Localization of Stem Rust Resistance Genes and Associated Molecular Markers in Cultivated Oat

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ABSTRACT

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Molecular markers have been identified in cultivated oat for the *Pg9* and *Pg13* loci conferring resistance to different races of the stem rust pathogen, *Puccinia graminis* f. sp. *avenae*. Near-isogenic lines and bulked segregant analysis were used to identify putative restriction fragment length polymorphism (RFLP) and random amplified polymorphic DNA markers. Linkage relationships were established in segregating populations derived from crosses of OT328 with Dumont (segregating for both *Pg9* and *Pg13*), Rodney 0 with Rodney 0-*Pg9* (segregating for *Pg9*), and Rodney 0 with Rodney 0-*Pg13* (segregating for *Pg13*). The 5 markers linked to *Pg9* exhibited from 0 to 2.7% recombination with the resistance locus, and the 11 markers linked to *Pg13* exhibited from 0 to 22.7% recombination. An oat avenin clone detected a RFLP marker linked to the *Pg9* locus and an oat globulin clone detected a RFLP marker linked to the *Pg13* locus. Comparative mapping with an existing molecular linkage map of cultivated oat permitted localization of the *Pg9* and *Pg13* stem rust resistance genes. This is the first report of the localization of stem rust resistance genes on the linkage map of cultivated oat.

Additional keyword: genetic mapping.

Stem rust caused by *Puccinia graminis* Pers. f. sp. *avenae* Eriks. & E. Henn. is an important disease of cultivated oat (*Avena sativa* L.) and has periodically caused severe losses in most places where oat is grown (17). Seventeen genes (*Pg1* to *Pg17*) that confer resistance to different races of the pathogen have been identified in hexaploid oat (7,43). Types of gene action include dominant to partially dominant, incomplete recessive, and recessive. Both adult plant and seedling resistance have been reported, and the expression of several of these genes is influenced by temperature and light (17,18,21,22,43). Some of these genes are clustered within the oat genome, and some are associated with genes conferring resistance to crown rust (*P. coronata* Corda. f. sp. *avenae* Eriks. & E. Henn.). For example, loci *Pg1*, *Pg2*, and *Pg8* form one group, and *Pg4* is associated with *Pg13* (30). Another important group consists of *Pg3*, *Pg9*, *Pc44*, *Pc46*, *Pc50*, *Pc68*, *Pc95*, and *PcX* (3,6,19,25,27,29,49). *Pg11*, *Pg12* (*Pg-a* complex), and *Pg15* segregate independently of these three groups (19,21,28). In most cases, it is unclear whether the observed clusters consist of distinct genes or represent alleles of the same locus, though there have been claims that *Pg1* and *Pg2* have been combined (15,26, 27).

One of the breeding strategies for responding to the changing race populations of the stem and crown rust pathogens has been to develop germ plasm carrying combinations of several effective resistance genes (3,17). To facilitate these breeding objectives, the search for markers for different rust resistance genes has been intensified in recent years. The development of protein and DNA marker technologies has permitted rapid progress. Penner et al. (36) identified a random amplified polymorphic DNA (RAPD) marker linked to the *Pg3* gene. *Pg13* has been linked to a 56.6-kDa polypeptide locus resolved by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (3,11), and *Pg9* has been linked to an avenin band resolved by acid-PAGE (3). Molecular markers also have been identified for the following crown rust resistance genes: *Pc68* (37), *PcX* (3), *Pc91*, and *Pc92* (41) and three *Pc* genes transferred from *A. sterilis* (2).

Cultivated oat is highly polymorphic at the DNA level (34). Though this feature of the oat genome facilitates the development of linkage maps and the identification of markers, it also means that a marker linked to a resistance allele in one background may be monomorphic or associated with a susceptibility allele in a different background. Wight et al. (48) demonstrated that RAPD markers for day-length insensitivity in oat identified in one cross could not be assumed to be found in all germ plasm carrying the same day-length insensitivity allele nor only in day-length insensitive lines. Because of this, breeders should have access to several marker loci linked to a given resistance gene to find one that will be useful for the particular breeding program they wish to monitor. One solution to this problem would be to locate the resistance genes on a map consisting of many potential markers. The recent development of a molecular linkage map of cultivated oat (33) allows us to do this.

All the oat cultivars currently recommended for the Canadian eastern prairies carry either the gene combination *Pg2* and *Pg13* or the combination *Pg2*, *Pg9*, and *Pg13*. Genes *Pg9* and *Pg13* have provided effective resistance against the *P. graminis* f. sp. *avenae* population since the first release of these cultivars during the early 1980s (8). Given the paucity of effective resistance genes to the stem rust pathogen in the eastern prairies (8), it is important to retain both *Pg9* and *Pg13* in the new cultivars developed for this region. Molecular markers can be used to transfer these genes without the need for disease testing. This would be particularly

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useful for *Pg9* because there are no available stem rust races that will detect the presence of *Pg9* in the presence of *Pg13* (3).

The objectives of the current study were to: (i) identify restriction fragment length polymorphism (RFLP) and RAPD markers for stem rust resistance genes *Pg9* and *Pg13* and (ii) locate these genes and the associated markers within the hexaploid oat genome by comparative mapping.

MATERIALS AND METHODS

Plant material and crosses. The pedigrees of the oat lines and cultivars used in this study are outlined in Table 1. Cultivar Dumont has both stem rust resistance genes *Pg9* and *Pg13* (3,24). Line OT328 has no known stem or crown rust resistance genes (3,11). Rodney 0-*Pg9* and Rodney 0-*Pg13* are near-isogenic lines (NILs) developed by backcrossing resistance genes *Pg9* and *Pg13*, respectively, into the susceptible line Rodney 0 (11). Gene *Pg9* (formerly known as gene H) is either tightly linked in coupling or is pleiotropic to a gene for crown rust resistance (25). This crown rust gene, recently designated *PcX*, is linked in coupling to *Pg9* in Dumont (3).

Determination of linkage relationships between genes *Pg9* and *Pg13* and putative markers (described below) were carried out on segregating F_3 families derived from three crosses: OT328 × Dumont, Rodney 0 × Rodney 0-*Pg9*, and Rodney 0 × Rodney 0-*Pg13*. The F_3 families of the OT328 × Dumont cross were taken from the same segregating population used in a previous linkage study of protein markers to *Pg9* and *Pg13* (3). All crosses were made in growth chambers at 18°C with an 18 h/6 h light/dark cycle.

Rust resistance evaluations. Segregations of the 92 F_3 families of the Rodney 0 × Rodney 0-*Pg9* cross for *Pg9* and *PcX* (tightly linked in coupling to *Pg9*) resistance were tested with stem rust race NA27 (avirulence/virulence formula = *Pg9*, *13*, *15*, *16*, *a/1*, *2*, *3*, *4*, *8*) (22) and crown rust isolate CR192 (Winnipeg Research Centre accession number) (*Pc45*, *48*, *50*, *54*, *56*, *58*, *59*, *60*, *61*, *62*, *64*, *X/35*, *38*, *39*, *40*, *46*, *63*), respectively. Approximately 24 seedlings from each family were inoculated with NA27 when the primary leaves were fully expanded (1-leaf stage) then with CR192 8 days later (2-leaf stage). Segregation of the 95 F_3 families of the Rodney 0 × Rodney 0-*Pg13* cross for *Pg13* resistance was tested by inoculating about 24 seedlings from each family with stem rust race NA55 (*Pg8*, *13*, *a/1*, *2*, *3*, *4*, *9*, *15*, *16*) at the 1-leaf stage. The absence of *Pg9* and *Pg13* resistance in cvs. Kanota and Ogle was determined by inoculation with race NA27.

The methodology and results of rust resistance evaluations of the 88 F_3 families from the OT328 × Dumont cross segregating for genes *Pg9*, *Pg13*, and *PcX* with NA27, NA25 (*Pg8*, *13*, *16*, *a/1*, *2*, *3*, *9*, *15*), and CR192 have been described elsewhere (3). Because there are no stem rust races that will detect the presence of *Pg9* when *Pg13* also is present, the tight linkage (in coupling) of *PcX* to *Pg9* allowed identification of *Pg9* in F_3 families that were homozygous resistant or segregating for *Pg13* resistance. Thus, for this cross, all families homozygous resistant or segregating for *PcX* resistance also were considered homozygous resistant or segregating, respectively, for *Pg9*.

TABLE 1. Pedigrees and presence or absence of stem rust resistance genes *Pg9* and *Pg13* in the cultivars and lines of hexaploid oat used in this study

Line or cultivar	*Pg9*	*Pg13*	Pedigree[a]	Reference
Dumont	Yes	Yes	Harmon HAM/Double Cross 7	3, 11, 24
OT328	No	No	S79107/Cascade	3
Rodney 0	No	No	Rodney*5/Exeter	11
Rodney 0-*Pg9*	Yes	No	Rodney 0*3/3/OT174*2//CI6792/Rodney	19, 27
Rodney 0-*Pg13*	No	Yes	Rodney 0*3/*Avena sterilis* CW490-2	30
Kanota	No	No	Selection from Fulghum	4
Ogle	No	No	Brave//Tyler/Egdolon 23	1

[a] Harmon HAM = OT184*5/4/OT182/3/OT183*2//*A. sterilis* CW490-2/2*Rodney 0; OT184 = Harmon*6//Rosens Mutant/Rodney; OT182 = OT174*5//CI6792/Rodney; T183 = Kelsey*6//Rosens Mutant/Rodney. Possible sources of resistance are underlined.

All plants were inoculated by applying a suspension of urediniospores in Dustrol (Ciba Canada Ltd., Winnipeg, MB) light industrial oil (4 mg/450 µl). The inoculated plants were incubated in a dew chamber (Percival model 160-D, Boone, IA) at 18°C for approximately 16 h. After incubation, the plants were placed in a greenhouse with supplemental fluorescent lighting. The temperature was maintained between 18 and 22°C because *Pg9* is less effective at temperatures above 25°C (20). Crown rust infection types (ITs) were scored at 12 days after inoculation by a 0 to 4 scale (32). ITs of 0, j, 1, and 2 were considered resistant, and ITs of 3 and 4 were considered susceptible. For stem rust, ITs were scored 16 days after inoculation by a 0 to 4 scale (45). ITs of 0 to 3 were considered resistant, and ITs of 3^+ and 4 were considered susceptible. Gene *PcX* is dominant, and plants carrying a resistance allele reacted with an IT of j to CR192. *Pg9* and *Pg13* are recessive genes. Plants homozygous for *Pg13* resistance reacted with an IT of 1 to NA25 and NA55, and plants homozygous for *Pg9* resistance exhibited an intermediate IT (range 1 to 3) to NA27. All F_3 families were classified as resistant, segregating, or susceptible.

Marker identification. ***Source of markers.*** A set of 174 oat (CDO) and barley (BCD) leaf cDNA, oat endosperm cDNA (UMN), and wheat genomic clones that had been mapped in cultivated oat by O'Donoughue et al. (33) were used to detect RFLPs. All four libraries have been described previously (9,33). These clones were selected for even coverage of the mapped portion of the cultivated oat genome. In addition, because of the known association between endosperm proteins and the *Pg9* and *Pg13* genes (3,11), two known sequence clones, provided by I. Altosaar (University of Ottawa, Ottawa, Canada) also were used. One of these clones, pOP6, is an oat prolamin (avenin) genomic clone (39), and the other, MOG12, is an oat globulin cDNA clone (46). RAPDs were detected with decamer primers obtained from J. B. Hobbs (Biotechnology Laboratory, University of British Columbia (UBC), Vancouver).

Polymorphism surveys. *RFLP.* To identify putative markers for *Pg9* and *Pg13*, survey filters carrying single digests (with *Dra*I, *Eco*RI, or *Eco*RV) of DNA from NILs Rodney 0, Rodney 0-*Pg9*, and Rodney 0-*Pg13*; cvs. Dumont, Kanota, and Ogle; and line OT328 were prepared. These survey filters were probed with 174 clones and the 2 endosperm protein clones described above. Clones exhibiting RFLPs between isoline pair Rodney 0/Rodney 0-*Pg9* or Rodney 0/Rodney 0-*Pg13* were considered putative markers (23). Dumont and OT328 were checked simultaneously for the presence of the same polymorphisms. DNA extractions and digestions, Southern blotting, and hybridizations were performed as described by O'Donoughue et al. (33,35).

RAPDs. DNA from NILs Rodney 0, Rodney 0-*Pg9*, and Rodney 0-*Pg13* was surveyed with 200 random primers. Any putative markers identified with the NILs were checked for polymorphisms between Dumont and OT328. Bulked segregant analysis (5,31) also was performed with 127 random primers and 2 pairs of differential bulks differing for resistance at the *Pg9* and *Pg13* loci (100 of these primers were different from those used in the NIL surveys). The pools consisted of DNA from 10 F_3 families (8 to 10 plants per family), each selected from the segregating OT328 × Dumont population. Primers detecting a polymorphism between the differential bulks were considered putative markers. The protocol used for detection of RAPDs was as described in Wight et al. (48).

Linkage between markers and stem rust resistance genes. Linkage between the stem rust resistance genes and markers was determined with DNA from individual F_3 families (pooled from 6 to 12 individuals per family) from the segregating populations

derived from the crosses of OT328 with Dumont (88 families), Rodney 0 with Rodney 0-*Pg9* (92 families), or Rodney 0 with Rodney 0-*Pg13* (95 families). The scores for the 56.6-kDa polypeptide locus and the avenin B2 band, linked to *Pg13* and *Pg9*, respectively, in the OT328 × Dumont population (3), were provided by J. Chong and coauthors (Winnipeg Research Centre, Agriculture and Agri-Food Canada, Winnipeg).

Linkage maps were obtained by the program Mapmaker, version 2.0 (16). Markers and rust resistance loci were first grouped together by two-point analysis with a LOD score of 8 and a maximum recombination level of 0.30 with the "Group" command. Multipoint analysis with a LOD threshold of 2 was used to order loci within the linkage group. Loci that could not be positioned precisely with this LOD threshold were assigned to the most likely interval and placed in parentheses (Figs. 1 and 2).

Localization of markers and associated stem rust resistance genes. Localization of the stem rust resistance genes and associated markers was achieved by comparative mapping with the *A. byzantina* 'Kanota' × *A. sativa* 'Ogle' map published by O'Donoughue et al. (33). Marker loci were assumed to be the same as the Kanota × Ogle loci when at least one of the Kanota × Ogle allelic fragments was identical to one of the allelic fragments found in OT328 × Dumont, Rodney 0 × Rodney 0-*Pg9*, and Rodney 0 × Rodney 0-*Pg13* (Tables 2 and 3). Other marker loci that did not meet the above criteria but that were detected by the same clone and mapped to similar positions in Kanota × Ogle and the current populations were tentatively assigned the same locus designation and placed in brackets (e.g., XpOP6(A) versus XpOP6A; Table 2; Figs. 1 and 2). The marker loci that had not been mapped previously or that had been mapped with a different restriction enzyme in Kanota × Ogle by O'Donoughue et al. (33) were mapped in Kanota × Ogle for this study. The methodology and mapping were performed as described in O'Donoughue et al. (33).

To further clarify the positions of the different loci, the Kanota × Ogle linkage groups involved were compared to those of the diploid oat map produced by O'Donoughue et al. (35) with a population derived from an *A. atlantica* × *A. hirtula* cross. Three new loci (Xcdo220, Xbcd342, and Xcdo395B) were added to the published linkage group A to facilitate comparisons. The methodology and mapping were performed as in O'Donoughue et al. (35).

RESULTS AND DISCUSSION

Identification of markers to stem rust resistance genes. *Pg9.* Segregation data for *Pg9* and *PcX* resistance in the F_3 families from crosses of Rodney 0 with Rodney 0-*Pg9* and OT328 with Dumont to stem rust race NA27 and crown rust isolate CR192 are shown in Table 4. In both F_3 populations, the chi-square test for goodness-of-fit indicated that the segregation data did not deviate significantly from the 1:2:1 ratio expected for a single gene. In addition, all F_3 families of the Rodney 0 × Rodney 0-*Pg9* cross that were resistant, segregating, or susceptible to NA27 showed the same reaction to CR192. These results further confirmed that *Pg9* is closely linked in coupling to *PcX*.

TABLE 2. Characteristics of markers linked to stem rust resistance gene *Pg9* in two F_3 populations segregating for *Pg9* resistance and comparison of these markers to loci detected in the 'Kanota' × 'Ogle' cross

		OT328 (OT) × Dumont (DU)				Rodney 0 (R0) × Rodney 0-*Pg9* (*Pg9*)				Kanota (KA) × Ogle (OG)[a]		
	Mapping enzyme/	Fragment (kb)		% Recom.		Fragment (kb)[d]		% Recom.		Fragment (kb)[d]		
Marker	primer sequence	DU	OT	with *Pg9*[b]	χ^{2c}	R0	*Pg9*	with *Pg9*[b]	χ^{2c}	KA	OG	Group
Xcdo1385F	*Eco*RV		7.20	2.3 ± 1.27	0.40	Monomorphic				8.75	7.20	4
Xacor458A	5′ CTCACATGCC 3′		0.90	1.2 ± 1.15	3.73	Monomorphic				Monomorphic		
XpOP6(A)	*Eco*RI	4.40		0.0	3.88*	15.50	4.40	2.7 ± 1.3	0.58	14.50		4
Xumn101A	*Eco*RI	6.80, 3.70		0.0	3.85*	Monomorphic				18.00	6.80, 3.70	4
Xacor195A	5′ GATCTCAGCG 3′	0.40		0.0	4.48*	Monomorphic				Monomorphic		

[a] O'Donoughue et al. (33).
[b] Recom. = recombination.
[c] The expected ratios for χ^2 calculations were 3:1 for dominant markers (1 df) and 1:2:1 for codominant markers (2 df). * indicates the probability of obtaining a larger χ^2 value by chance is $0.05 > P > 0.01$.
[d] Monomorphic indicates the locus was monomorphic with the enzymes *Eco*RI, *Eco*RV, and *Dra*I.

TABLE 3. Characteristics of markers linked to stem rust resistance gene *Pg13* in two F_3 populations segregating for *Pg13* resistance and comparison of these markers to loci detected in the 'Kanota' × 'Ogle' cross

		OT328 (OT) × Dumont (DU)				Rodney 0 (R0) × Rodney 0-*Pg13* (*Pg13*)				Kanota (KA) × Ogle (OG)[a]		
	Mapping enzyme/	Fragment (kb)[b]		% Recom.		Fragment (kb)		% Recom.		Fragment (kb)[b]		
Marker	primer sequence	DU	OT	with *Pg13*[c]	χ^{2d}	R0	*Pg13*	with *Pg13*	χ^{2d}	KA	OG	Group
Xmog12B	*Dra*I	8.40		10.0 ± 3.50	5.40*	10.05		0.0	0.20	8.40	10.05	3
Xcdo1242B	*Eco*RI	3.90	3.40	3.1 ± 1.34	2.72	3.40	3.90	3.4 ± 1.28	12.56**	Monomorphic		
Xcdo346A	*Dra*I	5.00	5.55	3.8 ± 1.55	0.33	5.55	5.00	3.4 ± 1.28	11.54**	7.80	5.55	3
Xcdo1420A	*Eco*RI		13.70	2.3 ± 1.24	0.47	13.70		0.0	0.61	13.20	13.70	3
Xcdo393A	*Eco*RV	5.30	3.90	4.3 ± 1.53	1.70	3.90	5.30	3.9 ± 1.46	14.83**	6.90	3.90	3
Xbcd1562A	*Eco*RV		9.40	1.0 ± 1.14	0.73	9.40		0.0	0.45	11.50	9.40	3
Xcdo270B	*Eco*RV	20.50	8.90	4.8 ± 1.75	2.66	8.90	20.50	4.7 ± 1.66	16.12**	13.50	8.90	3
Xbcd342B	*Eco*RV	Monomorphic				13.50	3.00	5.8 ± 1.78	9.19*	9.40	13.50	3
Xacor254C	5′ CGCCCCCATT 3′		0.35	ND[e]	0.02	0.35		8.5 ± 3.35	20.09**	Monomorphic		
Xacor372A	5′ CCCACTGACG 3′	Monomorphic				0.66	0.59	15.4 ± 4.03	2.18	0.47	0.66	3
Xcdo1385(D)	*Dra*I	Monomorphic				6.80		22.7 ± 4.96	0.72	<2.0		3

[a] O'Donoughue et al. (33).
[b] Monomorphic indicates the locus was monomorphic with the enzymes *Eco*RI, *Eco*RV, and *Dra*I.
[c] Recom. = recombination.
[d] The expected ratios for χ^2 calculations were 3:1 for dominant markers (1 df) and 1:2:1 for codominant markers (2 df). * indicates the probability of obtaining a larger χ^2 value by chance is $0.05 > P > 0.01$; ** indicates the probability of obtaining a larger χ^2 value by chance is $P < 0.01$.
[e] Not determined.

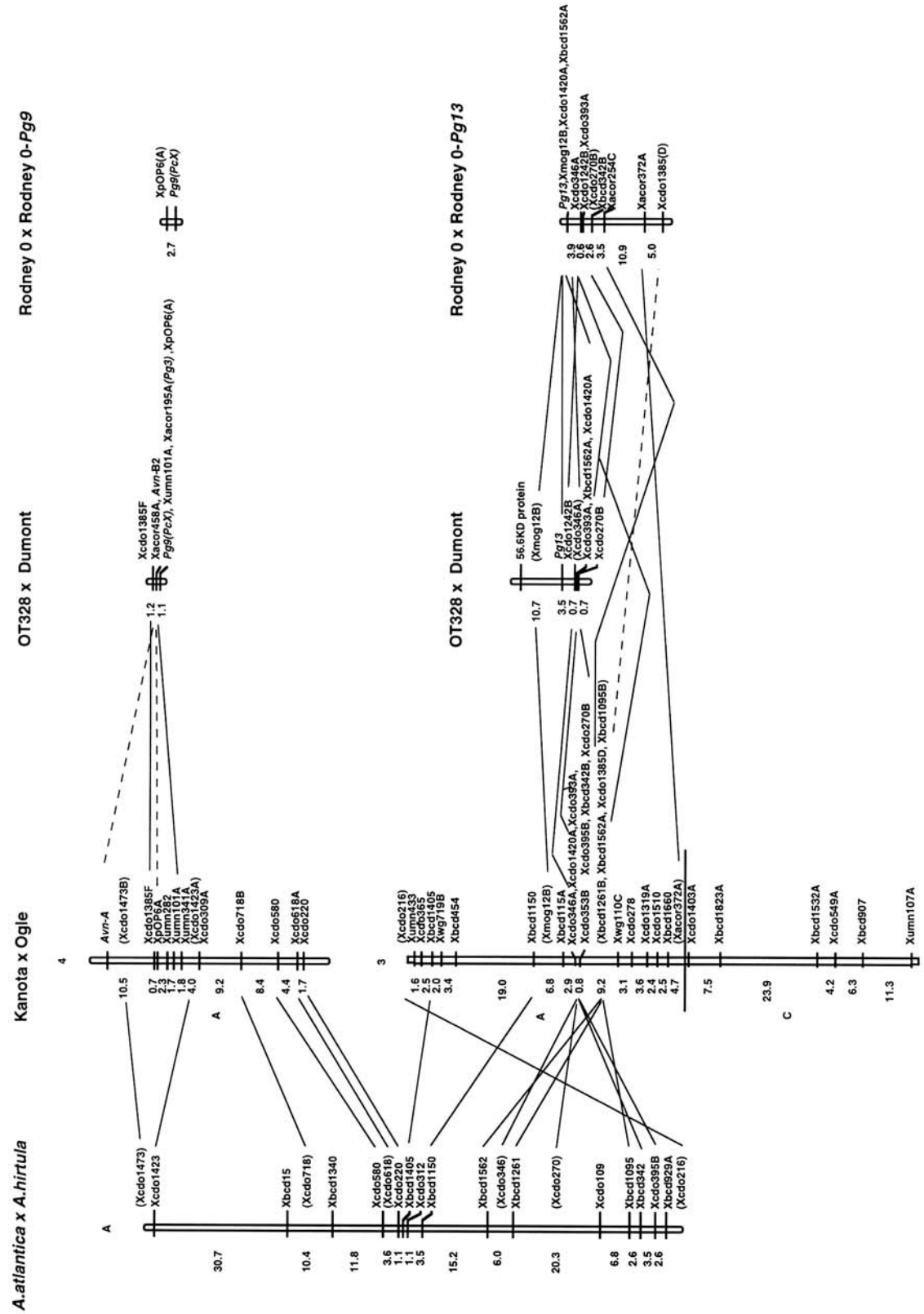

Fig. 1. Comparative maps of the rust resistance regions identified in OT328 × Dumont, Rodney 0 × Rodney 0-*Pg9*, and Rodney 0 × Rodney 0-*Pg13* with linkage groups 4 and 3 from the linkage map of cultivated oat based on the 'Kanota' × 'Ogle' cross (33) and linkage group A from the *Avena atlantica* × *A. hirtula* diploid oat map (35). Solid lines between the diploid and hexaploid oat map linkage groups indicate orthologous loci. Solid lines between the hexaploid oat maps indicate the same loci as determined by identical allelic fragments; dashed lines represent loci that may be the same based on map position only. Letters to the left of the Kanota × Ogle linkage groups refer to homoeologies with the linkage groups of the *A. atlantica* × *A. hirtula* map. Map distances are given in centimorgans (Kosambi function). Loci in parentheses have been assigned to intervals only (LOD < 2).

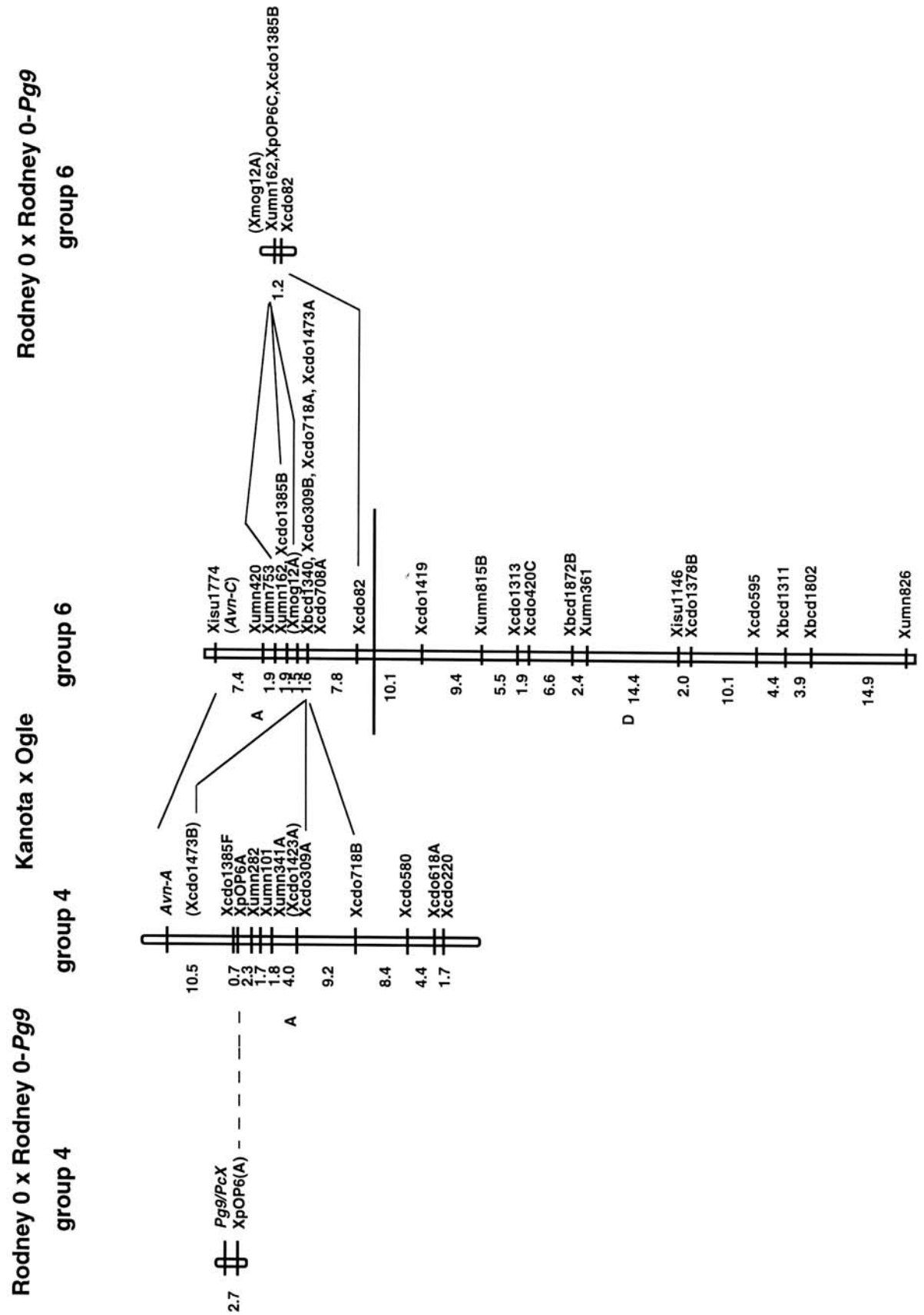

Fig. 2. The two polymorphic regions identified between the near isogenic lines Rodney 0 and Rodney 0-*Pg9* and their relationship to the linkage map of cultivated oat based on the 'Kanota' × 'Ogle' cross (33). Solid lines between the Kanota × Ogle linkage groups indicate orthologous loci. Solid lines between the Kanota × Ogle and Rodney 0 × Rodney 0-*Pg9* linkage groups indicate the same loci as determined by identical allelic fragments; dashed lines represent loci that may be the same based on position only. Letters to the left of the linkage groups refer to homoeologies with the linkage groups of the *Avena atlantica* × *A. hirtula* map (35). Map distances are given in centimorgans (Kosambi function). Loci in parentheses have been assigned to intervals only (LOD < 2).

In this study, five new markers were identified for the *Pg9-PcX* complex. In total, six markers are now available for *Pg9*, including the avenin B2 marker first reported by Chong et al. (3), three RFLPs, and two RAPDs (Fig. 3A; Table 2). Two of the three RFLP markers (Xcdo1385F and XpOP6(A)) were identified with NILs, and the RAPD marker Xacor458A was identified by bulked segregant analysis. The RFLP marker Xumn101A was discovered through comparative mapping with the Kanota × Ogle map (Fig. 1). Primer UBC195 (Xacor195A) was surveyed with DNA from all parental lines because of its known association with another stem rust resistance gene, *Pg3* (36).

The five new markers exhibited from 0 to 2.7% recombination with the *Pg9* locus (Table 2). Only XpOP6(A) was polymorphic in both the OT328 × Dumont and Rodney 0 × Rodney 0-*Pg9* populations; the others were monomorphic in Rodney 0 × Rodney 0-*Pg9*. With XpOP6(A), the allele associated with *Pg9* resistance was represented by a fragment of equal size (4.4 kb) in both Dumont and Rodney 0-*Pg9*. The source of *Pg9* in line Rodney 0-*Pg9* can be traced to accession CI 6792 (Table 1 [27]), whereas the source of resistance in Dumont could have originated from 'Rosen's Mutant' or CI 6792 (3,27) (Table 1 [27]).

The three markers, Xacor195A, Xumn101A, and XpOP6(A), all cosegregated with *Pg9* in the OT328 × Dumont population and exhibited a segregation ratio that deviated significantly from the 3:1 ratio expected from a single dominant locus in this population. Though the segregation of *Pg9* resistance did not deviate significantly from a 1:2:1 ratio in this population, the chi-square value was quite high, with a probability between 0.1 and 0.05 of obtaining a larger chi-square value (Table 4). The significant chi-square values obtained for the segregation of the three cosegregating markers, therefore, are consistent with the segregation of *Pg9*. Marker XpOP6(A), although exhibiting a deviant segregation ratio in the OT328 × Dumont population, segregated as a single locus in the Rodney 0 × Rodney 0-*Pg9* population. All markers either cosegregated with the *Pg9* locus or mapped to one side. Flanking markers were not identified.

Interestingly, marker Xacor195A (designated ACOpR-2 by Penner et al. [36]), which is linked to *Pg9* in the OT328 × Dumont cross, is the same fragment previously shown to be linked to *Pg3* (Fig. 3A). Penner et al. (36) established linkage in a population derived from the Rodney 0 × Rodney 0-*Pg3* cross. However, Xacor195A is linked in coupling to *Pg9*, whereas it was shown to be linked in repulsion to *Pg3* (Table 2; Fig. 3A). This is consistent with the previous report that *Pg3* and *Pg9* are either allelic or tightly linked in repulsion (27). This area of the oat genome appears to be important with respect to rust resistance, because at least six crown rust resistance genes (including *PcX*) also have been reported to be associated with either *Pg3* or *Pg9* (3,6,19,25,29). Penner et al. (37) identified a RAPD marker (with primer UBC269) to one of these crown rust resistance genes, *Pc68*. Unfortunately, no polymorphisms were detected with this primer in the current study using the OT328/Dumont and Rodney 0/Rodney 0-*Pg9* pairs, despite the known linkage of *Pc68* and *Pg9*.

The association of *Pg9* with an avenin marker in the OT328 × Dumont cross has been demonstrated by Chong et al. (3). In the current study, an oat avenin genomic clone, pOP6, also detected a RFLP linked to *Pg9*. Combining data from both studies, no recombination was detected between the Avn-B2 and XpOP6(A) loci in the OT328 × Dumont population. The dominant nature of markers such as XpOP6(A) and Xacor458A (Table 2) results in a lower resolution for detecting recombination especially when these markers are linked in repulsion. As a result, recombination could not be detected between XpOP6(A) and *Pg9* resistance nor between XpOP6(A) and the RAPD marker Xacor458A, which is linked in repulsion (1.2% recombination) to the *Pg9* resistance allele. Therefore, the data cannot resolve whether Avn-B2 and XpOP6 identify the same locus or different members of an avenin gene family.

Portyanko et al. (38) determined that avenin bands in cultivated oat were transmitted as blocks, within which they found no recombination in a segregating population of 208 F_2 kernels. Shotwell et al. (42) isolated and sequenced an oat genomic clone that contained four tightly linked avenin genes, indicating that the avenins occur as gene families in at least some areas of the oat genome. Close association of seed storage proteins with rust resistance loci has been reported in several species. Examples include close linkage of leaf and stem rust resistance loci to gliadins and glutenins in wheat (10,12,13,14), and of leaf, stem and stripe rust resistance to secalins in rye (44). These findings indicate that the association of seed storage proteins and rust resistance loci is highly conserved in grass genomes.

Pg13. The segregation data for *Pg13* resistance in the F_3 families from the OT328 × Dumont and Rodney 0 × Rodney 0-*Pg13* crosses are outlined in Table 4. The chi-square test for goodness-of-fit indicated that the segregation in the Rodney 0 × Rodney 0-*Pg13* population did not deviate significantly from the 1:2:1 ratio expected for a single gene. However, segregation of the 88 F_3 families of OT328 × Dumont did deviate significantly ($0.05 > P > 0.025$) from the expected 1:2:1 ratio, with an excess of segregating families. These 88 F_3 families were part of a larger population of 141 F_3 families used in a previous study to establish linkage between *Pg13* resistance and a protein marker (3). The segregation data of this larger population for *Pg13* gave a good fit to the expected 1:2:1 ratio ($\chi^2 = 1.95$; $0.50 > P > 0.30$). No conscious selection was carried out for the subset of 88 F_3 families, except for lines that were eliminated because of poor DNA quality.

Eleven new markers linked to the *Pg13* locus were identified in the current study. Nine of the new markers are RFLPs, and two are RAPDs (Fig. 3B, Table 3). In total, 12 markers are now available for this locus, including the 56.6-kDa protein marker previously identified by Chong et al. (3). All RFLP and RAPD markers for *Pg13* were identified with NILs, except for Xacor254C, which was identified by bulked segregant analysis. The markers exhibited from 0 to 22.7% recombination with the *Pg13* locus.

Seven of the new markers were mapped in both populations segregating for *Pg13* resistance, and for codominant markers (Xcdo1242B, Xcdo346A, Xcdo393A, and Xcdo270B; Table 3),

TABLE 4. Segregation for oat seedling reactions to an isolate (CR) of *Puccinia coronata* f. sp. *avenae* or stem rust races (NA) of *P. graminis* f. sp. *avenae* in F_3 families from three crosses

Population	Race or isolate	Resistance gene detected	Number of F_3 families				χ^{2a}	*P*
			Resistant	Segregating	Susceptible	Total		
Rodney 0 × Rodney 0-*Pg9*	NA27	*Pg9*	25	42	25	92	0.79	0.70–0.50
Rodney 0 × Rodney 0-*Pg9*	CR192	*PcX*	25	42	25	92	0.79	0.70–0.50
Rodney 0 × Rodney 0-*Pg13*	NA55	*Pg13*	29	37	29	95	4.64	0.10–0.05
OT328 × Dumont[b]	CR192	*PcX*(*Pg9*)[c]	27	47	14	88	4.25	0.10–0.05
OT328 × Dumont[b]	NA25	*Pg13*	19	56	13	88	7.36	0.05–0.02

[a] Ratio of 1:2:1 expected.

[b] The 88 F_3 families used in this study were part of the segregating population published in a previous linkage study of *Pg9* and *Pg13* protein markers (Chong et al. [3]).

[c] The crown rust resistance gene *PcX* is tightly linked in coupling to *Pg9* (Chong et al. [3]).

the alleles associated with *Pg13* resistance in Dumont and Rodney 0-*Pg13* were identical. *A. sterilis* accession CW490-2 is the source of *Pg13* resistance for both cultivars (Table 1 [3]). The three markers (Xbcd342B, Xacor372A, and Xcdo1385(D)) that were polymorphic only in the Rodney 0 × Rodney 0-*Pg13* cross were among the more distant loci from the *Pg13* locus (5.8, 15.45, and 22.7% recombination, respectively). Interestingly, RAPD marker Xacor254C, which had a 345-bp band associated in repulsion with *Pg13* resistance in Rodney 0 × Rodney 0-*Pg13*, detected the same band segregating in the OT328 × Dumont cross. However, in the latter cross all homozygous susceptible families exhibited the band, whereas most segregating families did not. The segregation for absence or presence of the band fitted the expected 3:1 ratio for a dominant marker. Nevertheless, because of the possibility of amplification in some families segregating for this marker, the recombination data were not included. This suggests that caution is needed when dominant RAPD markers are used, especially when there are different doses of the locus to be amplified in different individuals.

Three markers (Xmog12B, Xcdo1420A, and Xbcd1562A) cosegregated with the *Pg13* locus in the Rodney 0 × Rodney 0-*Pg13* cross (Table 3). However, the same markers exhibited 10.0, 2.3, and 1.0% recombination, respectively, with *Pg13* in the OT328 × Dumont cross (Table 2). Again, most markers identified in this study cosegregated with or mapped to one side of the *Pg13* locus. However, the 56.6-kDa protein identified by Chong et al. (3) and the RFLP marker identified with the globulin cDNA clone, MOG12, mapped to the other side of the locus in the OT328 × Dumont population (Fig. 1), thereby providing flanking markers for the *Pg13* locus. Two-percent recombination was detected between the 56.6-kDa protein and the Xmog12B RFLP loci. It appears that these markers are derived from two different loci, although they may represent separate members of a globulin gene family. This constitutes another example of the association of loci for seed storage proteins and rust resistance.

None of the markers for *Pg13*, except for the most distant one (Xmog12B) in the Dumont × OT328 population, deviated from the expected 3:1 or 1:2:1 segregation ratios for a dominant or codominant single gene, respectively (Table 3). However, several of the same markers (Xcdo1242B, Xcdo346A, Xcdo393A, and Xcdo270B) deviated significantly from the expected single gene segregation ratios in the Rodney 0 × Rodney 0-*Pg13* population (Table 3). Together with the reduced recombination compared to the OT328 × Dumont cross, these distorted segregation ratios indicate that some cytological difference between Rodney 0 and Rodney 0-*Pg13* may exist in this area of the genome.

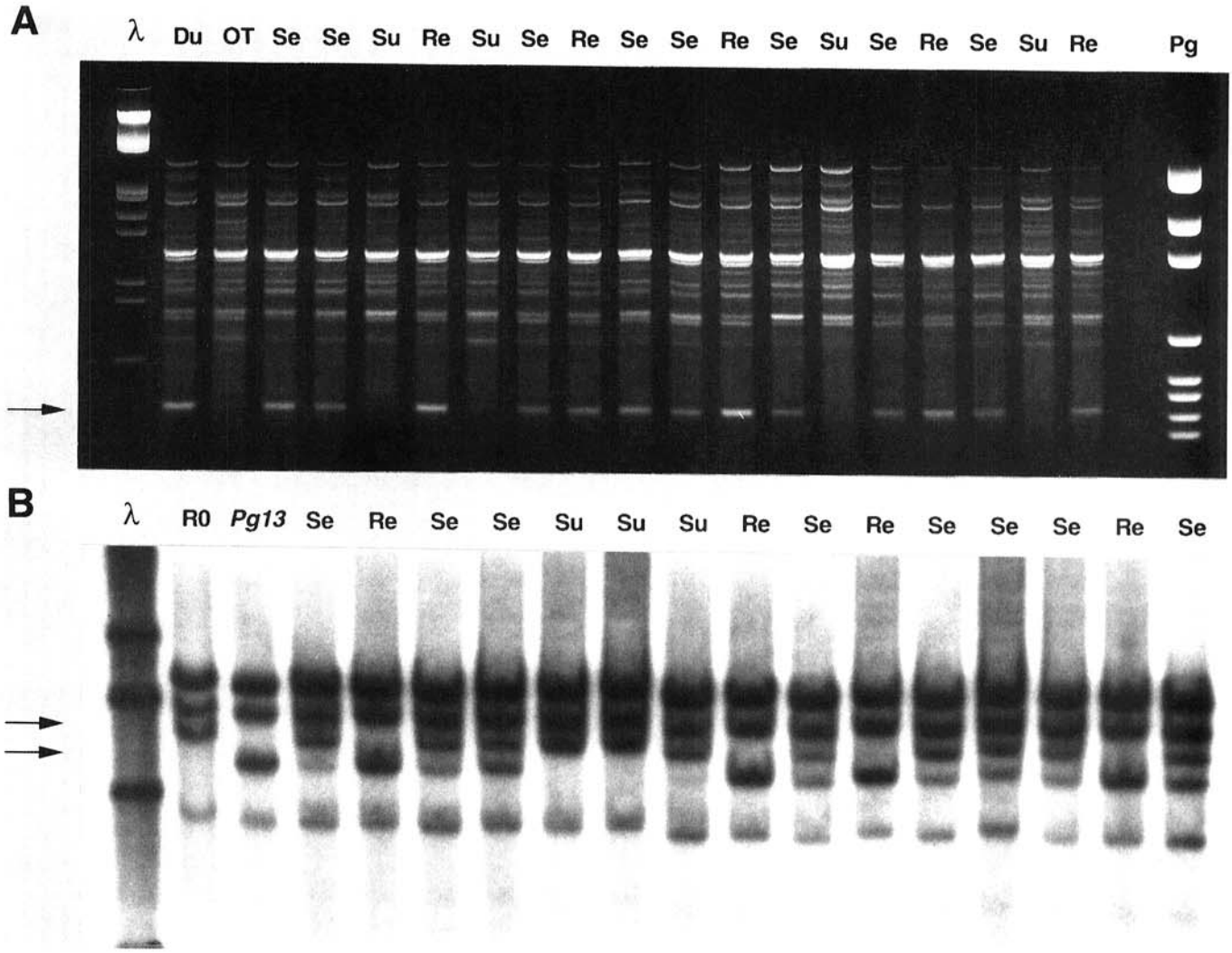

Fig. 3. Examples of marker patterns associated with stem rust resistance in segregating oat populations. **A,** Banding patterns obtained with *Pg9* marker Xacor195A on cvs. Dumont (Du), OT328 (OT), and segregating bulked F_3 families from the OT328 × Dumont cross. The first and last lanes are molecular weight markers λ-*Hin*dIII/*Eco*RI and pGem *Hin*fI/*Rsa*I/*Sin*I, respectively. **B,** Banding patterns obtained with *Pg13* marker Xcdo346A on Rodney 0 (R0), Rodney 0-*Pg13* (*Pg13*), and segregating bulked F_3 families from the Rodney 0 × Rodney 0-*Pg13* cross. The DNA was cut with the restriction enzyme *Dra*I. The first lane contains the molecular weight marker λ-*Hin*dIII. Arrows indicate marker fragments. Re: resistant family; Su: susceptible family; and Se: segregating family.

Localization of markers and associated stem rust resistance genes. The utilization of mapped probes in our initial surveys of NILs allowed us to identify markers that could be used for comparative mapping with the existing map of cultivated oat developed by O'Donoughue et al. (33). The fragment sizes of *Pg9* markers Xcdo1385F and Xumn101A, identified in the OT328 × Dumont population, were equal to the ones detected in the Ogle parent of the Kanota × Ogle mapping population (Table 2). Therefore, it is reasonable to assume that these two loci are the same in the two populations. By association with these loci, the *Pg9* locus can be mapped to group 4 of the Kanota × Ogle map (Fig. 1). Avenin clone pOP6 detected a locus linked to *Pg9* in OT328 × Dumont and Rodney 0 × Rodney 0-*Pg9*, as well as a locus in the same area of the Kanota × Ogle linkage group 4. However, because the fragment sizes did not correspond, it is not known if these fragments represent different alleles of the same locus or different loci possibly of the same gene family. It should be noted that the pair of fragments detected by clone UMN101 in resistant cv. Dumont also were found in cv. Ogle, which is known not to carry the *Pg9* resistance allele. This reinforces the notion that marker loci can be identified and used for marker-assisted breeding, but the level of polymorphism in oat precludes making the assumption that any given marker allele is always associated with the same allele of a given trait.

It was clear from our initial surveys that another area of the genome also was segregating in the Rodney 0 × Rodney 0-*Pg9* population. A number of polymorphic loci were identified that when mapped did not link to *Pg9*. Interestingly, this area was detected by at least two clones, pOP6 and CDO1385, that also detected loci linked to *Pg9*. Further mapping with additional clones and comparisons with the Kanota × Ogle map identified this region as part of group 6 (Fig. 2). This region is homoeologous to the region of group 4 where *Pg9* is located. Evidently, this homoloeogous region was inadvertently maintained during the development of the Rodney 0-*Pg9* backcross line.

It recently has been determined that the Rodney 0-*Pg9* line also carries a second crown rust resistance gene not linked to the *Pg9/PcX* complex (J. Chong, *unpublished data*). The group 6 region may be the site of this crown rust resistance gene. This possibility is currently being investigated. If this is the case, it would suggest that rust resistance loci can be found in homoeologous regions of the genome. It has already been demonstrated that endosperm proteins can follow this pattern, because the avenin clone pOP6 detects loci in both the group 4 and 6 regions.

Using the same comparative mapping methodology, *Pg13* was located in group 3 of the Kanota × Ogle map (Fig. 1). In this case, eight markers (including one RAPD marker) had fragment sizes corresponding to those detected in Kanota or Ogle (Table 3). Again, cv. Kanota exhibited the same allele as resistant cv. Dumont at the Xmog12B marker locus despite not having *Pg13* resistance. As for the Rodney 0-*Pg9* backcross-derived line, polymorphic loci that were not linked to the *Pg13* resistance locus were detected between Rodney 0 and Rodney 0-*Pg13*. The NILs Rodney 0 and Rodney 0-*Pg13* differed in at least one additional unlinked region that has no homoeology to the segment carrying *Pg13* (data not shown).

Unfortunately, the Kanota × Ogle map (33) has not yet been resolved into the 21 expected linkage groups. Therefore, to gain a better understanding of the organization of the *Pg9* and *Pg13* stem rust resistance genes within the oat genome, groups 3 and 4 were compared to a diploid oat map (35). Both groups 3 and 4 exhibit homoeology to group A of the diploid oat map (Fig. 1). However, the regions carrying the *Pg9* and *Pg13* genes are homoeologous to opposite ends of the diploid oat map group A. This indicates that groups 3 and 4 could be the two arms of one chromosome. However, it is believed that these two groups belong to two separate homoeologous (or partly homoeologous) chromosomes. Clone CDO220, situated at one end of group 3, and clone BCD1405, mapped near the end of group 4, are separated by only 1.1 centimorgans (cM) in the diploid oat map (Fig. 1). If groups 3 and 4 are part of the same chromosome, they would likely link to each other via these clones. These results indicate that both arms of group A chromosomes can carry rust resistance and endosperm protein loci. This is consistent with the organization found by Rayapati et al. (40) in an *A. strigosa* × *A. wiestii* diploid oat map. These authors (40) found that an avenin locus and a locus conferring resistance to nine crown rust isolates mapped to the same linkage group but were separated by 178 cM. It also is interesting that in the *Triticeae* the association between rust resistance genes and endosperm proteins is found in homoeologous group 1. Marker loci of group A of the *A. atlantica* × *A. hirtula* diploid oat map (35) recently have been shown to be mostly orthologous with loci of the group 1 consensus *Triticeae* map (47).

Comparison with the diploid oat map also reveals that recombination in the area carrying the *Pg13* resistance locus is drastically reduced with respect to recombination in the diploid (Fig. 1). O'Donoughue et al. (33) found this to be true for the Kanota × Ogle population, and the current study shows that it also may be true for two other hexaploid oat crosses. With the Rodney 0 × Rodney 0-*Pg13* cross, markers Xbcd1562A and Xbcd342B are separated by 2.6 cM, whereas the distance between the loci detected with the same clones in the diploid oat map spans 33.1 cM. Similarly, the 0.7 cM distance between Xbcd1562A and Xcdo270B detected in OT328 × Dumont suggests that recombination may be reduced in this cross as well. The *A. sterilis* source of the *Pg13* resistance could be implicated as the cause of reduced pairing and recombination in the Rodney 0 × Rodney 0-*Pg13* and OT328 × Dumont crosses but does not provide an explanation for the reduced recombination in Kanota × Ogle. It is interesting that O'Donoughue et al. (33) suspected that group 3 was implicated in a translocation difference between cvs. Kanota and Ogle. The distorted segregation ratios observed for some of the markers in the Rodney 0 × Rodney 0-*Pg13* cross (Table 3) indicate similar cytological differences between the parental lines of this population. This is consistent with the report, based on genetic transmission data, that the *Pg13* resistance locus in some crosses is likely to be involved in a translocation (30). Reduced recombination and translocation differences between different oat cultivars in this region of the genome implies that it may be more difficult to reduce linkage drag, break linkages, and transfer rust resistance genes located in this area.

General conclusions. The availability of a molecular linkage map of cultivated oat has allowed rapid identification and localization of several markers for two stem rust resistance genes. Breeders will now be able to select RFLP and RAPD markers for these genes, which exhibit polymorphisms better suited to their own germ plasm. Furthermore, the markers identified here and the other more distant loci selected from the Kanota × Ogle map will be useful for monitoring and reducing linkage drag in backcross breeding programs. The intimate association between loci detected with seed storage protein clones and both the *Pg9* and *Pg13* rust resistance loci indicate, based on homoeology and known conserved synteny across gramineae species (47), that these same clones may have potential as markers for other stem or crown rust resistance genes in *Avena* and even perhaps other grass species.

The information gained in this study and in other ongoing studies about the organization of rust resistance genes within the oat genome will prove useful for the long-term goal of combining several resistance genes into well-adapted common cultivars. The observation that the *Pg13* locus is located in an area of reduced recombination illustrates the kind of information that may help to devise strategies for manipulation and efficient transfer of areas carrying rust resistance genes. Furthermore, information on the organization of rust resistance genes may in time provide insight into how resistant genes have been evolving in *Avena*.

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