A restriction fragment length polymorphism based linkage map of a diploid *Avena* recombinant inbred line population

C.A. Kremer, M. Lee, and J.B. Holland

Abstract: A population of 100 F_6-derived recombinant inbred lines was developed from the cross of two diploid ($2n$ = 14) *Avena* accessions, CI3815 (*A. strigosa*) and CI1994 (*A. wiestii*). Restriction fragment length polymorphism (RFLP) probes previously mapped in other grass species were used to develop a framework linkage map suitable for comparative genetics. Nine linkage groups were identified among the 181 loci mapped, with an average interlocus distance of 5 cM, and a total genetic map length of 880 cM. A cluster of five tightly linked crown rust resistance genes (*Pca*) was localized on the map, as were five loci identified by disease resistance gene analogs from maize, sorghum, and wheat. None of the five loci identified by the gene analogs were linked to the *Pca* locus. The linkage map was compared with previously published diploid and hexaploid linkage maps in an attempt to identify homologous or homoeologous chromosomes between populations. Locus orders and linkage relationships were poorly conserved between the *A. strigosa* × *A. wiestii* map and other *Avena* maps. In spite of mapping complications due to duplications within a basic genome as well as the allopolyploid constitution of many *Avena* species, such map comparisons within *Avena* provide further evidence of substantial chromosomal rearrangement between species within *Avena*.

Key words: grasses, oat, genetic mapping, homoeology.

Résumé : Une population constituée de 100 lignées recombinantes fixées dérivées en F_6 a été produite à partir d'un croisement entre deux accessions diploïdes ($2n$ = 14) de l'*Avena* : CI3815 (*A. strigosa*) et CI1994 (*A. wiestii*). Des sondes RFLP précédemment utilisées pour la cartographie chez d'autres graminées ont été employées pour développer une carte cadre comparer l'organisation des génomes. Neuf linkats ont été identifiés parmi les 181 locus étudiés. La densité moyenne des marqueurs était de 5 cM et la taille totale de la carte était de 880 cM. Un îlot dense de cinq gènes conférant la résistance à la rouille couronnée (*Pca*) a été localisé sur la carte tout comme cinq locus correspondant à des analogues de gènes de résistance en provenance du maïs, du sorgho et du blé. Aucun des cinq locus identifiés n'était lié au locus *Pca*. La carte génétique a été comparée à des cartes publiées antérieurement pour des avoines diploïdes ou hexaploïdes afin d'identifier des chromosomes homologues ou homéologues entre populations. L'ordre des locus et les distances étaient très mal conservés entre la carte tirée du croisement *A. strigosa* × *A. wiestii* et les autres cartes pour le genre *Avena*. En dépit des complications qu'entraînent les duplications au sein du génome de base et l'allopolyploïdie de plusieurs espèces d'*Avena*, de telles comparaisons de cartes génétiques procurent des évidences additionnelles quant à l'existence d'importantes différences au niveau de l'arrangement des chromosomes parmi les espèces du genre *Avena*.

Mots clés : graminées, avoine, cartographie génétique, homéologie.

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Introduction

The genus *Avena* consists of diploid, tetraploid, and hexaploid species, all of which share the basic chromosome number x = 7. The most important cultivated *Avena* species are the hexaploid ($2n = 6x = 42$) oats, *A. sativa* L., and *A. byzantina* C. Koch. Rajhathy and Thomas (1974) suggested that the hexaploid *Avena* genome is comprised of three subgenomes, A, C, and D, each of which represents modifications of a common homoeologous chromosome se-

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ries. The genomes of diploid and tetraploid *Avena* species are also classified into A, B, C, and D genome groups and into further subdivisions, based on karyotypes and chromosome pairing in interspecific hybrids (Rajhathy and Thomas 1974). The diploid taxa *A. wiestii* Steud., *A. strigosa* Schreb., *A. hirtula* Lag., and *A. atlantica* Baum et Fedak are classified as AsAs, where As is a subdivision of the A genome group that the hexaploid *Avena* possess (Leggett and Thomas 1995; Rajhathy and Thomas 1974). The amount of chromosomal rearrangement among homoeologues in different genome groups both within hexaploid *Avena* and between hexaploid *Avena* and related diploid and tetraploid species is not certain, although Rajhathy and Thomas (1974) suggested that chromosomal rearrangement was an important factor in evolution and speciation within *Avena*. Molecular cytogenetic studies of *A. sativa* confirmed that the chromosomes of hexaploid *Avena* represent segmentally rather than completely homoeologous groups (Kianian et al. 1997).

Comparative mapping using linkage maps of different *Avena* species based on a common set of restriction fragment length polymorphism (RFLP) probes may help elucidate the relationships between the genomes of different species and between the different genomes within the cultivated hexaploid *Avena*. The diploid relatives of cultivated *Avena* could represent model species for *Avena* genetics because of their close taxonomic relationship to *A. sativa* and their simpler genomes. RFLP linkage maps have been developed in diploid *Avena* F_2-derived populations developed from crosses between *A. atlantica* and *A. hirtula* (O'Donoughue et al. 1992; Van Deynze et al. 1995) and between *A. strigosa* and *A. wiestii* (Rayapati et al. 1994*a*). Comparisons between the *A. atlantica* × *A. hirtula* RFLP map and a map based on the cross of the hexaploid *Avena* cultivars 'Kanota' and 'Ogle' revealed that there were numerous hexaploid *Avena* chromosomes that contain segments homoeologous to different diploid *Avena* chromosomes. This result provides further evidence for considerable structural rearrangement between the chromosomes of hexaploid *Avena* and its diploid relatives (O'Donoughue et al. 1995).

Populations of recombinant inbred lines (RILs) offer several advantages over F_2-derived mapping populations. All genotypes within a line are nearly genetically identical, allowing replication of the line for phenotypic evaluation and for distribution to a user community. New DNA markers can be mapped relative to the existing map, because the RILs represent a permanent population. Also, the additional meioses associated with the generations of self-fertilization during RIL development provide additional opportunity for recombination between tightly linked genes. As an example, a single locus, *Pca*, conferring resistance to at least eight isolates of *Puccinia coronata* Corda var. *avenae* W.P. Fraser and Ledingham, the causal agent of crown rust disease in *Avena*, was identified and mapped in the *A. strigosa* × *A. wiestii* $F_{2:3}$ population (Rayapati et al. 1994*a*, 1994*b*). Evaluation of the F_6-derived RILs for resistance to *P. coronata* identified a cluster of five tightly linked loci conferring resistance to specific isolates of *P. coronata* (Wise et al. 1996).

The objectives of this experiment were to develop a framework RFLP linkage map of a diploid oat RIL population and to map candidate genes for resistance to crown rust in the population. The framework map will be useful for other *Avena* researchers, because the population on which it is based is a permanent population of nearly homozygous lines. To permit comparisons with genetic maps of other grass species, we primarily used "grass anchor" clones that have already been mapped in other species as probes (Van Deynze et al. 1998). Mapping of known genes and RFLP probes that have been mapped in other species will allow comparative mapping between the diploid *Avena* As genome, the hexaploid *Avena* genomes, and the genomes of other grass species. Furthermore, the *Pca* cluster of crown rust resistance genes in this population may serve as a model for the genomic organization of crown rust resistance genes in the cultivated hexaploid *Avena*. A high-resolution genetic map of the *Pca* region will be helpful to understanding the relationships between crown rust resistance genes in diploid and hexaploid *Avena*. Wise et al. (1996) reported that the closest DNA marker loci linked to the *Pca* region were an RFLP locus 24 cM proximal to *Pca* and a randomly amplified polymorphic DNA (RAPD) locus 11 cM distal to the region. Therefore, we mapped clones of candidate genes known or presumed to confer resistance to rust or other diseases in other grass species in hope of identifying homologous loci within or near the *Pca* gene cluster.

Materials and methods

Mapping population

A population of 100 $F_{6:8}$ progeny was produced by single seed descent from a cross between *Avena strigosa* (CI 3815) and *Avena wiestii* (CI 1994). The recombinant inbred lines used herein are a different sample of progeny from the same population used to produce an earlier map for diploid *Avena* (Rayapati et al. 1994*a*). CI 3815 is resistant to at least 40 isolates of crown rust (*Puccinia coronata*) and CI 1994 is susceptible to these same isolates (Wise and Gobelman-Werner 1993). Three F_1 seeds from a single panicle were used to produce the F_2 generation. The recombinant inbred lines (RILs) were developed by successive generations of self-pollination of individual plants descended from 100 F_2 plants through the F_6 generation. The panicles were bagged to prevent cross-pollination.

DNA preparation

Genomic DNA was isolated from lyophilized leaf and stem tissue harvested from plants before flowering using a modified CTAB (cetyltrimethylammonium bromide) extraction (Saghai-Maroof et al. 1984). A minimum of five plants per line was sampled, and the tissue was bulked before grinding. DNA quantification, digestion, fragment separation, Southern blotting, and probe preparation and hybridization were described by Veldboom et al. (1994). Southern blots used to screen the parents for polymorphism were made with nuclear DNA (10 μg/lane) from the two parental lines digested with each of six different restriction endonucleases, *Bam*HI, *Dra*I, *Eco*RI, *Eco*RV, *Hin*dIII, and *Sst*I. Southern blots used to collect segregation data from the mapping population were constructed with the genomic DNA (10 μg/lane) from the two parental lines, and each of the 100 RILs from the mapping population was digested with a single restriction enzyme.

Table 1. Number of clones screened, their detected polymorphism, and average number of polymorphic loci detected per clone for cDNA and genomic clones from barley, oat, rice, wheat, maize, and sorghum used to construct the genetic linkage map of the *A. strigosa* × *A. wiestii* RIL population.

Clone designation	Type	Source	No. screened	Polymorphism detected (no.)	Average no. of polymorphic loci per clone
BCD	Barley cDNA	Cornell "Anchor" Set	20	8	1.1
CDO	Oat cDNA	Cornell "Anchor" Set	60	22	1.0
RZ	Rice cDNA	Cornell "Anchor" Set	65	13	1.2
WG	Wheat genomic	Cornell "Anchor" Set	1	0	0.0
PSR	Wheat cDNA and genomic	John Innes Center	66	9	1.1
UMN	Oat cDNA	University of Minnesota	1	1	1.0
pZmISU	Maize cDNA	Iowa State University	52	17	1.2
Putative disease resistance gene clones					
PIC	Maize genomic	Kansas State University	4	1	2
pLRK10A	Wheat genomic	University of Zurich	1	1	1.0
PLW25	Wheat genomic	University of Zurich	1	0	0.0
rRGH	Rice genomic	Purdue University	1	0	0.0
sRGH	Sorghum genomic	Purdue University	4	1	1.0
mr505	Maize genomic	Purdue University	1	0	0.0

RFLP probes

In order to make comparisons between the linkage map of diploid oat and the linkage maps of other grass species, we primarily used cDNA clones that were previously mapped in other species. A set of cDNA "grass anchor" clones (Van Deynze et al. 1998) was provided by Susan McCouch of Cornell University. The grass anchor clone set contained cDNA clones from *Avena*, barley (*Hordeum vulgare* L.), and rice (*Oryza sativa* L.), and one wheat (*Triticum aestivum* L.) genomic clone (Table 1). In addition, we screened five BCD and six CDO clones that O'Donoughue et al. (1992) mapped in the *A. atlantica* × *A. hirtula* population. Mike Gale of the John Innes Center provided a set of 73 wheat genomic and cDNA clones (described by Sharp et al. 1989). Eighty-nine oat cDNA clones (OISU0003, OISU0441–OISU2285) and 52 maize (*Zea mays* L.) cDNA clones (ISU032A–ISU151) from Iowa State University that were described by Rayapati et al. (1994*a*) and Pereira et al. (1994), respectively, were also used. The *Avena* DNA clones with "ISU" designations in Rayapati et al. (1994*a*) are referred to here as "OISU" clones to distinguish them from the maize "ISU" clones. One *Avena* genomic clone (UMN145) that O'Donoughue et al. (1995) mapped in hexaploid *Avena* was provided by Ron Phillips of the University of Minnesota. An additional *Avena* genomic clone (OG176) was provided by Mark Sorrells of Cornell University and was described by Rayapati et al. (1994*a*).

Four maize genomic clones (PIC), possibly derived from disease resistance genes, were provided by Scot Hulbert of Kansas State University (Collins et al. 1998). PIC13 and PIC20 are clones from, or closely linked to, the *rp3* and *rp1* common rust (*Puccinia sorghi* Schwein.) resistance loci. The PIC21 clone is also linked to the *rp3* locus. A locus detected by PIC19 is located near *rhm1* and *wsm1* loci, which confer resistance to northern corn leaf blight (*Exserohilum turcicum* (Pass.) K.J. Leonard & E.G. Suggs) and wheat streak mosaic virus, respectively. Two wheat genomic clones, possibly derived from disease resistance genes, were provided by Catherine Feuillet from the University of Zurich. The pLRK10A clone is a putative leaf rust (*Puccinia recondita* Roberge ex Desmaz) resistance gene clone (Feuillet et al. 1997) and the PLW25 clone is a polymerase chain reaction (PCR) product obtained from conserved regions of the nucleotide binding site of a gene with a nucleotide binding site and a leucine-rich repeat motif sequence (C. Feuillet, personal communication). Five genomic clones of disease resistance gene analogs from rice (rRGH8), maize (mr505), and sorghum (*Sorghum bicolor* (L.) Moench.; sRGH8, sRGH25, sRGH32) were provided by Jeff Bennetzen of Purdue University. The RFLP clones used herein are low-copy sequences that generally produced one to three intense bands on the autoradiograms. The autoradiograms of the Southern hybridizations with probes derived from the putative disease resistance genes generally exhibited one to two intense bands and five or more faint bands. The intense bands identified the polymorphism used in linkage analysis.

Sequence-tagged-site marker

A PCR primer pair was designed to amplify the second intron of a 12S globulin seed storage protein, GenBank accession X17637 (Schubert et al. 1990). The primer sequences are TGTTACC-AGCCAACAAGAACTC and TTCGACGTAAACAACAACGC. PCR amplification, fragment separation, and scoring methods followed Senior et al. (1996).

Data analysis

Autoradiograms from the mapping population were scored twice and independently verified for parental allele patterns. Phenotypic scores of resistance or susceptibility to five isolates *Puccinia coronata* and genotypic data from a RAPD marker locus (*ISUC18*) reported by Wise et al. (1996) were used to map crown rust resistance loci and the RAPD locus on our map.

Chi-square tests were performed to determine if loci segregated in the expected F_6 ratio of 48.4375 : 3.125 : 48.4375. RFLP loci that did not segregate as expected for a single locus at $p < 0.01$ were eliminated from the data set because they may lead to false-positive linkage (Lorieux et al. 1995). RFLP loci that did not segregate as expected for a single locus at $0.01 < p < 0.05$ were added to the map only after the initial linkage group assignments had been made.

MAPMAKER/EXP version 3.0b (Lander et al. 1987) was used for linkage analysis. Loci linked with a minimum LOD of 3.0 and a maximum distance of 40 centimorgans (cM, Haldane) were placed into linkage groups using the two-point "group" command. Multipoint analysis was then performed to determine linear arrangements by using the "order" command with a LOD of 3.0 and a maximum distance of 40 cM. The LOD was then lowered to 2.0

Table 2. Number of loci, map length, and average map distance between loci for each linkage group of the *A. strigosa* × *A. wiestii* RIL map.

Linkage group	No. of marker loci	Map length (cM)	Average map distance between loci (cM)
A	30	105.0	3.5
B	32	141.0	4.4
C	10	32.4	3.2
D	42	185.9	4.4
E	20	82.4	4.1
F	11	64.6	5.9
G	17	145.4	8.6
H	10	79.0	7.9
I	5	44.1	8.8
Unlinked loci	4	—	—
Total	180	879.8	
Average per linkage group	19.6	97.8	5.0

to place additional markers once a linear order was established. The "ripple" command was used to verify the linear arrangement. The "compare" command was required to generate a linear order for smaller linkage groups containing eight or fewer loci. Two loci (OISU1410, OISU1464) were discarded because of presumably spurious linkage to more than one linkage group. Loci that did not segregate as expected for a single locus at $0.01 < p < 0.05$ were added using the "assign" (LOD 3.0, max. distance 40 cM), "place," and "together" commands after the linkage groups were framed. Map distances in centimorgans were calculated using the Kosambi function (Kosambi 1944) to simplify comparisons to other grass genetic maps, which were constructed with the Kosambi function (Van Deynze et al. 1995).

The multipoint analysis of the "order" command separated one linkage group into two. The four possible combinations of terminal loci from each new linkage group were tested for linkage by attempting to force an order and using the "map" command. The two linkage groups could not be joined without creating a gap of more than 50 cM. Therefore we considered these to be separate linkage groups. We attempted to link smaller linkage groups together by testing their terminal loci for linkage based on χ^2 tests. Where such linkage was found at $p < 0.01$, the linkage groups were combined and remapped.

Comparative mapping

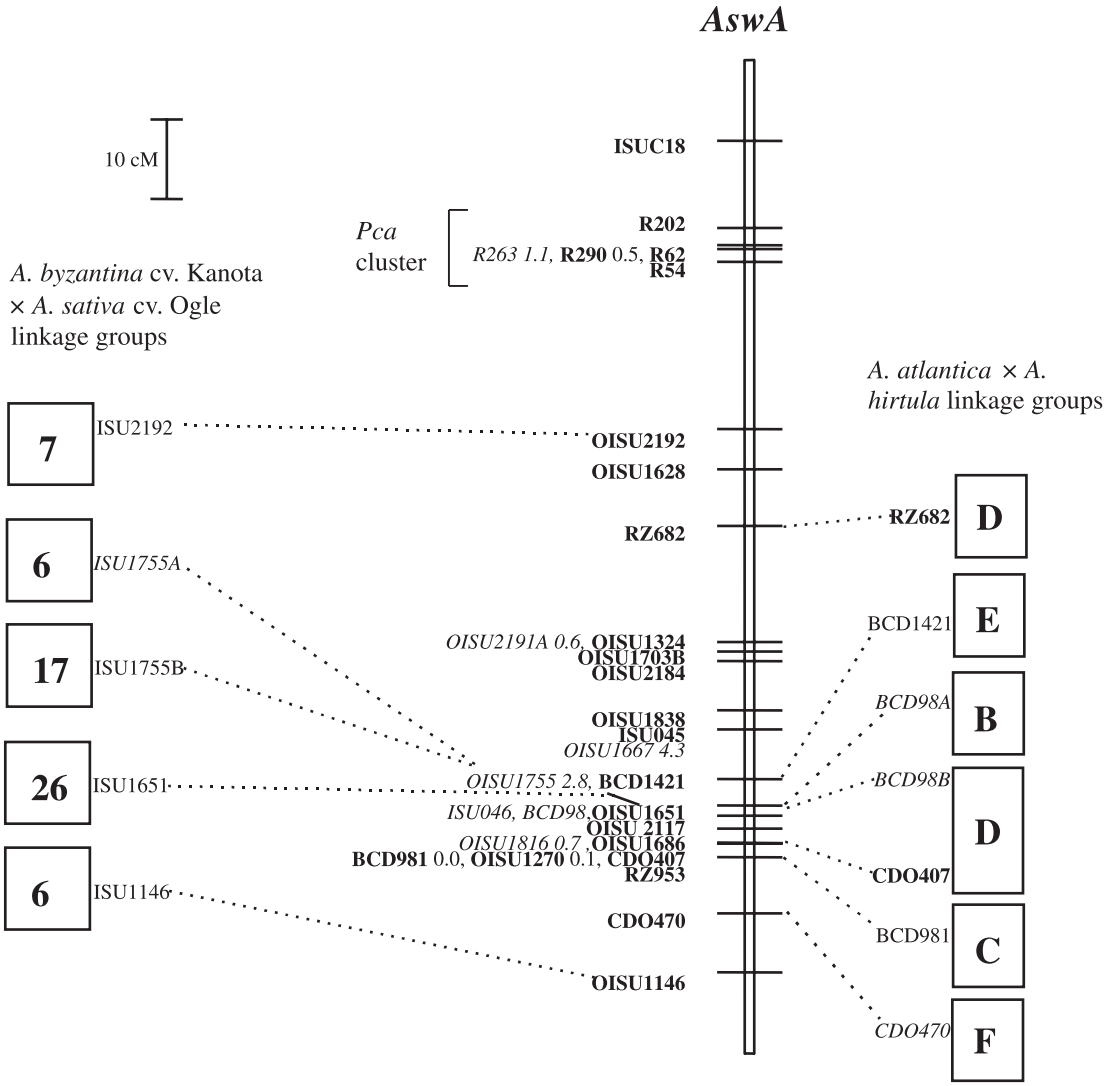
We compared the *A. strigosa* × *A. wiestii* RIL map (i.e., *Asw*RI) to the *A. atlantica* × *A. hirtula* (*Aah*) map (O'Donoughue et al. 1992; Van Deynze et al. 1995) and to the *A. byzantina* cv. Kanota × *A. sativa* cv. Ogle map (O'Donoughue et al. 1995). For comparison with a specific map, we first defined a skeletal *Asw*RI map containing only loci identified by markers shared with the map to which comparisons were being made. Next, we identified intervals defined by adjacent loci on the skeletal map. If the loci defining the interval were also located on the same linkage group of the other map, the interval was considered to be conserved between the two maps. Otherwise, the interval was considered to be rearranged between the two maps. The number of conserved intervals was divided by the total number of intervals to obtain the percentage of conserved intervals. This method provides a liberal assessment of linkage conservation between maps, because intervals were considered conserved when the flanking loci were on the same linkage group in both maps, irrespective of locus order.

Results and discussion

The DNA clones and sequences identified 203 loci; 177 of which were assigned to nine linkage groups of the *Asw*RI linkage map. Twenty of 203 loci were not included in the map because they did not segregate in expected ratios (at $p < 0.01$). Ten loci exhibited excess of the CI 3815 homozygous class and 10 exhibited excess of the CI 1994 homozygous class. One locus (*OISU1364*) had an excessive number of heterozygotes, and also an excessive number of homozygotes from one parental class. Most clones detected a single polymorphic locus, although 14 clones (PIC20, ISU107, ISU136, OISU1389, OISU1537, OISU1635, OISU1798, OISU1913, OISU2191, RZ421, CDO520, CDO1502, BCD348, and PSR920) detected two loci, and two clones (OISU1703, and RZ390) detected three polymorphic loci (Table 1).

The average percentage of detected polymorphism varied among the groups of clones, from 13.6% for the PSR wheat cDNA clones to 40% for the BCD barley cDNA clones (Table 1). The wheat clones (WG and PSR) had the lowest detected polymorphism percentage (Table 1). The low polymorphism detected by the PSR clones was due in part to the negligible hybridization signal given by numerous clones. The PSR clones had only been used to map in close relatives of wheat (Sharp et al. 1989) and were not selected based on ability to hybridize across distantly related grass species, as was the Cornell grass anchor clone set (Van Deynze et al. 1995). Twenty percent of the rice (RZ) clones detected polymorphism (Table 1). This low frequency may be attributed to rice probes often not hybridizing to *Avena* DNA because of the distant evolutionary relationship between oat and rice. The pZmISU clones (ISU032A–ISU151) from maize cDNAs were used to map RFLP loci in maize and sorghum (Pereira et al. 1994) and 33% of these clones detected polymorphism in this *Avena* population (Table 1). The oat cDNA (CDO) clones and the barley cDNA (BCD) clones from the Cornell grass anchor set detected a polymorphism percentage rate of 37% and 40%, respectively (Table 1). The barley cDNA (BCD) and *Avena* cDNA (CDO) clones used by Rayapati et al. (1994*a*) to create the F_2-generation map (i.e., *Asw*F2) of this oat population exhibited higher percentages of polymorphism using only two restriction enzymes compared with the BCD and CDO clones included in the Cornell grass anchor clone set. Rayapati et al. (1994*a*) selected their BCD and CDO clones based on poly-

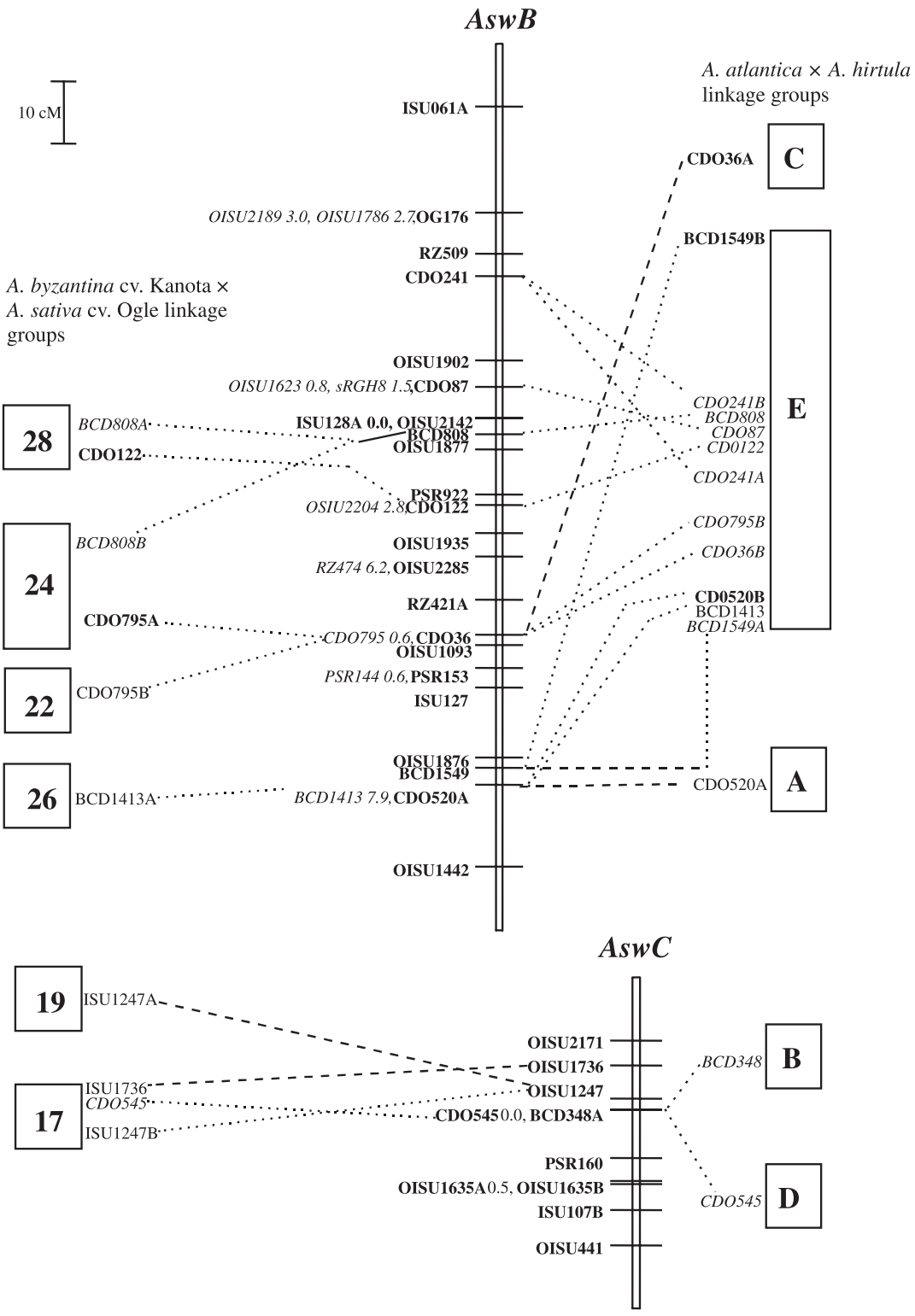
Fig. 1. The genetic linkage map of the *A. strigosa* × *A. wiestii* RIL population (*Asw*RI) and comparisons with other *Avena* maps. The *Asw*RI linkage group designations are given above each linkage group (e.g., *Asw*A). The legend indicates scale of map in cM (Kosambi function). Loci in bold were uniquely placed on the map at LOD ≥ 2.0. Where loci are tightly clustered in an interval, map distances between loci are indicated after the locus name. Loci in italics were placed in the most likely interval at LOD < 2.0. The darkened interval on *Asw*RI-G indicates an interval defined by loci according to the χ^2 test. Dashed lines connecting loci to boxes to the right of the *Asw*RI linkage groups indicate the position of the homologous marker locus in the *A. atlantica* × *A. hirtula* (*Aah*) linkage map (Van Deynze et al. 1995). Dashed lines connecting loci to boxes to the left of the *Asw*RI linkage groups indicate the position of the homologous marker locus in the *A. byzantina* cv. Kanota × *A. sativa* cv. Ogle (KO) linkage map (O'Donoughue et al. 1995). Genetic distances between loci on *Aah* and KO maps are drawn to scale. Figure 1 continues on following pages.



morphism detected in the *A. atlantica* × *A. hirtula* population (O'Donoughue et al. 1992). A single clone from each of the maize, wheat, and sorghum putative disease resistance gene clone sets detected polymorphism in this population (Table 1). One of these three clones (PIC20) identified two loci, providing a total of four putative disease resistance gene homologues that were mapped herein.

After discarding 20 loci because of segregation distortion and two loci because of aberrant linkage patterns, 181 loci remained for linkage mapping. Nine linkage groups were created from 177 loci with 4 remaining unlinked (Table 2, Fig. 1). Linkage groups were designated *Asw*RI-A through *Asw*RI-I to distinguish them from linkage groups from other *Avena* mapping studies. Average map distance was 97.8 cM per linkage group, and each linkage group contained an average of approximately 20 loci (Table 2). The average distance between loci was 5.0 cM. The linkage groups ranged in size from 32.4 cM (linkage group *Asw*RI-C) to 185.9 (*Asw*RI-D), and the total genetic map length was 880 cM (Fig. 1). The number of loci per linkage group ranged from five loci in linkage group *Asw*RI-I to 42 loci for *Asw*RI-D. Marker loci were generally uniformly distributed along the linkage map. The largest gaps between loci were a 24.4-cM gap near the end of linkage group *Asw*RI-H, a 26.5-cM gap on *Asw*RI-F, and a gap of 35.9 cM on *Asw*RI-G (Fig. 1). The loci that flank the gap on *Asw*RI-G were tested for linkage

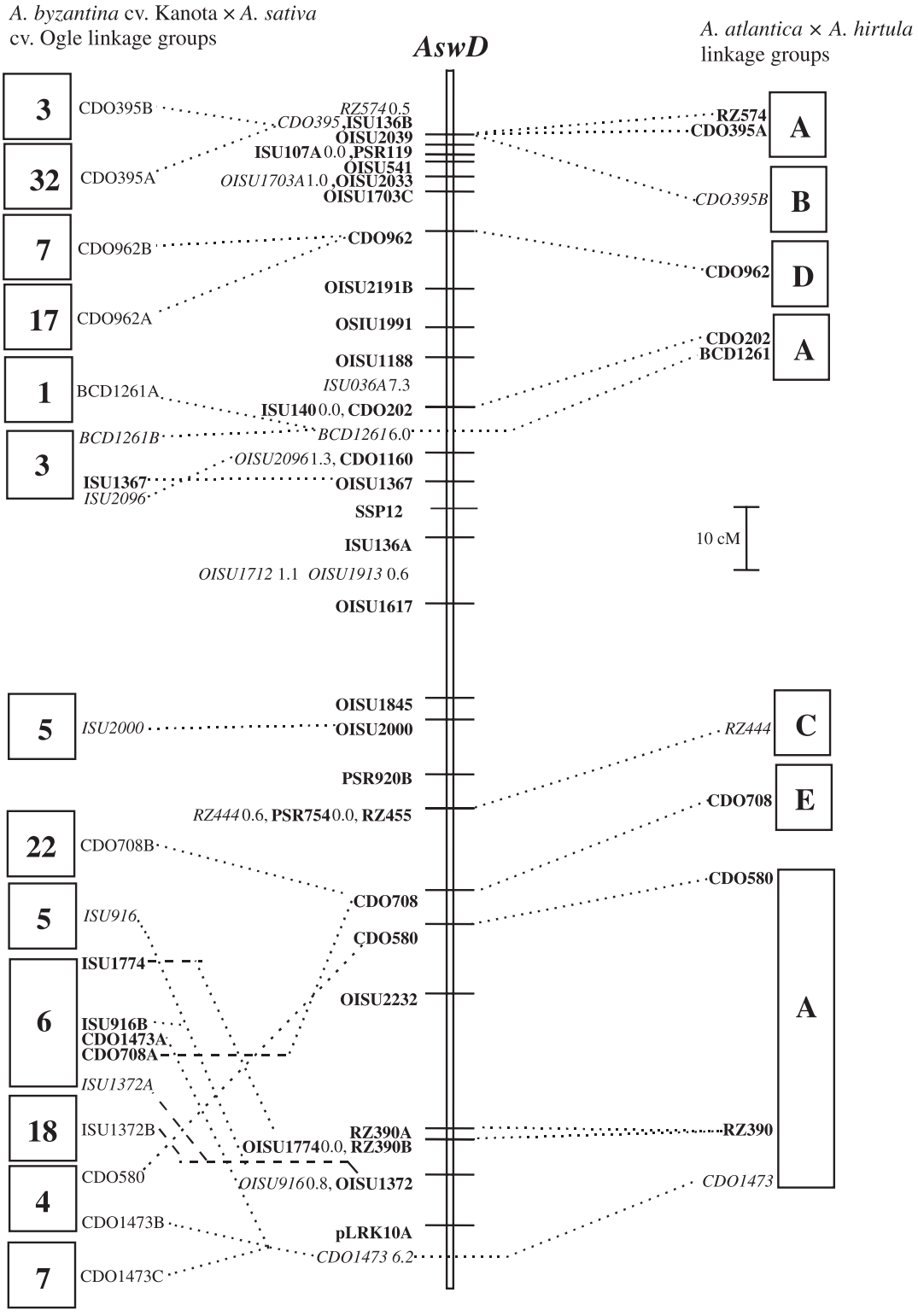
Fig. 1 (*continued*).



using a χ^2, test and the null hypothesis of no linkage was rejected with $p < 0.05$.

Linkage groups *Asw*RI-A and *Asw*RI-B were initially grouped together. By analyzing the two-point data, it was found that one locus (*CDO520A*) from *Asw*RI-B was apparently linked to two loci (*R54, R263*) within the *Pca* cluster on *Asw*RI-A but not linked to the loci that closely flanked *R54* and *R263*. Multipoint analysis placed the two groups at

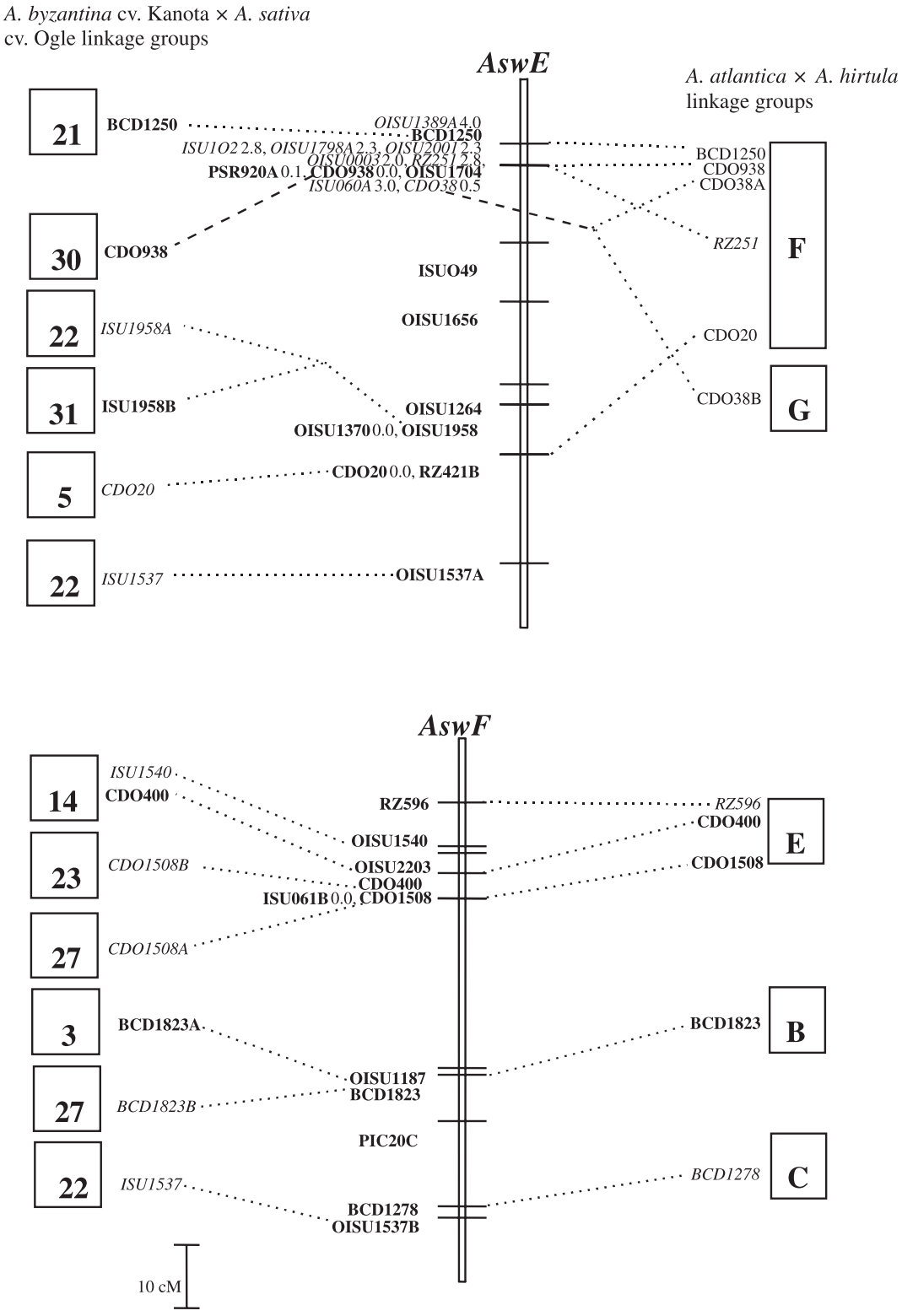
Fig. 1 (*continued*).



a distance greater than 50 cM, and there was no evidence for linkage of the loci flanking this gap between the two groups. Therefore they were established as separate linkage groups.

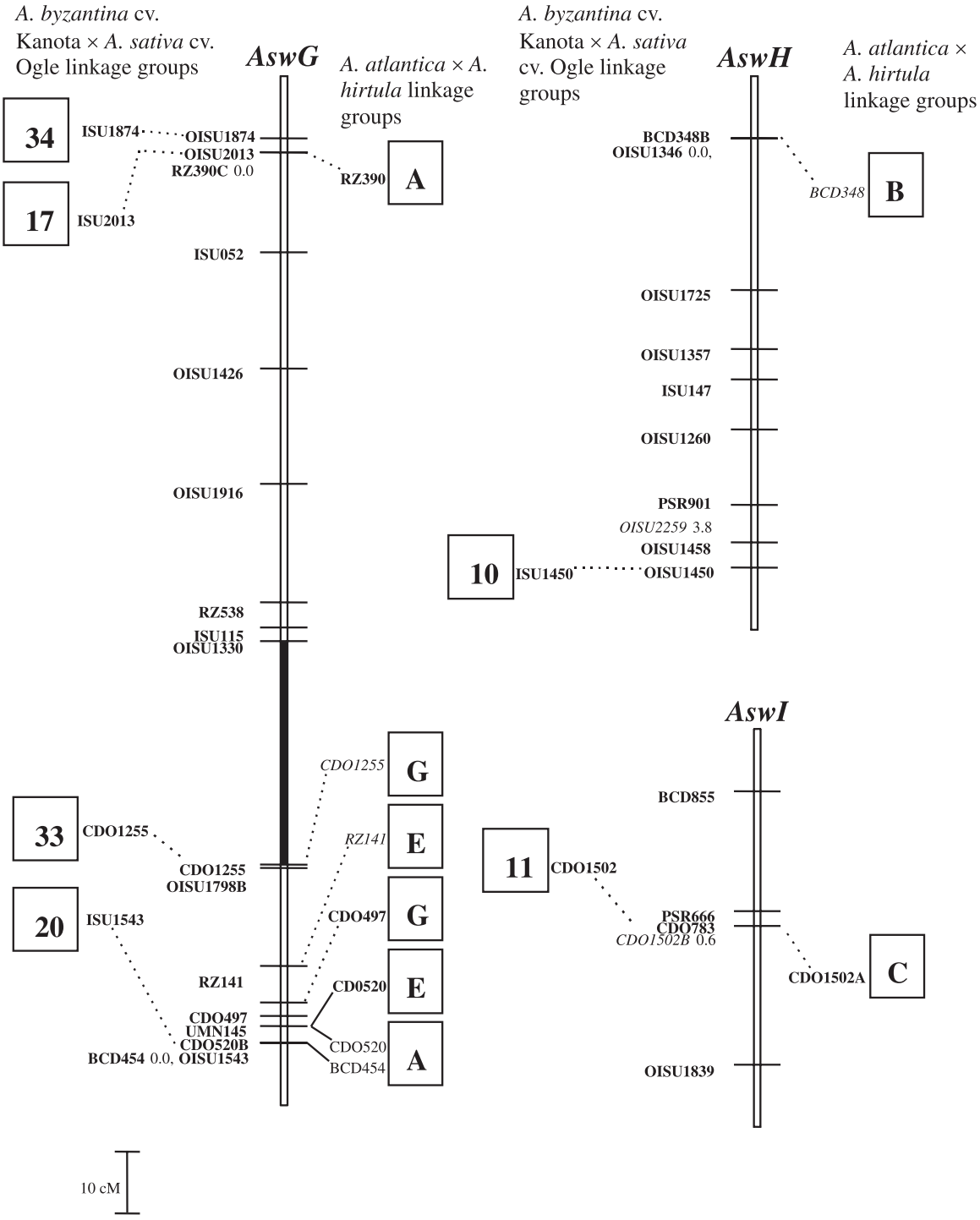
Total length of the *Asw*RI map was much smaller than that of the map constructed by Rayapati et al. (1994*a*) based on the F_2 generation of the same population (i.e., *Asw*F2). The *Asw*RI map shared 83 loci with the *Asw*F2 map, and 39% of the intervals were rearranged. The rearranged intervals occurred at gaps exceeding 30 cM in the *Asw*F2 map. Such gaps, which contributed to the greater size of the *Asw*F2 map, resulted from technical errors and misinterpretation of segregation data (Rayapati et al. 1994*a*). The *Asw*RI map replaces the *Asw*F2 map. One reason for the smaller size of the *Asw*RI map might be that terminal re-

Fig. 1 (*continued*).



gions of chromosomes were not adequately covered. Eleven loci that Rayapati et al. (1994*a*) mapped to terminal positions were not used in this map owing to skewed segregation ratios. Another reason that the *Asw*F2 map was larger may be the numerous large gaps (>30 cM) therein that were not found in this study. Finally, four loci were unlinked, and the number of linkage groups was higher than the basic chromosome number of the species, providing evidence that the *Asw*RI map did not cover the entire genome. The *Asw*RI map has greater total map length than the *A. atlantica* × *A. hirtula* (*Aah*) diploid oat map (737.4 cM; O'Donoughue et al. 1992; Van Deynze et al. 1995). O'Donoughue et al.

Fig. 1 (*concluded*).



(1992) and Rayapati et al. (1994*a*) suggested that the shorter map length of the *Aah* map was due to a reduction in recombination frequency in that population resulting from the more distant evolutionary relationship of the parental lines.

The PCR primers targeting the 12S seed storage protein produced a single amplification product from CI1994 and produced no product from CI3815. The presence or absence of the PCR product from the RILs was repeatable in two independent tests and segregated as a single locus. The locus, *SSP12*, mapped to linkage group AswRI-D (Fig. 1). The absence of the PCR product from CI1994 perhaps was due to a large insertion in the targeted intron of that genotype.

None of the candidate genes for disease resistance mapped to the same linkage group as the *Pca* cluster (linkage group *Asw*RI-A). The locus (*sRGH8*) detected by the sorghum clone mapped to linkage group *Asw*RI-B, the locus (*pLRK10A*) identified by the wheat clone mapped near the end of *Asw*RI-D, one of the two loci (*PIC20C*) identified by

Table 3. Standard deviations of two-point map distance estimates from a population of 100 RILs (used to make the *A. strigosa* × *A. wiestii* RIL map) or 44 F_2-derived lines (used to make the *A. atlantica* × *A. hirtula*

Recombination frequency (%)	Map distance (cM)	Standard error of map distance estimates	
		44 $F_{2:3}$ families (cM)	100 RIL families (cM)
0.1	0.1	0.3	0.2
1	1	1.1	0.7
5	5	2.4	1.7
7.5	7.6	2.9	2.2
10.0	10.1	3.4	2.7
15.0	15.5	4.2	3.6
20.0	21.2	4.9	4.4
25.0	27.5	5.5	5.3
30.0	34.7	6.1	6.2
40.0	54.9	7.2	8.1

Note: Standard deviations of recombination frequency estimates were computed using formulas of Liu et al. (1996), then the estimates and their standard errors were converted to cM using the Kosambi mapping function.

the maize clone mapped near the end of linkage group *Asw*RI-F, and the *PIC20B* locus remained unlinked. It is possible that these clones of putative resistance genes detected loci near genes that confer resistance to rust but are not segregating or have not been identified in the population. Wise et al. (1996) suggested that at least one locus unlinked to *Pca* was involved in resistance to crown rust in this population.

RFLP known to be linked to genes for resistance to crown rust in hexaploid *Avena* also did not map to the same linkage group as *Pca*. *UMN145*, which was linked to crown rust resistance gene *Pc91* in hexaploid *Avena* (Rooney et al. 1994), mapped to *Asw*RI-G, while *CDO783* and *CDO1502*, which were tightly linked to the *Pc71* gene in hexaploid *Avena* (Bush and Wise 1998), mapped to *Asw*RI-I. The sRGH8 probe identified a locus on *Asw*RI-B, 28 cM from *OG176*, which Rooney et al. (1994) reported to be loosely linked (14 cM) to the *A. strigosa* derived crown rust resistance gene, *Pc92*, in hexaploid *Avena*. The *pLRK10A* locus mapped to *Asw*RI-D, 13 cM from *OISU1774*, which was tightly linked to a gene conferring to *Puccinia coronata* isolate Pc203 in hexaploid *Avena* (Bush et al. 1994). The *PIC20C* locus was linked (13.3 cM) on the *Asw*RI map to *BCD1278*, which was closely linked to a leaf rust resistance gene in wheat (Autrique et al. 1994).

Twelve loci (the *Pca* cluster, *OISU2192*, *OISU2191A*, *OISU2117*, *BCD98*, *OISU1146*, *CDO1473*, *OISU916*, *OISU1774*, *OISU2232*, *CDO580*, and *CDO708*) on the *Asw*RI map were also mapped by Yu et al. (1996) in a population of $F_{8:9}$ RILs descended from the $F_{6:8}$ RILs used in this study. The linear order of the loci in that map is identical to that shown here, except that Yu et al. (1996) placed all 12 loci on a single linkage group, whereas the loci *Pca* through *OISU1146* mapped to *Asw*RI-A, while *CDO1473* through *CDO708* mapped to *Asw*RI-D (Fig. 1). *OISU1146* is a terminal locus on *Asw*RI-A, and *CDO1473* is a terminal locus on *Asw*RI-D; so, it is possible that they are linked, but there was no substantial evidence for their linkage (a LOD support of only 0.9 and two-point map distance of 39 cM). Yu et al. (1996) also observed that the evidence for linkage of *OISU1146* and *CDO1473* was weak (LOD 0.6 and map distance of 35 cM) but assigned the loci to the same linkage group with a large gap in order to remain consistent with the results of Rayapati et al. (1994*a*). We suggest that the two loci should be considered unlinked until stronger evidence for linkage is found.

The *Asw*RI map and the *A. atlantica* × *A. hirtula* (*Aah*) map shared 46 markers, and 56% of the marker intervals were conserved between the maps (Fig. 1). The *Asw*RI linkage groups shared from 1 (*Asw*RI-H and *Asw*RI-I) to 10 (*Asw*RI-B and *Asw*RI-D) markers with the *Aah* map. The colinear regions tended to have similar locus orders and genetic distances in both maps. Colinear segments tended to consist of only 2 or 3 markers, but a few larger segments were observed. One segment of five RFLP loci from linkage group *Asw*RI-E was colinear with linkage group *Aah*F. Four of the five loci (*BCD1250*, *CDO938*, *CDO38*, and *RZ251*) were clustered within a 5-cM interval, and the fifth locus (*CDO20*) was located 45 cM distally on the *Asw*RI map. Three of the corresponding loci on the *Aah* map clustered tightly, while the fourth locus (*RZ251*) and *CDO20* mapped 10 cM and 30 cM from the cluster, respectively. The largest colinear segment contained nine loci from *Asw*RI-B that also mapped to *Aah*E. The linear order was maintained except for rearrangements of tightly linked loci. Seven clones (CDO36, CDO38, CDO241, CDO395, CDO795, BCD98, BCD1549) each identified a single locus on the *Asw* map but they detected multiple loci on the *Aah* map. Therefore, some duplicated regions of the *Aah* map were not detected in the *Asw*RI map. *Asw*RI-G had adjacent loci which mapped to linkage groups *Aah*G and *Aah*E. *Asw*RI-D had three segments that were colinear with *Aah*A, but were separated by shared markers that mapped to other linkage groups on the *Aah* map. There were no groups of loci that were linked on the *Aah* map that mapped to linkage groups *Asw*RI-C, *Asw*RI-H, or *Asw*RI-I.

The *Asw*RI and hexaploid *Avena* maps shared 39 markers, and 39% of marker intervals were conserved between the two maps (Fig. 1). The number of shared loci with the hexaploid map ranged from 12 loci for linkage group *Asw*RI-D to 1 locus for *Asw*RI-H and *Asw*RI-I. Most of the colinear segments involved only 2 or 3 loci in sequence. The largest colinear segment was 5 loci that spanned a 55-cM portion of *Asw*RI-D and mapped to a 20-cM segment of

linkage group 6 in hexaploid *Avena*. *CDO708* and *OISU1372* defined the ends of the 55-cM segment in the *Asw*RI map, but were tightly linked in the hexaploid *Avena* map, indicating that finer-scale genomic rearrangements likely exist even in regions that are relatively well conserved. Pairs of loci on *Asw*RI linkage groups A, E, and F mapped to hexaploid *Avena* linkage groups 6, 22, and 27, respectively, but these pairs were each separated by 1 or 2 loci that mapped to other hexaploid *Avena* linkage groups, disrupting the linear order. These could be the result of insertion events or more simply, the result of not identifying the appropriate duplicate locus in the hexaploid population owing to lack of polymorphism. No groups of loci that were linked on the hexaploid map detected homoeologous loci on *Asw*RI linkage groups A, E, G, H, or I. The amount of genetic rearrangement between hexaploid *Avena* and diploid relatives observed in this study was similar to that reported by O'Donoughue et al. (1995).

We found that genomic organization of RFLP loci was considerably rearranged between closely related diploid *Avena* species and between diploid and cultivated hexaploid *Avena*, and that clones of putative genes for disease resistance did not detect loci near known resistance genes in this population. Leister et al. (1998) reported that disease resistance gene homologues of rice and barley identified using degenerate PCR primers designed to amplify homologues of disease resistance genes of dicots did not exhibit conserved linkage relationships between the two species, despite previous reports that the rice and barley genomes are highly colinear (Saghai-Maroof et al. 1995). Leister et al. (1998) suggested that disease resistance genes evolve rapidly and their chromosomal positions are rearranged more frequently than other genes. If this is true, then one might expect that heterologous clones such as those used herein (e.g., sRGH8, pLRK10A, PIC20C) will frequently map to locations unlinked to disease resistance genes identified by phenotype, as we observed.

The high degree of genomic rearrangement between diploid and hexaploid *Avena* is not surprising, either, in light of the classical and molecular cytogenetic work that has shown that the chromosomes of *Avena* have been more highly rearranged than those of *Triticum*. Segmental homoeology, rather than whole-chromosomal homoeology, is likely the rule rather than the expectation in *Avena* (Kianian et al. 1997; Leggett and Thomas 1995; McMullen et al. 1982; Rajhathy and Thomas 1974). Lack of extensive homology between chromosomes of different *Avena* species contributes to the difficulty that has been encountered in transferring crown rust resistance genes from *A. strigosa* to *A. sativa* (Thomas 1992).

The most surprising result of this study is that more colinearity between the *A. strigosa* × *A. wiestii* and *A. atlantica* × *A. hirtula* linkage maps was not found. All four species involved in these mapping populations are interfertile and are considered to be members of the same genomic subgroup (As) and the same biological species (Leggett and Thomas 1995). The population size of the *Asw*RI map was more than twice as large as that of the *Aah* map. Therefore, precision of linkage distance estimates is expected to be higher for the *Asw*RI map for recombination frequencies of less than 30% (Table 3) and we expect that the *Asw*RI map is robust. It is possible that duplication in the As diploid *Avena* genome is sufficiently common to complicate assignment of homology between different species of the As genome group. Our results and those of O'Donoughue et al. (1992) indicate that the diploid *Avena* species studied exhibit only a low frequency (9% and 7%, respectively) of polymorphic duplicated RFLP loci. This is likely an underestimate of the true level of genetic duplication, however, because clones identifying single loci on the maps may identify duplicated loci that were not polymorphic in the populations tested. It is also possible that genomic rearrangements have occurred on a scale that is detectable by RFLP linkage maps, but does not completely prevent pairing of partially homologous chromosomes or substantially reduce hybrid fertility. Leggett (1987) noted that small cryptic chromosomal differences occur between *Avena* species, and these are not large enough to prevent regular chromosome pairing at meiosis, although they may reduce fertility of interspecific hybrids. Leggett (1987) also observed that hybrids between *A. atlantica* and *A. strigosa* had reduced fertility, and some hybrids exhibited quadrivalent pairing at meiosis, indicating that at least one large chromosomal interchange exists between the species. Finally, it is well known that structural rearrangements exist among cultivars of the domesticated hexaploid *Avena* without noticeably reducing fertility of the hybrids between such cultivars (Chen and Armstrong 1994; Jellen et al. 1993, 1994; O'Donoughue et al. 1995; Singh and Kolb 1991; Zhou et al. 1999). Therefore, it is possible that diploid *Avena* species that are related based on gross chromosomal morphology and interspecific fertility may yet possess quite different chromosomal arrangements, although diploids do not have the potential for chromosomal buffering provided by homoeologous chromosomes in polyploids (Leggett 1988). The results of this study provide some exceptions to the reports of colinearity among some grass species (Bennetzen and Freeling 1993; Gale and Devos 1998; Moore et al. 1995; Saghai-Maroof et al. 1995). Rather, they support the contention that colinearity among species must be assessed on a case-by-case basis (Tanksley et al. 1988).

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