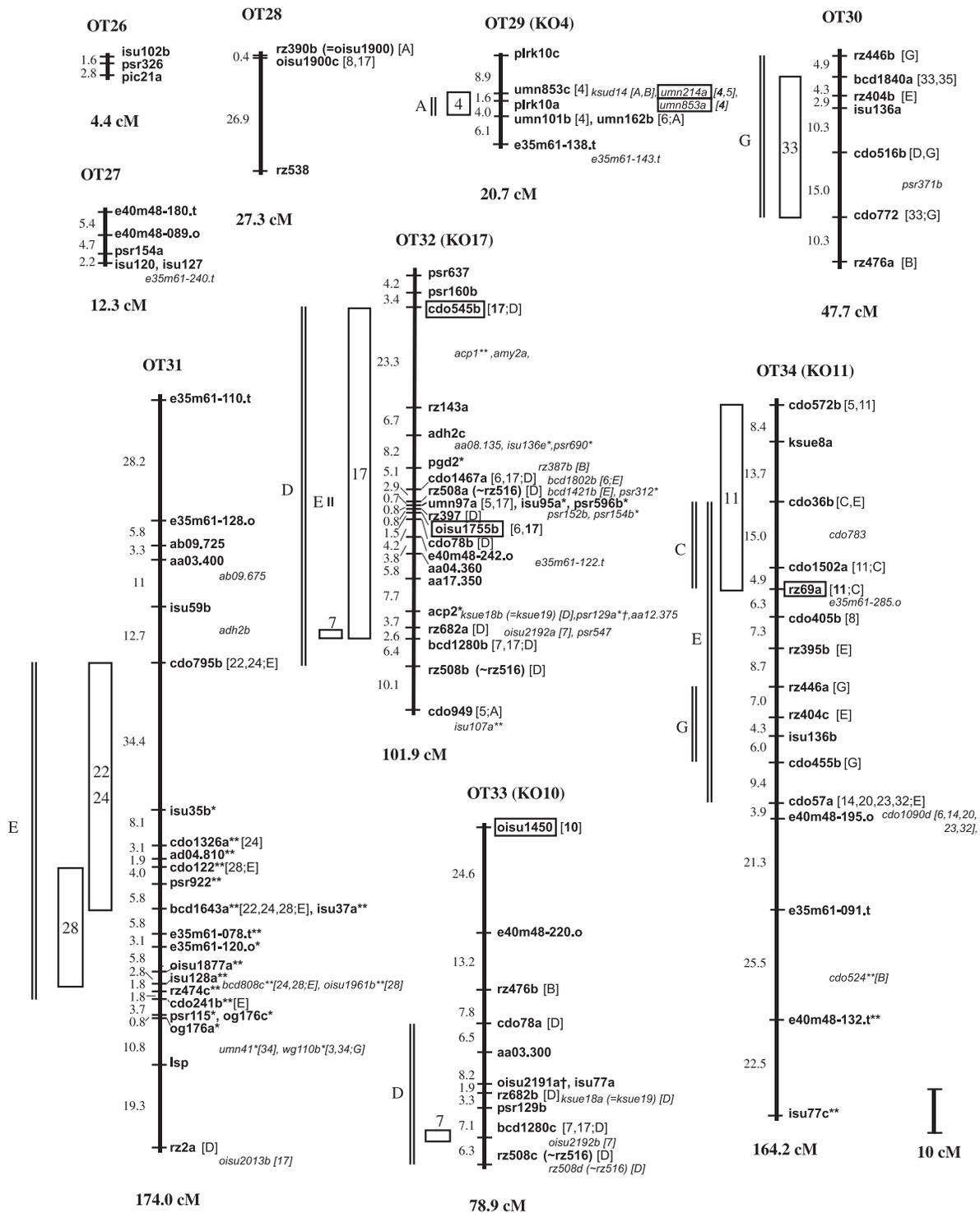
OT5

- umn97b [5,17]
- 21.5 — *e40m48-118.o*
- bcd1235a [25]
- 15.6
- cdo708a [6,22;E]
- 17.1
- e40m48-087.o** — *aa02.100*

54.2 cM

OT6

- e35m61-238.o*
- 27.2 — *cdo460b [13,15;C]*
- 2.4 — rz543 [C] *cdo460a [13,15;C]*
- isu54b
- 9.0
- psr598
- 24.0
- bcd1235c [25]
- 16.6 — *bcd828b [C], bcd855, cdo470a [F]*
- cdo470b* [F]

C

79.2 cM

OT7 (KO21)

- rz672 — bcd1250f *[21;F], rz251 [F]*
- 6.6
- rz574a [A]
- 23.4 — *e35m61-130.t*
- umn51a [5]

30.0 cM

OT8 (KO6)

- glav3.1
- 17.8 — *glav3.2*
- e40m48-310.o — *e35m61-214.t, umn101a, umn101c [4],*
- 9.4 — umn853b [4] — *umn214b [4,5]*
- 1.1 — isu54c — oisu916b *[5,6], umn162a [6;A]*
- 1.1 — cdo718a [A], cdo718c [4,6;A], isu72a*,
- 2.7 — srgh25a* — *oisu1774a [6]* — ksug9
- 4.4 — rz390a (~oisu1900) [A]
- estc [5,6,34] — *nag*
- 19.7 — *bcd1495b**[13;B]*
- 0.8 — umn815†† [5,6]
- 2.7 — psr119a
- 2.8 — psr160a
- 3.9 — cdo545a† [17;D]
- aa17.810
- 24.7 — *rz144*
- 3.7 — isu136d
- 2.0 — cdo1467b [6,17;D]
- 1.5 — rz387a [B]
- 2.8 — srgh32, ac06.480 — *cdo17a [D]*
- cdo57b [14,20,23,32;E]
- 6.6
- 2.9 — psr128
- 2.2 — bcd98 [B,D], bcd147 [D]
- psr596a — *aa02.110, aa17.400*
- 7.8
- bcd1802c [6;E]
- 11.5
- bcd1421a [E]
- 28.8
- spg*

A

5

6

17

D

E

162.0 cM

OT9

- cdo17b [D]
- 10.0
- kv1.9c
- 2.3 — cdo1466 [2;D]
- 1.6 — bcd149 [2;D]
- 7.0
- rz892a (=rz900) [A]
- 3.4 — cdo962c [7,17;D]
- 15.0 — *isu47a*
- e40m48-144.o
- 9.2
- aa17.550

D [2]

48.5 cM

OT10 (KO28)

- isu36a
- 5.0 — cdo1326b [24]
- 1.8 — pgm2 — *bcd808a [24,28;E]*
- 3.0 — bcd1643b [22,24,28;E]
- 6.4
- bcd808b [24,28;E]
- 19.1
- cdo1437 [22,24]
- 4.3 — og176b
- 10.8
- bcd1802a [6;E]

E 22 24 [28]

50.4 cM

OT11

- 3.0 — cdo580 [4;A]
- 1.2 — cdo216f [3;A]
- rz574b [A]
- 1.4 — bcd1823a [3,15;B]
- 33.3 — *e35m61-050.t, e35m61-148.t*
- cdo244 [B]
- 30.8 — *cdo118 [C], isu150a, rast1_4, e35m61-195.t*
- idh [15]
- 7.4
- cdo328 [C] — *isu150b, e40m48-238.t*
- 3.5 — bcd828a [C]
- 4.6 — rz444b [C]
- 12.7
- pic20a

A 15 B C

97.9 cM

OT12

- srgh25b
- 18.7
- bcd1823b† [3,15;B]
- 14.0
- psr161 (=bcd1072) [A]
- 3.1 — isu73* — *bcd207*
- 8.4
- aa04.850 — *isu81***

44.2 cM

10 cM

Fig. 1 (*continued*).

OT 13

ksue8b

oisu2287b [11]

11.3

11

bcd1829b [2,37;B,E,G]

12.8

E

rz395a [E]

4.2 isu102c, psr666

1.1 cdo1502b [11;C]

7.8 e40m48-245.t

37.2 cM

OT14

oisu2287c [11]

4.4 bcd348 [B]

6.5 e40m48-285.o

10.9 cM

OT17

cdo1160 [C]

18.6

psr162

18.6 cM

OT18

e40m48-117.t

15.2

0.8 rz2b [D]
0.8 wg110a [3,34;G]
ac11.675

16.8 cM

OT20

cdo216b [3;A]

13.9 *psr119b*

1.4 cdo941 [B,C]
cdo56†† [6]

8.1

6

3.4 cdo1467c [6,7;D]
oisu582a [23,27]

6.2 cdo1508a [23,27;E]

23, 27

14.9

rz404a [E]

17.6

aa04.700

65.5 cM

OT15 (KO23)

G "

3.7 amy2d
cdo455a*[G]
0.8 cdo516a [D,G]

D

30.7

6.4 rz892b (=rz900) [A]
umn97c [5,17]
4.8 isu47b *cdo962a [7,17;D]*

17

26.3

1.6 cdo412b† [23,25;E] *bcd926*
3.8 cdo57d [14,20,23,32;E]
3.5 rz596 [E] *cdo1090b [6,14,20,23,32]*
3.3 isu37b
oisu563a [23,26]

E 23

10.2 oisu2203

12.8 *aa10.625*

oisu582b [23,27]
cdo1508b†† [23,27;E]

107.9 cM

OT19

4.2 cdo1396 [5]
rz444a [C]

4.2 cM

OT21

cdo1473a* [4,6,7;A]

8.5

srgh1

8.5 cM

OT23 (KO14)

cdo400 [14;E]

12.6

rz69b [11;C]

12.6 cM

OT25

cdo482 [18;G]

7.6 oisu2013a [17]

7.6 cM

OT16 (KO30)

oisu2287a [11]

12.6

cdo36a [C,E]

23.7

isu102a

17.4

srgh8a
2.1 isu37c *e40m48-147.o**
5.1 cdo1090a* [6,14,20,23,32]

14.4

kv1.9a

aa12.105*, cdo344a**[30;F]
e35m61-210.o, e35m61-124.t,
isu95b,

24.4

3.0 rz413 *cdo344b [30;F]*
2.1 cdo459 [F] *isu54a**
1.5 psr131, psr628
2.8 isu113
cdo938* [30;F]
6.9 *px5 [30]*
e35m61-082.t

F 30

23.1

e35m61-170.t

139.1 cM

OT22

1.2 oisu2191b, isu77b
3.3 cdo534[B]
2.4 cdo204*
psr371a, pic21b††

6.9 cM

OT24

bcd1829a [2,3,7;B,E,G]

12.7

rz143b

5.4 e35m61-197.t

6.5 psr112†
4.3 psr953
1.1 cdo385 [B], isu84
2.3 e35m61-079.o

B

24.1 *e35m61-065.t*

cdo405a†† [8]

56.4 cM

10 cM

primer pair to amplify the first intron of the *A. sativa* 11S globulin seed storage protein, Glav3 (GenBank accession X74741; Tanchak et al. 1995). Primer sequences for Glav3 are 5′-TGATGGGGA-TCAAGAAGAGC-3′ and 5′-ATATCGTCGACCTTCCCCAT-3′. Primer pairs were also developed to amplify introns of alpha amylase genes alpha-*Amy2A* (GenBank accession AJ010728) and alpha-Amy2D (GenBank accession AJ010729; Willmott et al. 1998) originally sequenced from *A. fatua* L. Primer sequences are 5′-GCTACGCCTACATCCTCACC-3′ and 5′-CGCGATCTCTTC-CTTGAATC-3′ for locus *Amy2A*, and 5′-TGATGGGGA-TCAAGAAGAGC-3′ and 5′-ATATCGTCGACCTTCCCCAT-3′ for locus *Amy2D*. PCR amplification, fragment separation, and scoring methods followed Senior et al. (1996).

SSR

A simple-sequence repeat (SSR) was discovered within the mRNA coding region of a thaumatin-like pathogenesis-related protein of

Fig. 1 (*concluded*).

OT26

1.6 isu102b
psr326
2.8 pic21a

4.4 cM

OT27

5.4 e40m48-180.t
e40m48-089.o
4.7 psr154a
2.2 isu120, isu127
e35m61-240.t

12.3 cM

OT31

e35m61-110.t

28.2

e35m61-128.o
5.8 ab09.725
3.3 aa03.400
11 *ab09.675*

isu59b

12.7 *adh2b*

cdo795b [22,24;E]

E 22 24

34.4

28

isu35b*
8.1 cdo1326a** [24]
3.1 ad04.810**
1.9 cdo122**[28;E]
4.0 psr922**
5.8 bcd1643a**[22,24,28;E], isu37a**
5.8 e35m61-078.t**
3.1 e35m61-120.o*
5.8 oisu1877a**
2.8 isu128a**
1.8 rz474c** *bcd808c**[24,28;E], oisu1961b**[28]*
1.8 cdo241b**[E]
3.7 psr115*, og176c*
0.8 og176a*
10.8 *umn41*[34], wg110b*[3,34;G]*
lsp
19.3
rz2a [D]
oisu2013b [17]

174.0 cM

OT28

0.4 rz390b (=oisu1900) [A]
oisu1900c [8,17]

26.9

rz538

27.3 cM

OT32 (KO17)

4.2 psr637
3.4 psr160b
cdo545b [17;D]

23.3 *acp1**,amy2a,*

6.7 rz143a
adh2c
8.2 *aa08.135, isu136e*,psr690**
pgd2*
5.1 cdo1467a [6,17;D] *rz387b [B]*
2.9 rz508a (~rz516) [D] *bcd1802b [6;E]*
0.7 umn97a [5,17], isu95a*, psr596b* *bcd1421b [E], psr312**
0.8 rz397 [D] *psr152b, psr154b**
0.8 oisu1755b [6,17]
1.5 cdo78b [D]
4.2 e40m48-242.o
3.8 aa04.360 *e35m61-122.t*
5.8 aa17.350
7.7
3.7 acp2* *ksue18b (=ksue19) [D],psr129a*†,aa12.375*
2.6 rz682a [D] *oisu2192a [7], psr547*
6.4 bcd1280b [7,17;D]

10.1 rz508b (~rz516) [D]

cdo949 [5;A]
*isu107a***

101.9 cM

OT33 (KO10)

oisu1450 [10]

24.6

e40m48-220.o

13.2

rz476b [B]
7.8
cdo78a [D]
6.5
aa03.300
8.2 oisu2191a†, isu77a
1.9 rz682b [D] *ksue18a (=ksue19) [D]*
3.3 psr129b
7.1 bcd1280c [7,17;D]
oisu2192b [7]
6.3 rz508c (~rz516) [D]
rz508d (~rz516) [D]

78.9 cM

OT29 (KO4)

plrk10c
8.9
A 4 umn853c [4] *ksud14 [A,B]* umn214a [4,5]
1.6 plrk10a *umn853a [4]*
4.0 umn101b [4], umn162b [6;A]
6.1
e35m61-138.t
e35m61-143.t

20.7 cM

OT30

4.9 rz446b [G]
bcd1840a [33,35]
4.3 rz404b [E]
2.9 isu136a
10.3
G 33 cdo516b [D,G]
15.0 *psr371b*
cdo772 [33;G]
10.3
rz476a [B]

47.7 cM

OT34 (KO11)

cdo572b [5,11]
8.4
ksue8a
13.7
11 cdo36b [C,E]
15.0 *cdo783*
cdo1502a [11;C]
4.9 rz69a [11;C]
C *e35m61-285.o*
6.3 cdo405b [8]
7.3
rz395b [E]
E 8.7
rz446a [G]
7.0
rz404c [E]
4.3
isu136b
6.0
G cdo455b [G]
9.4
cdo57a [14,20,23,32;E]
3.9 e40m48-195.o *cdo1090d [6,14,20, 23,32],*
21.3

e35m61-091.t

25.5 *cdo524**[B]*

e40m48-132.t**

22.5

isu77c**

164.2 cM

10 cM

A. sativa sequenced by Lin et al. (1996; GenBank accession L39777). A pair of primers was designed to target the SSR region in this gene, and the resulting polymorphic locus was designated *Rast1–4*. The primer sequences are 5′-CTTCTGCCCATGAAACCCTA-3′, and 5′-GAGGGTGCATGTGCTGAGT-3′. PCR amplification, fragment separation, and scoring methods followed Senior et al. (1996)

Isozymes

Plant culture, enzyme extractions, and electrophoresis were conducted as described in Hoffman and Goates (1990). Gel slices for esterase (EST), peroxidase (PER), and phosphoglucomutase (PGM) assays were from a 10.9% w/v starch (Sigma Chemical Corp., St. Louis, Mo.) gel run on a discontinuous tris-citrate lithium borate system described in Hoffman and Goates (1990). Isocitrate dehydrogenase (IDH), 6-phosphogluconate dehydrogenase (PGD), and shikimate dehydrogenase (SKDH) were assayed on slices from histidine-HCl pH 6.5 gels (Cardy et al. 1980). The citric acid gel system of Clayton and Tretiak (1972) was used to produce slices for assays of acid phosphatase (ACP), malate dehydrogenase (MDH), and beta-*n*-acetyl glucosaminidase (NAG).

Staining schedules for all enzymes except NAG followed that of Soltis et al. (1983) with minor modifications. The staining assay used for NAG is described in Weeden and Marx (1987).

Morphological traits

Plumule color (Plc), growth habit (gh), and leaf sheath pubescence (Lsp) were scored on five to eight $F_{6:8}$ plants per recombinant inbred line grown in 15-cm pots in the greenhouse. Plc was scored immediately after emergence, whereas gh and Lsp were scored at the four- to five-leaf stage. Seed pigmentation (Spg) was scored on bulk samples of seed harvested from these plants.

Map construction

Each locus was examined for goodness-of-fit to the expected 1:1 segregation ratio, ignoring heterozygotes, using the χ^2 test. Loci that deviated significantly ($P < 0.01$) from the expected segregation ratio were checked for influence on map order. Distorted loci that caused ambiguous map order were removed from the frame sequences and assigned only to intervals. Codominant RFLP were also examined for goodness-of-fit to the expected ratio of 31:1 homozygotes to heterozygotes using the χ^2 test.

A linkage map was constructed using MAPMAKER 3.0 (Lander et al. 1987). Linkage groups were established using a minimum LOD score of 3.0 and maximum map distance of 34.7 cM, using the Kosambi (1944) mapping function (equal to 30% recombination frequency), with the "join haplotypes" option. Marker loci were ordered within linkage groups using the "order" command with a LOD score of 2.0. Loci placed at LOD < 2.0 were assigned to intervals only.

Of the 30 linkage groups initially produced at LOD 3.0, two (OT1 and OT8) were much larger than the other groups and included 114 and 72 loci, respectively. These linkage groups included about 50% of the distorted loci, therefore we suspected that some of the linkages within these groups were spurious. Furthermore, following multipoint analyses, fewer than half of the markers in each of the two groups were placed uniquely. We increased the LOD score for linkage among markers within these groups until the majority of loci in the resulting smaller linkage groups could be placed uniquely as framework loci. This required a LOD score of 5.7 for OT1, and of 3.5 for OT8. Next, six to eight loci defining the ends of each of the subgroups were tested for linkage using the "near" command. When linkage between flanking loci of a pair of subgroups was significant at LOD > 2.0 and recombination fractions between loci in different subgroups were consistent with the hypothesis that they formed a single linkage group, subgroups were combined into larger linkage groups. Finally, most likely orders of loci determined by multipoint analyses were verified manually by comparison with two-point linkage estimates. Following this procedure, OT1 produced three additional linkage groups (OT31, OT32, and OT33); and two segments separated from OT8 were joined to OT1 and OT34, respectively, using the "assign" command.

Linkage group OT32 remained problematic: it was the only one in which more than half of the loci were assigned only to intervals. This linkage group, therefore, was ordered by first choosing a starting framework of five loci that were well distributed along the length of the linkage group and had consistent two-point and multi-point recombination fraction estimates. The remaining loci were added using the "build" command.

Extension of *A. strigosa* × *A. wiestii* diploid oat map

A diploid oat mapping population was developed from a cross between *A. strigosa* Schreb. (CI 3815) and *A. wiestii* Steud. (CI 1994) (Rayapati et al. 1994). A map based on 100 $F_{6:8}$ recombinant inbred lines derived from this cross and previously developed by Kremer et al. (2001) was extended by including 23 additional RFLP loci. Segregation distortion appeared to cause spurious link-

ages in this population as well, and these were dealt with as in the hexaploid population. Linkage groups referred to as *Asw*BF and *Asw*AC were initially linked together by a strongly distorted region, and this resulted in conflicting two-point and multipoint linkage estimates. Therefore, they were separated at LOD 4.0 into groups with consistent two-point and multipoint map distances.

Map comparisons

The 'Ogle' × 'TAM O-301' (OT) map was compared with the 'Kanota' × 'Ogle' (KO) map developed by O'Donoughue et al. (1995), and with the maps of diploid taxa *A. atlantica* × *A. hirtula* (*Aah*; O'Donoughue et al. 1992; Van Deynze et al. 1995*b*) and *A. strigosa* × *A. wiestii* (*Asw*; Kremer et al. 2001; and updated here). For each comparison, the percent of genome examined was calculated as the proportion of the total map length in the base species flanked by probes mapped in the other species. The percent of genome conserved was calculated as the proportion of the genome examined that could be considered homeologous following Van Deynze et al. (1995*b*). If a locus flanking a comparative segment was mapped to an interval at LOD < 2.0, then the map length of the comparative segment was considered to equal half of that interval plus the remaining map distance to the locus defining the other end of the comparative segment.

'Ogle' was a common parent to both OT and KO populations, therefore some homologous RFLP loci could be identified between the two maps. Homologous loci were identified by comparing fragment sizes of bands from 'Ogle', 'Kanota', and 'TAM O-301' produced by the same restriction enzyme – probe combination and mapped in both KO and OT. The sizes of bands mapped in KO were obtained from the GrainGenes website <http://wheat.pw.usda.gov/ggpages/kxo_autorads.html>. Where such homologies were identified, loci in the OT map were given the same name as their homologous loci in the KO map.

Results

'Ogle' × 'TAM O-301' linkage map

Of 471 DNA clones screened, 70% (329) detected DNA fragment size polymorphism between 'Ogle' and 'TAM O-301' digested with at least one of six restriction enzymes. Two-hundred eight DNA clones used for mapping had an average of 1.7 polymorphic loci per clone (range 1–7), producing a total of 355 segregating RFLP loci. In addition, 40 AFLP, 22 RAPD, 7 STS, 1 SSR, 12 isozyme, and 4 discrete morphological trait loci were mapped, providing a total of 441 loci. Three-hundred twenty-one (73%) loci were codominant. Fifty-nine marker loci (13%) demonstrated segregation distortion ($P < 0.05$) with 24 of them (5%) being distorted at $P < 0.01$. Out of 441 marker loci, 426 formed 34 linkage groups (containing from 2 to 43 loci each) spanning 2049.2 cM of the oat genome (from 4.2 to 174.0 cM per group; Fig. 1). Two-hundred eighty-three loci (64%) were placed uniquely at LOD > 2.0; 25 loci (6%) co-segregated with other mapped loci; and 14 loci remained unlinked. Average map density was 7.2 cM between loci.

A. strigosa × *A. wiestii* linkage map

The addition of 23 marker loci to the diploid oat map previously developed by Kremer et al. (2001) resulted in the combining of their linkage groups *Asw*B and *Asw*F into a single linkage group, designated here as *Asw*BF (Fig. 2). Similarly, linkage groups *Asw*A and *Asw*C were combined into *Asw*AC (Fig. 3). A total of 204 marker loci produced eight linkage groups spanning 861.9 cM (range 2.9–201.1

Fig. 2. Linkage groups of 'Ogle' × 'TAM O-301' (OT) homoeologous to linkage group *Asw*BF of *A. strigosa* × *A. wiestii* map. Regions conserved with respect to *A. atlantica* × *A. hirtula* (*Aah*) linkage groups are superimposed as boxes with *Aah* linkage group designations (D, E, and F) indicated within. Comparative anchor loci on conserved segments showing colinearity to the *Aah* map are underlined. Markers exhibiting segregation distortion at *P* = 0.05 are marked with *, those exhibiting segregation distortion at *P* = 0.01 are marked with **.
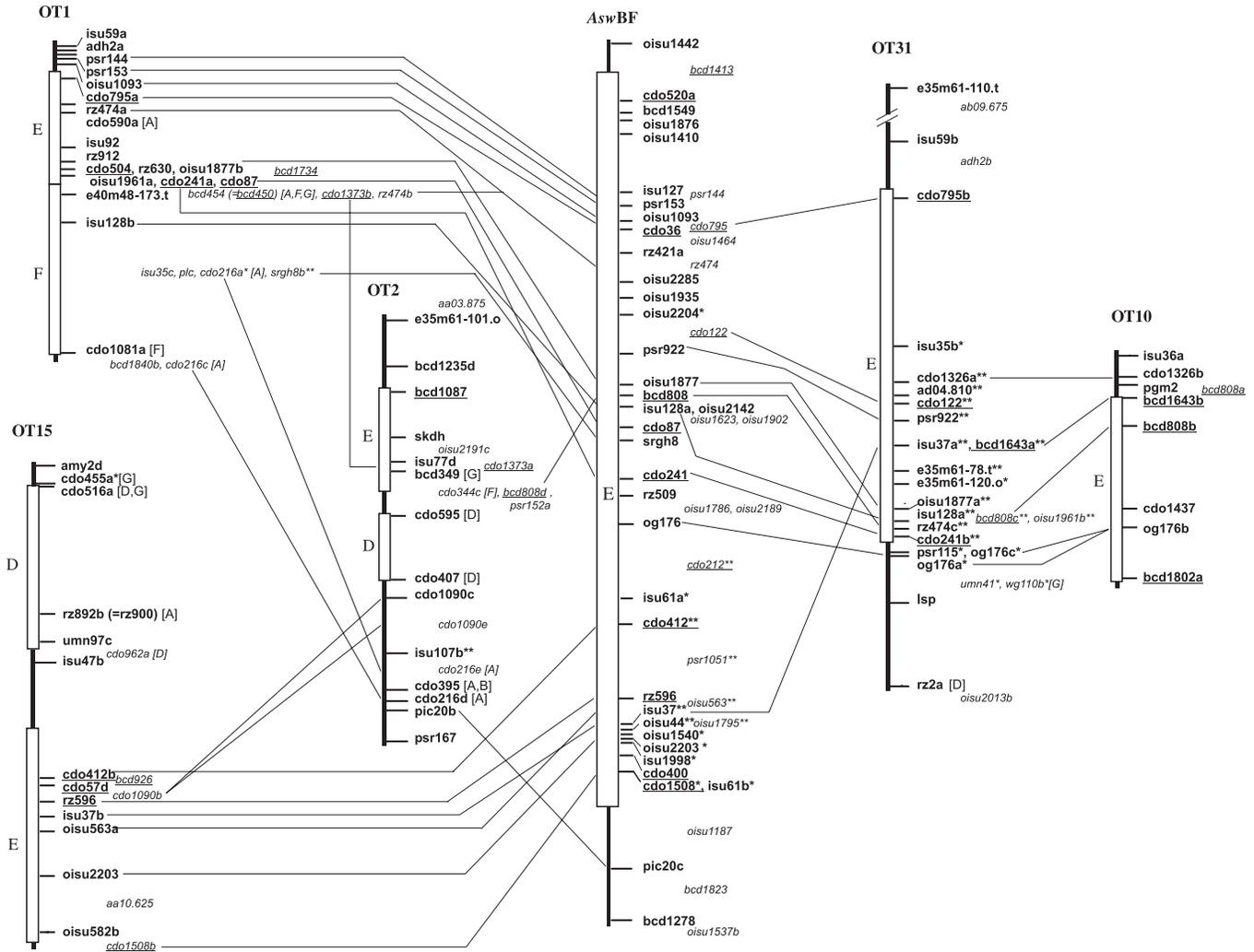
cM) with an average density of 6.5 cM per locus (Figs. 2–5). This is similar to the original map published by Kremer et al. (2001), which had a total map length of 880 cM and average density of 5 cM. The major changes were the grouping of four linkage groups into two for a total of seven major linkage groups equal to the haploid chromosome number of diploid oat (*n* = 7), plus the addition of a very small linkage group (*Asw*J) consisting of only two tightly linked loci, each of which was highly distorted (Fig. 5).

Segregation distortion

Out of 59 distorted markers in the hexaploid map, only 12 (20%) were not linked to at least one other distorted locus. The majority of distorted loci formed extended regions (of 2–19 loci; Fig. 1) within which all distorted loci exhibited an excess of the same parental genotype. Segments showing segregation distortion on linkage groups OT8 (ISU72A–sRGH25A), OT16 (flanked by AA12.105 and e40m48–147.o), and OT34 (CDO524–ISU77C) had an excess of the

'Ogle' genotype (Fig. 1). The longest and most severely distorted segment covered 52 cM on OT31 (flanked by ISU35B and WG110B) and had an excess of the 'TAM O-301' genotype. A second region with excess of the 'TAM O-301' genotype was on OT12 (ISU73–ISU81). Three regions of OT32 (ISU136E–*Pgd2*; PSR312–PSR154B; *Acp2*–PSR129A) also had an excess of the 'TAM O-301' genotype.

The observed mean frequency of heterozygotes among 321 codominant loci was 3.5%, in good agreement with the expectation of 3.1% heterozygosity. The observed exceeded the expected frequency of heterozygotes at the 5% probability level at 15 loci and at the 1% level at 6 loci. The greatest frequency of heterozygotes observed at a single locus was 10% at UMN815. Two loci with excess heterozygosity were observed on each of linkage groups OT4, OT8, OT15, and OT24 (Fig. 1).

In the *Asw* map, only 6 of 38 loci with segregation distortion (16%) were not linked to other distorted loci. The other

Fig. 3. Linkage groups of 'Ogle' × 'TAM O-301' (OT) homoeologous to linkage group *Asw*AC of *A. strigosa* × *A. wiestii* map. Regions conserved with respect to *A. atlantica* × *A. hirtula* (*Aah*) linkage groups are superimposed as boxes with *Aah* linkage group designations (A, D, E, and F) indicated within. Comparative anchor loci on conserved segments showing colinearity to the *Aah* map are underlined. Markers exhibiting segregation distortion at *P* = 0.05 are marked with *, those exhibiting segregation distortion at *P* = 0.01 are marked with **.
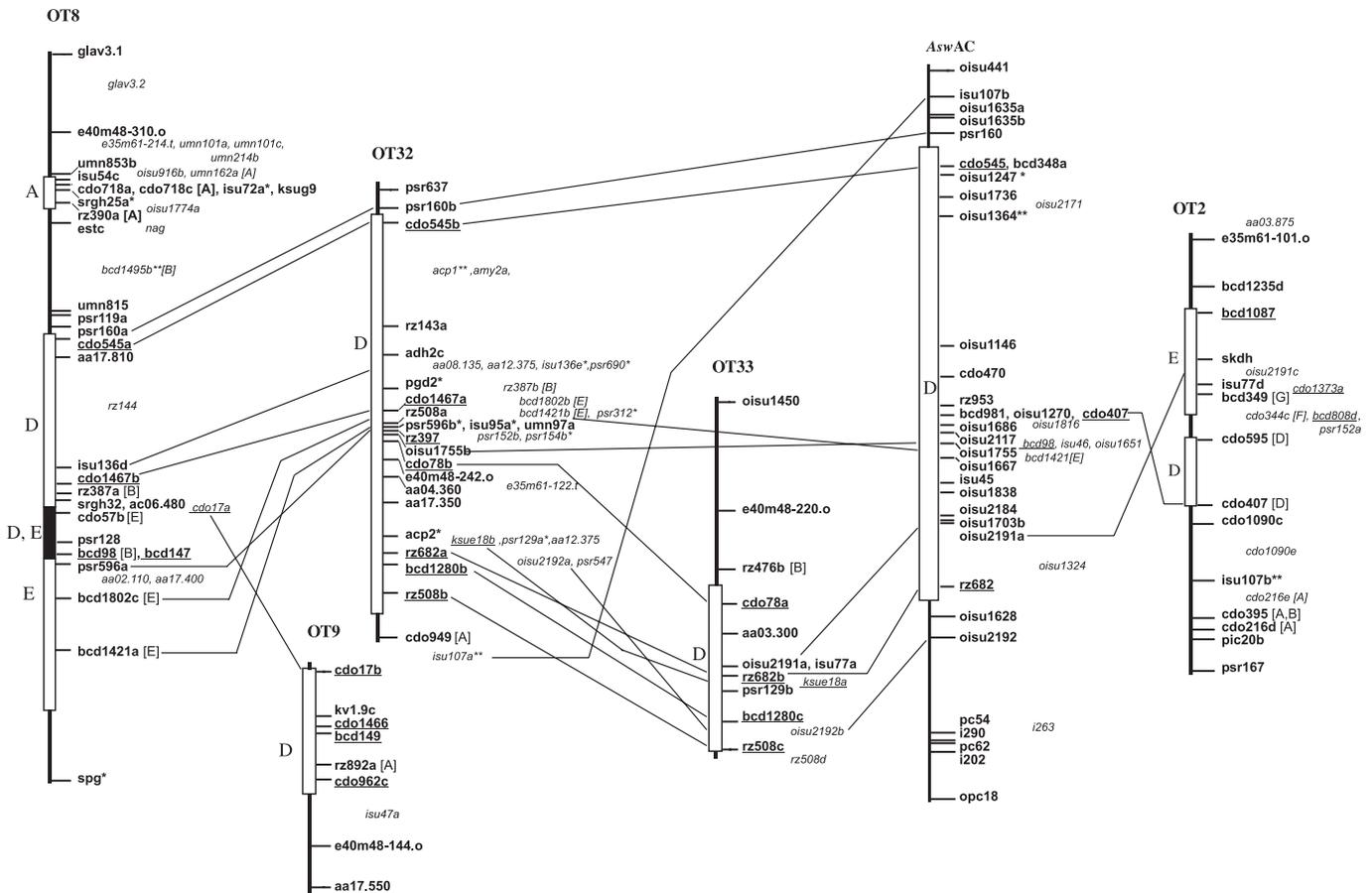
distorted loci mapped to four areas with two of the regions skewed toward the *A. strigosa* parent and two toward the *A. wiestii* parent. The most heavily distorted segment was on linkage group *Asw*BF (Fig. 2).

Distribution of duplicated loci on the 'Ogle' × 'TAM O-301' map

All but one linkage group of the 'Ogle' × 'TAM O-301' map contained markers that cross-hybridized to sequences on another linkage group of the same map. Markers from any one linkage group collectively cross-hybridized to 1 to 15 other linkage groups (Table 2). Thirteen linkage groups contained markers that cross-hybridized to more than 6 other linkage groups (Table 2). Some duplications could be unambiguously classified as paralogous because they mapped to the same linkage group. Such paralogous pairs were detected on 11 linkage groups, and were revealed by 17 probes.

Alignment of hexaploid oat maps

The 'Ogle' × 'TAM O-301' (OT) map and the 'Kanota' × 'Ogle' (KO) map shared 88 polymorphic probes, which identified 135 loci covering 38% of the KO map and 159 loci covering 39% of the OT map (Table 3). Of these 159 markers, 109 were considered to map to syntenic regions

and 50 were distributed among nonsyntenic segments (Table 3). Marker order within the syntenic segments was nearly identical with some minor exceptions, and the correlation between map distances on the two maps was *r* = 0.73 (*P* < 0.0001; Table 3). In most (22 of 35) cases the lengths of OT syntenic genome regions exceeded the corresponding ones of the KO map. The discrepancies were generally not very large, but in seven cases the distance of the segment in the OT map, and in one instance the distance on the KO map exceeded the distance of the homologous segment from the other map by more than two times.

Direct evidence for some syntenous relationships between the OT and KO maps was provided by homologous RFLP loci mapped in both populations. Homologous loci revealed the following linkage group homologies: OT1 = KO22, OT3 = KO3, OT4 = KO5, OT7 = KO21, OT8 = KO6, OT10 = KO28, OT15 = KO23, OT16 = KO30, OT23 = KO14, OT29 = KO4, OT32 = KO17, OT33 = KO10, and OT34 = KO11 (Fig. 1). Only one discrepancy was observed among such homologies; BCD1261 mapped to OT3, near BCD1150A and CDO590B, both of which were also mapped on KO3. The fragment corresponding to BCD1261 on the OT map was named BCD1261A on the KO map and was localized to KO1. This discrepancy could be due to complications intro-

Fig. 4. Linkage groups of 'Ogle' × 'TAM O-301' (OT) homoeologous to linkage group *Asw*D of *A. strigosa* × *A. wiestii* map. Regions conserved with respect to *A. atlantica* × *A. hirtula* (*Aah*) linkage groups are superimposed as boxes with *Aah* linkage group designations (A, D, E, and F) indicated within. Comparative anchor loci on conserved segments showing colinearity to the *Aah* map are underlined. Markers exhibiting segregation distortion at *P* = 0.05 are marked with *, those exhibiting segregation distortion at *P* = 0.01 are marked with **.
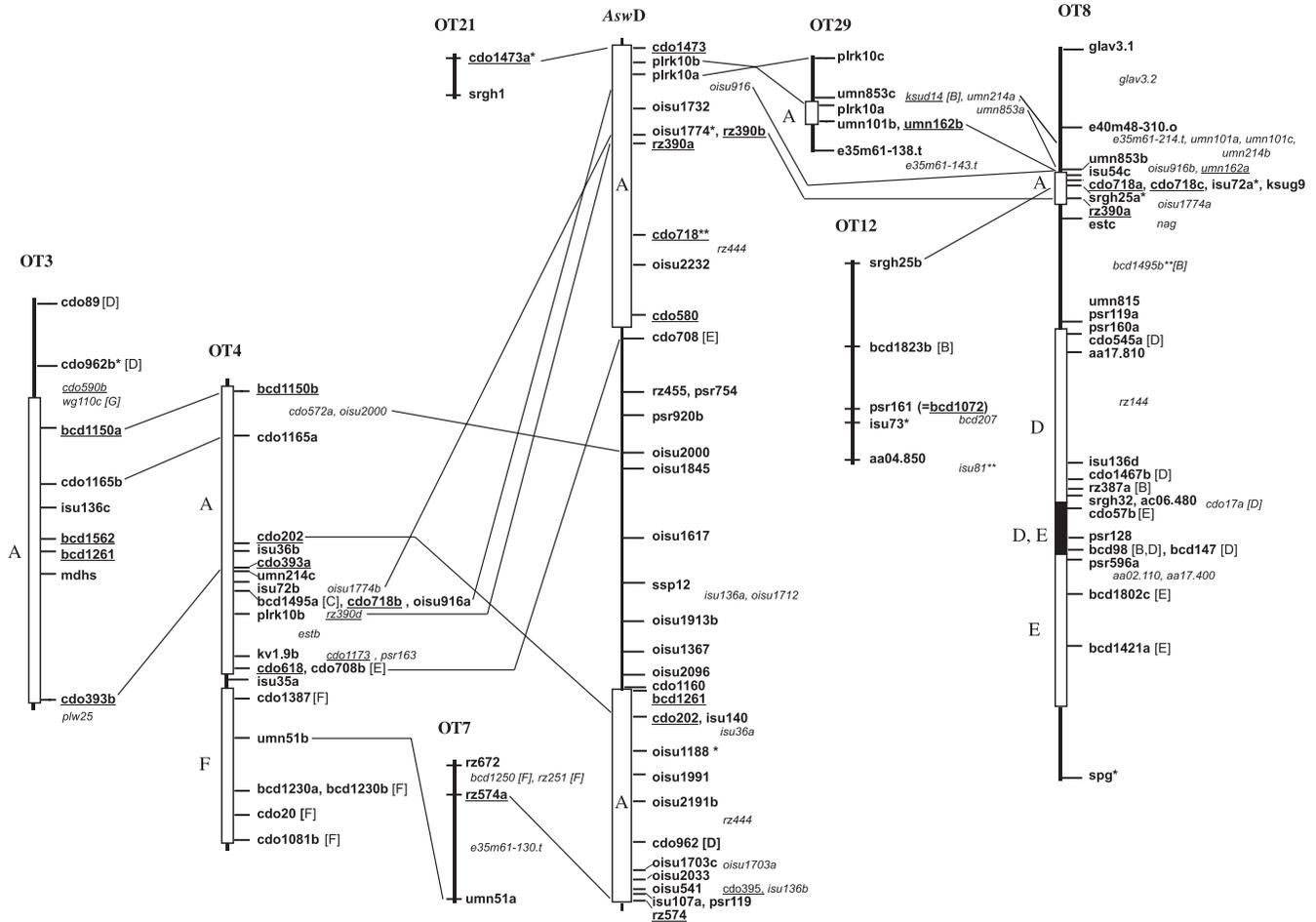
duced by the chromosome 7C-17 translocation, corresponding with linkage groups KO3 and KO24, which differed between 'Kanota' and 'Ogle' parents (Zhou et al. 1999). It is also possible that the KO loci or banding patterns were mislabeled, because the BCD1261B locus mapped to KO3.

Alignment of hexaploid and diploid *Avena* maps

The 'Ogle' × 'TAM O-301' map and *A. atlantica* × *A. hirtula* (*Aah*) map shared 97 polymorphic probes, which identified 162 loci covering 45% of the OT map and 108 loci covering 68% of the *Aah* map (Table 3). The OT and *A. strigosa* × *A. wiestii* (*Asw*) maps shared 70 polymorphic probes, which identified 120 loci covering 28% of the OT map and 80 loci covering 55% of the *Asw* map (Table 3). Thirty-four syntenic segments, ranging in size from 1 to 78 cM, were identified between the OT map and the *Aah* map. Sixteen syntenic segments, ranging in size from 1 to 83 cM, were identified between the 'Ogle' × 'TAM O-301' map and the *Asw* map. Correlations between the lengths of the conserved regions in diploid and OT maps were moderately high for the *Aah* map (*r* = 0.56) and high for the *Asw* map (*r* = 0.86) (Table 3).

Among the linkage groups of the *Aah* diploid map, segments of the A, D, and E linkage groups were the best conserved on the OT map (Fig. 1). The three largest of eight linkage groups of the updated *Asw* map (*Asw*AC, *Asw*D, and *Asw*BF) shared large syntenic regions with the OT and *Aah* maps (Table 3; Figs. 2–4). As expected, the greatest amount of synteny was observed between the two diploid As-genome maps, *Aah* and *Asw* (Figs. 2–5). *Asw*BF had nearly complete identity to *Aah*E, based on 13 markers with nearly identical orders in the two maps (Fig. 2). *Asw*AC and *Aah*D shared 4 markers, arranged in similar but not identical orders (Fig. 3). *Asw*D and *Aah*A shared 8 markers, arranged in identical order on the two maps (Fig. 4).

Several putatively syntenous regions differed strikingly for map lengths between the OT and *Asw* maps. The distance between BCD808D and PIC20B on OT2 was 50 cM, whereas loci identified by the same two probes were separated by 109 cM on *Asw*BF. A region delimited by ISU37 and CDO795 was 43 cM on OT31 and 114 cM on *Asw*BF (Fig. 2). Similarly, the distance between OISU1774B and CDO708B was 19 cM on OT4 and 46 cM on *Asw*D (Fig. 4). These differences were sometimes associated with putative

Fig. 5. Linkage groups *Asw*E, *Asw*G, *Asw*H, *Asw*I, and *Asw*J of the updated linkage map of diploid *A. strigosa* × *A. wiestii* population constructed using 100 F_6-derived recombinant inbred lines from the cross CI3815 × CI1994. Numbers to the left of linkage group intervals refer to map distances of the intervals (in cM). Numbers at the bottom of the linkage groups refer to cumulative lengths of each linkage group. Marker loci with tick marks were assigned as framework loci at LOD > 2.0. Marker loci shown in italics without tick marks were assigned to intervals only (at LOD < 2.0). Loci demonstrating segregation distortion at $P = 0.05$ or $P = 0.01$, are marked with * and **, respectively. Double solid lines to the left of linkage groups demonstrate segments conserved with the indicated *A. atlantica* × *A. hirtula* linkage groups.
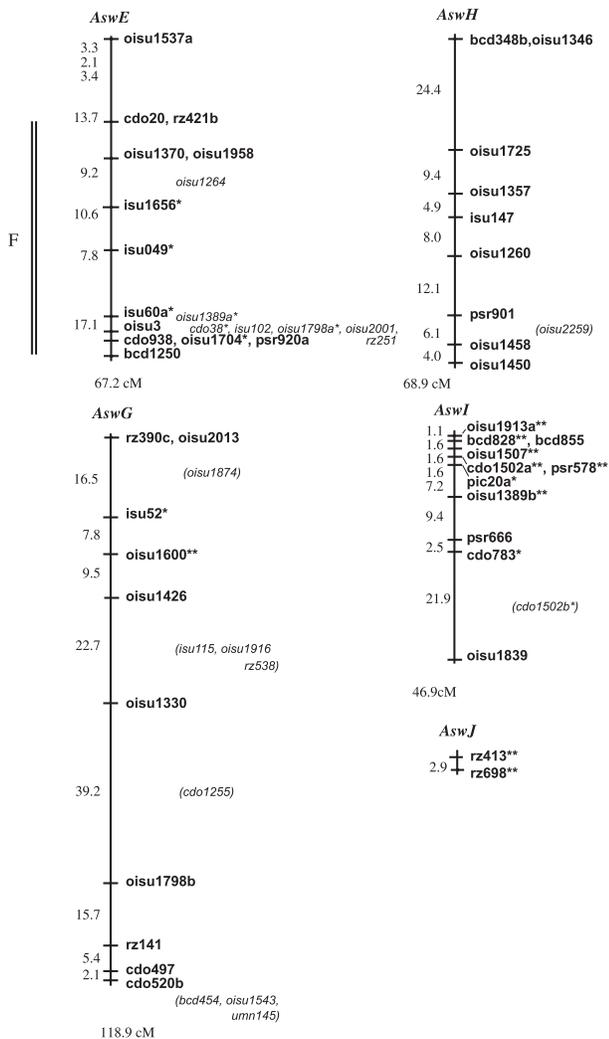
genome rearrangements. For example, a segment flanked by loci identified by PLRK10 and CDO708 and a segment defined by OISU2000 and CDO202 was inverted on OT4 relative to *Asw*D (Fig. 4). A similar inversion may have occurred between *Asw*BF and OT31 (Fig. 2).

Duplications, homoeology, and rearrangements

Alignment of the four *Avena* maps revealed several putative homoeologous linkage group sets as well as some linkage groups composed of segments from different homoeologous groups (Table 4). We declared linkage groups

to be homoeologous if they had syntenous regions defined by at least three markers in common. Homoeology was defined transitively. For example, OT1 and OT10 shared no markers (Table 2). Nevertheless, OT1 was clearly homoeologous to OT31 and *Asw*BF, and both of these linkage groups were, in turn, substantially homoeologous to OT10 (Fig. 2). Therefore, OT1, OT10, OT31, and *Asw*BF were considered to be members of a common homoeologous set (Table 4). Furthermore, we allowed some linkage groups (e.g., OT8) to be considered members of more than one homoeologous set (Table 4). This was necessary because there is substantial evidence that homoeology in *Avena* can be defined for segments of chromosomes, but not necessarily for whole chromosomes (Kianian et al. 1997; Kremer et al. 2001; O'Donoughue et al. 1995).

Discussion

Hexaploid oat map

Most (85%) of the 441 loci used in this study were mapped previously in other cereal species as well as in hexaploid and diploid *Avena*. The resulting hexaploid oat map was 2049 cM in length, significantly longer than the 1482-cM 'Kanota' × 'Ogle' map originally published by O'Donoughue et al. (1995), but similar to the updated version of the KO map, which expanded to 2351 cM following the inclusion of 263 AFLP loci (Jin et al. 2000). The OT map was based on a population that was approximately twice as large as the KO mapping population and included a greater proportion of codominant markers than the KO map. Furthermore, probe selection for the OT map was aided by previous studies, including the KO map (O'Donoughue et al. 1992). These advantages contributed to a greater proportion of loci being mapped to unique positions at LOD 2.0 or greater (64% in OT vs. 52% in KO). Furthermore, the OT map had a lower proportion of loci that co-segregated with the framework loci (6% vs. 13%) and a slightly lower proportion of unlinked loci (3% vs. 5%). Finally, the chromosome 7C–17 translocation may have complicated linkage analysis in the 'Kanota' × 'Ogle' population (O'Donoughue et al. 1995; Zhou et al. 1999), but would not have introduced complications into this map, because both parental lines possess the same form of the interchange (E.N. Jellen, personal communication).

Segregation distortion

Segregation distortion may have been caused by chromosomal microrearrangements related to introgression of alien segments carrying desirable genes from wild germplasm. For example, crown rust resistance genes were introgressed from *A. sterilis* into 'TAM O-301' (McDaniel 1974). Another possible cause of segregation distortion is natural selection, although we minimized this as much as possible. Of 140 F_2 seeds from the cross that were originally chosen to initiate the population, only four failed to germinate. A recombinant inbred line was developed from each of the remaining 136 F_2 plants, suggesting that natural selection was largely avoided. Finally, genes causing gametic or zygotic lethality may have caused segregation distortion. The heavily distorted region on OT31 was homoeologous to a region on *Asw*BF that was also strongly distorted (Fig. 2). These re-

Table 2. Duplications between linkage groups of the 'Ogle' × 'TAM O-301' linkage map identified based on linkage groups sharing cross-hybridizing markers.

Reference linkage group	Linkage groups sharing cross-hybridized markers with reference linkage group	No. of cross-hybridized linkage groups
OT1	OT2 (3; 40.3 cM; 56%), OT3, OT4 (2; 15.9 cM; 22%), OT11, OT16, OT20, OT30, OT31 (9; 30.1 cM; 42%), OT32	9
OT2	OT1 (3; 54.3 cM; 56%), OT5, OT6, OT10, OT11 (2), OT15, OT16 (2; 25.0 cM; 18%), OT20, OT22 (2; 2.8 cM; 3%), OT32, OT33, OT34 (2; 38.9 cM; 40%)	12
OT3	OT1, OT4 (3; 61.0 cM; 69%), OT8, OT9, OT15, OT18, OT30, OT31, OT32, OT34	10
OT4	OT1(2; 36.2 cM; 36%), OT3 (3; 39.2 cM; 39%), OT5, OT7, OT8 (9; 14.6 cM; 14%), OT9, OT10, OT16, OT28, OT29 (3; 9.7 cM; 10%), OT31, OT34	12
OT5	OT2, OT4, OT6, OT15, OT32	5
OT6	OT2, OT5, OT8, OT11, OT16	5
OT7	OT4, OT11	2
OT8	OT3, OT4 (9; 15.1 cM; 9%), OT6, OT9, OT10, OT12, OT15, OT16, OT20 (2; 37.8 cM; 23%), OT28, OT29 (4; 5.2 cM; 3%), OT30, OT32 (8; 53.1 cM; 33%), OT34 (2; 10.0 cM; 6%)	14
OT9	OT2, OT3, OT4, OT8, OT15 (3; 10.9 cM; 22%), OT16	6
OT10	OT2, OT3, OT4, OT8, OT31 (4; 34.6 cM; 69%), OT32	6
OT11	OT1, OT2 (2), OT6, OT7, OT12, OT19, OT20	7
OT12	OT8, OT11	2
OT13	OT14, OT16 (2; 28.3 cM; 76%), OT24, OT26, OT34 (3; 29.4 cM; 79%)	5
OT14	OT13, OT16	2
OT15	OT2, OT3, OT5, OT8, OT9 (3; 11.2 cM; 10%), OT16 (2; 7.3 cM; 7%), OT20 (2), OT30, OT31, 0T32 (2; 41.6 cM; 38%), 34 (3; 72.5 cM; 67%)	11
OT16	OT1, OT2 (2; 26.6 cM; 19%), OT4, OT6, OT8, OT9, OT13 (2; 36.3 cM; 26%), OT15 (2; 5.1 cM; 4%), OT26, OT31, OT32, OT34 (2; 48.5 cM; 35%)	12
OT18	OT31 (2; 0.8 cM; 5%)	1
OT19	OT11	1
OT20	OT1, OT2, OT8 (2; 16.4 cM; 25%), OT11, OT15 (2; 6.2 cM; 9%), OT30, OT32, OT34	8
OT22	OT2 (2), OT26, OT30, OT33 (2), OT34	5
OT23	OT34	1
OT24	OT13, OT32, OT34	3
OT25	OT31	1
OT26	OT13, OT16, OT22	3
OT27	OT32	1
OT28	OT4, OT8	2
OT29	OT4 (2; 0.8 cM; 4%), OT8 (4; 5.2 cM; 25%)	2
OT30	OT1, OT3, OT8, OT15, OT20, OT22, OT32, OT33, OT34 (3; 12.1 cM; 25%)	9
OT31	OT1 (9; 89.3 cM; 51%), OT2, OT3, OT4, OT10 (4; 40.4 cM; 23%), OT15, OT16, OT18 (2; 24.7 cM; 14%), OT20, OT32	9
OT32	OT1, OT2, OT3, OT5, OT8 (8; 50.3 cM; 49%), OT10, OT15 (2; 35.2 cM; 34%), OT16, OT20, OT24, OT27, OT30, OT31, OT33 (8; 34.8 cM; 34%), OT34	15
OT33	OT2 (2), OT22 (2), OT32 (8; 33.3 cM; 42%), OT34	4
OT34	OT2 (2; 71.2 cM; 43%), OT3, OT4, OT8 (2; 15.4 cM; 9%), OT13 (3; 47.2 cM; 29%), OT15 (3; 11.4 cM; 7%), OT16 (2; 70.8 cM; 43%), OT20, OT22, OT23, OT24, OT30 (3; 11.3 cM; 7%), OT32, OT33	14

Note: If the cross-hybridizing markers identify syntenic regions, the number of shared markers, and the map lengths (in cM) and proportions (%) of syntenic segments on the reference linkage group are given in parentheses.

gions are homoeologous to *Aah*E, which in turn is related to chromosomes 5 of Triticeae and 3 of rice (Van Deynze et al. 1995*b*). Segregation distortion in the corresponding chromosomal regions of *Triticum tauschii* and rice due to genes causing gametophytic lethality has been documented (Boyko et al. 1999; Causse et al. 1994; Faris et al. 1998; Xu et al. 1997).

Comparative mapping of putative disease resistance genes

We observed numerous correspondences between regions containing resistance gene analogs on different linkage groups and on different *Avena* maps. For example, markers located in regions homoeologous to one end of *Aah*A were linked to resistance gene analogs in both the OT and *Asw* maps (OT21, OT29, OT8, and *Asw*D; Fig. 4) and in other cereals (O'Donoughue et al. 1996; Van Deynze et al. 1995*b*; Wise et al. 1996; Yu et al. 1996). Similarly, the sRGH8 probe detected a locus on syntenic segments of *Asw*BF and OT1 (Fig. 2). Furthermore, the sRGH8 locus on *Asw*BF was linked to OG176, which Rooney et al. (1994) localized near an *A. strigosa* derived crown rust resistance gene in hexaploid oat. Finally, loci detected by the probe ISU102

Table 3. Comparisons between the 'Ogle' × 'TAM O-301' (OT) map and linkage maps of 'Kanota' × 'Ogle', *A. atlantica* × *A. hirtula*, and *A. strigosa* × *A. wiestii* populations based on shared markers.

Mapping population	Map coverage by OT (%)	Map coverage in OT (%)	Map conservation (%)	No. of syntenic segments	Range of no. of loci per syntenic segment	Range of syntenic segment size (cM)	Correlations[a] between map distances of syntenic segments
'Kanota' × 'Ogle'	38	39	100	35	2–13	1.6–96.1	$r=0.73$***
A. atlantica × *A. hirtula*	68	45	95	34	2–7	1.2–77.8	$r=0.56$***
A. strigosa × *A. wiestii*	55	28	89	16	2–10	1.1–82.9	$r=0.86$***

[a]Spearman rank correlations.
***Significant at the 0.001 probability level.

Table 4. Suggested homoeologous set assignments of linkage groups and linkage group segments from 'Ogle' × 'TAM O-301', 'Kanota' × 'Ogle', *A. atlantica* × *A. hirtula*, and *A. strigosa* × *A. wiestii* maps.

'Ogle' × 'TAM O-301'	'Kanota' × 'Ogle'	*A. atlantica* × *A. hirtula*	*A. strigosa* × *A. wiestii*
OT3, OT4 (BCD1150B–CDO618), OT8 (UMN214B–RZ390A), OT29	KO1, KO3, KO4, KO5 (ACOR254A–UMN51A), KO6 (EstA–CDO82)	*Aah*A	*Asw*D
OT8 (PSR160A–BCD1421A), OT9, OT15 (RZ892B–ISU47B), OT32, OT33	KO6 (CDO1419–UMN826), KO7, KO10[a], KO17	*Aah*D	*Asw*AC
OT1, OT2, OT10, OT13, OT14[a], OT15 (CDO412B–OISU582B), OT16 (CDO1090B–OISU2287B), OT31, OT34	KO11, KO22, KO23, KO24, KO27, KO28, KO32	*Aah*E	*Asw*BF
OT6, OT11 (RZ444B–ISU150A)	KO15	*Aah*C	*Asw*I
OT4 (CDO1387–CDO1081B), OT16 (CDO344B–CDO398)	KO5 (BCD1897A–AvnB), KO30	*Aah*F	*Asw*E
OT30	KO33	*Aah*G	—

[a]Assignment to homoeologous set based on only a single homologous locus mapped in both 'Ogle' × 'TAM O-301' and 'Kanota' × 'Ogle' maps.

mapped close to sRGH8a on OT16 and PIC21 on OT26 (Fig. 1). However, no putative resistance genes were mapped in OT segments homoeologous to the end of *Asw*AC, to which a cluster of known crown rust resistance genes map (Kremer et al. 2001; Wise et al. 1996). Kremer et al. (2001) also mapped five such putative disease resistance genes directly in the *Asw* population, and found that none were linked to this resistance gene cluster. If the position of disease resistance genes remains consistent across *Avena* species, however, we would predict that disease resistance genes exist at the ends of OT32, OT33, and KO17, in regions that are homoeologous to the *Asw*AC segment that contains the rust resistance genes (Fig. 3), and is in turn also homeologous to *Aah*D.

Conservation and rearrangements among the *Avena* genomes

Adding 22 loci that exhibited segregation distortion to the *Asw* map allowed us to combine linkage groups *Asw*A and *Asw*C into *Asw*AC and *Asw*B and *Asw*F into *Asw*BF. This updated map exhibited improved colinearity with the diploid *A. atlantica* × *A. hirtula* map (Van Deynze et al. 1995*b*) relative to the original *Asw* map (Kremer et al. 2001). For example, combining *Asw*A and *Asw*C into a single group increased the length of a segment that seems to be homolo-

gous to *Aah*D (Fig. 3). Similarly, both *Asw*B and *Asw*F were syntenous to portions of *Aah*E (Fig. 2), and therefore *Asw*BF is consistent with the expected homology of diploid As-genome *Avena* taxa. Furthermore, these new groupings exhibited good colinearity with some OT linkage groups (Figs. 2 and 3).

Most of the OT map that could be considered to be conserved relative to the diploid *Aah* map corresponded with linkage groups *Aah*A, *Aah*D, and *Aah*E. Of the total conserved OT map length, 38% was syntenic with *Aah*E, 28% with *Aah*D, and 17% with *Aah*A. Most other regions of the OT map with comparative loci exhibited interchanges and rearrangements relative to the *Aah* map (Fig. 1). For example, OT2, OT15, and OT34 were highly rearranged relative to the *Aah* map (Fig. 1). In summary, conservation of the diploid and hexaploid oat linkage maps was restricted primarily to segments of chromosomes, and rearrangements prevailed at the level of whole chromosomes. We found greater conservation between the OT and KO hexaploid maps than between diploid and hexaploid maps (Table 3).

Following the definition of genomic conservation given by Van Deynze et al. (1995*b*), we found that the hexaploid oat maps demonstrated 100% genome conservation. Yet substantial uncertainty about the relationships of the linkage groups of the two maps remains. We proposed assignments

of some linkage groups to homoeologous sets, but these assignments are not definite, and even so account for only 18 of 34 OT linkage groups, 18 of 38 KO linkage groups, and 5 *Asw* linkage groups (Table 4). Comparisons across maps helped to define the homoeologous sets, but the relationships between linkage groups in different maps will remain uncertain until the relationships among linkage groups within the same hexaploid map are determined. The number of different OT linkage groups to which the markers on a single OT linkage group cross-hybridized was often greater than 10 (Table 2), demonstrating the difficulty of determining homoeology of linkage groups within the OT map.

A major complication in assigning linkage groups to homoeologous sets in oat is the simultaneous existence of orthologous genomic duplications that resulted from polyploidy and those that resulted from other mechanisms. The latter represent nonhomoeologous paralogous duplications, but may not be distinguishable from homoeologous (orthologous) duplications on the basis of mapping data alone. Until linkage groups can be assigned to chromosomes, assignments to homoeologous sets must remain putative. Additional complications arise because paralogous duplications may have occurred at either the diploid, tetraploid, or hexaploid levels. There is considerable evidence of paralogous duplications within diploid *Avena* genomes (Hoffman 1999; Kremer et al. 2001; Van Deynze et al. 1995*b*). Both orthologous and paralogous duplications may be further rearranged by translocations, inversions, or deletions. An example of a translocation between the *Aah* and OT maps that occurs three times was observed on OT2, OT8, and OT15 (Fig. 1), each of which contains segments syntenous to both *Aah*D and *Aah*E. Possible explanations for this are that the translocation occurred three times independently at the hexaploid level or once in a diploid species that was an ancestor of all three hexaploid genomes but not an ancestor of the diploid As-genome species *A. hirtula* and *A. atlantica*. Circumstantial evidence for the latter hypothesis is the observation that apparent translocation differences exist between the *Asw* and the *Aah* As-genome diploid maps, although not necessarily in this region (Kremer et al. 2001).

Mapping data from a single population will likely not resolve the uncertainties about which duplications are paralogous and which are homoeologous, although more mapping data could help to some extent. Assignment of additional mapped loci to chromosomes, through the use of nullisomic stocks (Kianian et al. 1997), for example, would greatly increase understanding of genome organization in *Avena*. The lack of a complete nullisomic set for hexaploid oat limits the potential of cytological analysis, although monosomic stocks can also be used to assign loci to chromosomes (Kianian et al. 2001). A combined approach of comparative mapping within and across ploidy levels and molecular cytogenetic analysis may offer the greatest hope of elucidating the chromosomal relationships among species and genomes in *Avena*.

In addition to its utility in understanding genomic relationships within *Avena*, the development of the OT map based primarily on anchor grass clones will allow comparisons between hexaploid oat and other grass species. To date, such comparisons have been limited to diploid oat (Van Deynze et al. 1995*b*). Interpretations of comparisons between the OT map and genetic maps of other grass species should be made with the understanding that not all of the chromosomal relationships within *Avena* are clear yet. Nevertheless, in the same way that comparisons between hexaploid and diploid *Avena* maps were helpful in better understanding the relationships within hexaploid maps, cross-genera comparisons may also help to improve our understanding of genomic relationships within *Avena*. Finally, the OT map is based on reproducible recombinant inbred lines that can be evaluated phenotypically in multiple environments. The parents of the population are divergent for growth habit, region of adaptation, and crown rust resistances. Therefore, the genes controlling these traits can be mapped in the population. The larger size of the OT mapping population relative to the KO population should provide better ability to detect and localize genes affecting both discrete characters and quantitative traits of agronomic importance.

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