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ORIGINAL PAPER

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A genetic linkage map of grape, utilizing *Vitis rupestris* and *Vitis arizonica*

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Abstract A genetic linkage map of grape was constructed, utilizing 116 progeny derived from a cross of two *Vitis rupestris* × *V. arizonica* interspecific hybrids, using the pseudo-testcross strategy. A total of 475 DNA markers —410 amplified fragment length polymorphism, 24 inter-simple sequence repeat, 32 random amplified polymorphic DNA, and nine simple sequence repeat markers—were used to construct the parental maps. Markers segregating 1:1 were used to construct parental framework maps with confidence levels >90% with the Plant Genome Research Initiative mapping program. In the maternal (D8909-15) map, 105 framework markers and 55 accessory markers were ordered in 17 linkage groups (756 cM). The paternal (F8909-17) map had 111 framework markers and 33 accessory markers ordered in 19 linkage groups (1,082 cM). One hundred eighty-one markers segregating 3:1 were used to connect the two parental maps' parents. This moderately dense map will be useful for the initial mapping of genes and/or QTL for resistance to the dagger nematode, *Xiphinema index*, and *Xylella fastidiosa*, the bacterial causal agent of Pierce's disease.

Introduction

Grape is one of the most important horticultural crops in the world. The world's grape industry is primarily based on *Vitis vinifera* L. cultivars, which are highly susceptible to a wide range of pests and diseases. Classical breeding programs have produced rootstock and scion cultivars with pest and disease resistance; however, such breeding programs often take decades to fully evaluate and release new cultivars. Placement of resistance genes and loci on a genetic linkage map could greatly accelerate the breeding process by allowing the use of markers to screen and select resistant individuals in early growth stages. For example, Dalbo et al. (2001) found two molecular markers tightly linked with a powdery mildew resistance QTL and utilized them for marker-assisted selection in a breeding population. Pre-screening seedlings with these markers reduced the number of susceptible individuals an average of 24%, thereby reducing time and cost of caring for the vines in the field. Resistance loci could also be isolated and fully characterized with the intention of genetically transforming current *V. vinifera* L. cultivars with resistance genes.

A first step toward characterizing and cloning resistance genes is the construction of a genetic linkage map derived from a family segregating for the resistance. The development of reliable and efficient polymerase chain reaction (PCR)-based markers, such as amplified fragment length polymorphism (AFLP) (Vos et al. 1995), has greatly reduced the time required to create genetic linkage maps (Marques et al. 1998). Combined with the two-way pseudotestcross strategy (Grattapaglia and Sederoff 1994), PCR-based markers have led to the development of useful genetic maps in many outcrossing species (Hemmat et al. 1994; Marques et al. 1998; Cristofani et al. 1999; Debener and Mattiesch 1999; Remington et al. 1999; Cervera et al. 2001). In a pseudotestcross, dominant molecular markers segregate in a 1:1 ratio because many alleles in highly heterozygous species are present in only one copy in one parent. These informative markers are used to build separate molecular maps for each parent. Markers heterozygous in both parents or codominant markers, such as simple sequence repeats (SSR), can be used to combine the two parental maps (Dalbo et al. 2000; Cervera et al. 2001).

To date, five genetic linkage maps for grape have been published (Lodhi et al. 1995; Dalbo et al. 2000; Doligez et al. 2002, Grando et al. 2003; Riaz et al. 2004). These maps have been utilized to map horticultural traits such as seedlessness and berry weight, and disease-resistance traits such as powdery mildew resistance. However, the grape

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species utilized for construction of these maps are not resistant to two important grape diseases: fanleaf degeneration and Pierce's disease (PD). Fanleaf degeneration is caused by *Grapevine fanleaf virus* (GFLV) and is vectored from root to root by the dagger nematode *Xiphinema index*. This virus/nematode disease complex affects flower pollination, causing yield reductions of up to 80% and is widely spread across the world's viticultural regions (Martelli and Savino 1988). PD is caused by the bacterium *Xylella fastidiosa* and is vectored by xylem-feeding insects such as sharpshooter leafhoppers. This disease is endemic across the warmer parts of the Americas and, although relatively limited in distribution, continues to cause severe economic loss in California (Hopkins 1989).

In previous studies, an interspecific grape population designated as '8909' (provided by H.P. Olmo), located in the University of California, Davis vineyards, has been shown to segregate for resistance to both *X. index* (Walker and Jin 2000) and *X. fastidiosa* (Krivanek and Walker 2001). Two selections—D8909-15, resistant to both *X. index* and *X. fastidiosa* and F8909-17, susceptible to *X. index* but resistant to *X. fastidiosa*—were crossed to produce a mapping population. This paper presents a genetic linkage map derived from the cross of D8909-15 and F8909-17 and is a first step towards genetically mapping these and other important resistance traits.

Materials and methods

Plant material

The mapping population utilized in this study consisted of a total of 116 progeny, randomly selected from a family of vines derived from the cross of two half-sib genotypes, D8909-15 and F8909-17. The female parent D8909-15 was selected from a cross of *V. rupestris* 'A. de Serres' × b42-26 (a *V. arizonica* selection from Baja California, Mexico). The pollen parent F8909-17 was selected from a cross of *V. rupestris* 'A. de Serres' × b43-17 (a *V. arizonica* type from Monterrey, Mexico). Parental relationships were confirmed via SSR marker analysis (data not shown). The entire family derived from the cross D8909-15 × F8909-17 was designated as "9621."

DNA extraction

Genomic DNA was extracted from the young leaves and shoot tips of 116 individuals in the 9621 family and from the two parents using the CTAB procedure from Lodhi et al. (1995).

Marker development

Random amplified polymorphic DNA markers

Nineteen random 10-mer primers from Operon Technologies (Alameda, Calif., USA) were used to generate random amplified polymorphic DNA (RAPD) markers. Reactions were performed as described by Jin (1997). Amplified products were run on agarose gels, and DNA bands were visualized by ethidium-bromide staining.

SSR markers

The SSR markers included those that have been published (Thomas et al. 1994; Bowers et al. 1999) and some that have been developed within the Vitis Microsatellite Consortium (VMC). Twenty-seven SSR primer pairs (Thomas et al. 1994; Bowers et al. 1999) were tested on six samples from the 9621 population (including the parents). Fourteen primer pairs successfully produced amplified products. Nine primer pairs produced informative markers that were easily scored for our population: VVS2 (Thomas et al. 1994), VVMD25, VVMD27, VVMD28, VVMD31, VVMD32 (Bowers et al. 1999), VMC16F3, VMC4d4, and VMC1c10 (VMC). PCR amplifications were performed in 20-µl reactions consisting of 2.25 mM $MgCl_2$, 250 µM each dNTP, 10 pmol each primer, 1× *Taq* Gold buffer, 0.6 U *Taq* Gold polymerase, and approximately 50 ng DNA. PCR conditions were 34 cycles of a 1-min denaturation at 94°C, a 1-min annealing at 56°C, and a 2-min extension at 72°C. PCR products were mixed with 2× sequencing dye (98% formamide, 10 mM EDTA, 0.05% bromphenol blue and xylenecyanol), denatured at 94°C for 2 min and 3 µl was loaded onto 6% polyacrylamide gels. Gels were visualized by silver staining with a commercial kit from Promega (Madison, Wis., USA).

ISSR markers

Thirty-seven inter-short sequence repeat (ISSR) primers (Wu et al. 1994) were tested on six samples (including the parents). Six primers with high numbers of polymorphic markers were chosen (Table 1). For each primer, two independent reactions were performed. Only unambiguous markers consistent in both reactions were scored. PCR reactions and cycling conditions were as described above for the microsatellites, except the annealing temperature was 50°C instead of 56°C. Five microliters of amplified product was mixed with 3 µl sequencing dye and visualized by silver staining on 4% polyacrylamide gels.

AFLP analysis

AFLP markers were generated using the protocol described by Vos et al. (1995) with the following modifications. About 1 µg genomic DNA was used for the *Mse*I–*Eco*RI digest and ligation of adapters. Pre-selective amplification was performed using standard *Eco*RI and *Mse*I primers (E0 and M0, respectively) without any additional selective nucleotides. Three microliters template DNA (20 ng based on absorbance at OD260) was added to a mixture containing 2.0 mM $MgCl_2$, 250 µM each dNTP, 10 pmol E0 primer, 20 pmol M0 primer, 1× *Taq* buffer, and 0.7 U *Taq* polymerase, for a total reaction volume of 20 µl. The PCR conditions were 26 cycles of a 30-s denaturation at 94°C, a 1-min annealing at 56°C, and a 2-min extension at 72°C. The PCR product was diluted five times with distilled DNAse-free water. Selective amplification was performed using primers with three additional selective nucleotides. The conditions were the same as the preselective reaction, except

Table 1 Summary of inter-short sequence repeat (ISSR) markers used in the genetic linkage map

Marker name	Sequence[a]
ISSR41	TGTGTGGTGGTGGGTGGG
ISSR43	$(GT)_8$YA
ISSR54	YC$(AG)_7$
ISSR55	YC$(AC)_7$
ISSR201	$(GA)_7$GY
ISSR203	$(GA)_7$GYG

[a] *Y* pYrimidine

15 pmol each primer and 2 μl diluted pre-amplification product were used. PCR amplification consisted of 38 cycles of a 30-s denaturation at 94°C, a 30-s annealing, and a 60-s extension at 72°C. The annealing temperature was 60°C for the first cycle and reduced by 0.7°C for the next 11 cycles. Annealing temperature was 56°C for the last 26 cycles. PCR product was mixed with sequencing dye in a 1:1 ratio, denatured at 94°C for 2 min, and loaded onto a 6% polyacrylamide gel. Gels were silver stained as described above.

To examine the reproducibility of the AFLP process, two researchers independently ran the preamplification and the selective amplification for two primer pairs on all 116 genotypes. Genotypes scored by each researcher matched 100%.

Segregation analysis

All marker types were scored visually for presence/absence (1/0) of the band. Scoring for each marker was double checked, and any ambiguous genotypes were rerun, reamplified, or left as unknown. The data were converted to Plant Genome Research Initiative (PGRI) and JoinMap, version 3.0, file formats by PERL scripts (available upon request). Chi-square goodness-of-fit tests to expected 1:1 or 3:1 segregation ratios were performed in Microsoft Excel 2000.

Map construction

Framework maps for each parent were created with markers heterozygous in only one parent and segregating with the expected 1:1 ratio ($P>0.05$). Linkage groups were built with JoinMap at LOD≥3 and confirmed with the free program PGRI (Liu 1998), with an approximate linkage significance value $\alpha=0.001$ and recombination frequency $\theta=0.3$. PGRI was used to establish the order of framework markers. PGRI uses a resampling, or bootstrapping, method to determine the confidence level of a particular gene ordering. This bootstrapping method combined with jackknifing analysis also evaluates the effect of individual loci on the overall locus ordering, allowing quick detection of unreliable markers (Liu 1998). All markers segregating 1:1 were ordered using the "manually interactive" option and the simulated annealing/sum of adjacent recombination fraction algorithm. Markers with low probability of correct order (PCO) and high jackknife values (the average PCO of the linkage group after the marker is removed) were removed until an average PCO>98% was obtained. Then, markers were added back individually. The marker that maintained the highest PCO average was kept on the framework map. The process of adding back the most confident markers was repeated until the PCO average went below 90%. The remaining markers were added as accessory markers.

Markers segregating 1:1 with significant segregation distortion ($P<0.05$) were then positioned on the framework map. If these markers grouped together (≥2 markers) and increased the confidence interval of the other markers, the markers were added to the framework map. If not, the markers showing segregation distortion were added as accessory markers.

Heterozygous markers present in both parents that did not significantly deviate from the expected 3:1 ratio ($P>0.05$) were used to combine the two parental maps with JoinMap. A file containing only framework markers and the 3:1 markers was loaded into JoinMap. LOD values, ranging from 3.0 to 6.0, were chosen to maintain the original linkage groups. Each 3:1 marker was added individually to the framework map, while the order of the framework markers was fixed. As with the 1:1 accessory markers, the Kosambi map distance from the closest framework marker was recorded.

Estimated and observed genome length and map coverage

A method-of-moments type estimator (Hulbert et al. 1988) as proposed in method 3 by Chakravarti et al. (1991) was used to estimate genome length (G_e) for each parent by the formula $N(N-1)X/K$, where N is total number of framework markers, X is the observed maximum distance between two marker pairs at LOD=3.5, and K is the number of locus pairs with minimum LOD score. The genome length was calculated twice, using only the framework markers and using all 1:1 markers (framework and accessory). To prevent an overestimation of genome length due to marker clustering, only framework markers were included in this analysis. A minimum LOD score of 3.5, and Kosambi map distances were used.

Two estimates of observed genome length (G_{on}) were calculated for each parent: the total length of the framework map (G_{of}) and the length estimated by the formula $G_{on}=G_{of}+X(L-R)$ (Nelson et al. 1994). In this formula, X is the maximum distance between two framework markers, L is the total number of linkage groups (including doublets, triplets, and unlinked markers), and R is the haploid number of chromosomes. Observed map coverage (C_{of}) was calculated by G_{on}/G_e. Estimated map coverage (C_e) was calculated by the equation: $C_e=1-e^{-XN/1.25Ge}$ (Lange and Boehnke 1982). Only framework markers and G_e calculated with only the framework markers were used in these estimates because these equations assume a random distribution of markers.

Table 2 Summary of markers generated for the genetic map of the 9621 (D8909-15 × F8909-17) hybrid population

Marker[a]	Maternal[b]	Paternal[b]	Markers segregating in both parents	Total
Average AFLP markers per primer	2.5	2.4	3.2	
Total AFLP markers scored before χ^2 analysis ($P<0.05$)	133	128	173[c]	434
AFLP markers included on map	129	120	161[c]	410
RAPD markers scored/included on map	12	8	12[c]	32
SSR scored/included on map	1	1	7	9
ISSR markers scored	13	13	2[c]	28
ISSR markers included on map	12	11	1[c]	24
Total markers scored	159	150	194[c]	496
Total markers mapped	154	140	181	475
Distorted markers χ^2, $df=1$, $P=0.05$ (%)	18 (11)	8 (5)	18 (9)	44
Distorted markers χ^2, $df=1$, $P=0.001$ (%)	7 (4)	3 (2)	0 (0)	

[a] *AFLP* Amplified fragment length polymorphism, *RAPD* random amplified polymorphic DNA, *SSR* simple sequence repeat, *ISSR* inter-simple sequence repeat
[b] Markers segregating 1:1
[c] Markers segregating 3:1

Results

Analysis of AFLP markers

Fifty-four selective primer pairs with three additional nucleotides each were used. A total of 434 AFLP markers were scored with 261 heterozygous in one parent and absent in the other and 173 markers heterozygous in both parents (Table 2). After chi-square analysis, 129 maternal markers, 120 paternal markers, and 161 markers heterozygous in both parents were added to the map. The number of polymorphic bands per primer pair varied from 1 to 16 with an average of 8.

Analysis of RAPD, ISSR, and microsatellite markers

Nineteen different RAPD primers produced 32 markers (1.6 markers per primer): 12 heterozygous in the female parent, eight heterozygous in the male parent, and 12 heterozygous in both parents (Table 2). Six ISSR primers with high numbers of polymorphic markers were chosen. When faint bands were discarded, the reactions were highly reproducible between two researchers. Twenty-four ISSR markers were scored: 12 heterozygous in the female parent, 11 were heterozygous in the male parent, and one heterozygous in both parents (Table 2).

Nine out of 27 of the microsatellite (SSR) primers tested produced useful markers. Marker VMC4d4 segregated in the female parent only, and marker VMC1c10 segregated in the male parent only (Table 2). The remaining seven SSR markers were fully informative as they segregated in both parents (progeny segregation in a 1:1:1:1 or 1:2:1 ratio) and could therefore be placed on both male and female linkage maps (Table 2).

Segregation analysis

A chi-square test was performed to test for significant distortion from the expected segregation ratios of 1:1 and 3:1. Forty-four markers (9%) had significant segregation distortion at P=0.05 (Table 2). Alleles from the female parent showed segregation distortion twice as frequently as those from the male parent.

Linkage analysis and framework map construction

Female map

The map for female parent D8909-15 consisted of 105 framework markers and 55 accessory markers in 17 linkage groups and one doublet at LOD≥3. There were four unlinked markers (3%). The linkage groups in the framework map were ordered with an average confidence level of 93%. Clusters of markers showing significant segregation distortion at P=0.05 (prefaced with a "d" in Fig. 1) could be ordered with high confidence (≥96%) on groups 9, 11, and 12 (female map in Fig. 1). The 17 linkage groups and one doublet cover a total of 756 cM (Fig. 1). The linkage groups ranged from 10.1 cM to 75.0 cM, with an average of 39.8 cM. The average distance between framework markers was 8.7 cM.

Male map

The map for the male parent F8909-17 consisted of 111 framework markers and 33 accessory markers in 19 linkage groups at LOD≥3. There were three (2%) unlinked markers and one marker (E9M1415) that successfully linked with a group, but could not be ordered. Two markers showing significant segregation distortion at P=0.05 grouped with one of the doublets and could be ordered with a high confidence level (92%, group 5 in male map in Fig. 1). Four other distorted markers were interspersed among other markers. The linkage groups in the framework map were ordered with an average confidence level of 96%. The 19 linkage groups covered a total of 1,082 cM (Fig. 1). Ten linkage groups were larger than 60 cM (62.1–85.7 cM). The other seven ranged from 19.7 cM to 55.0 cM. The average distance between markers was 11.7 cM.

Map comparisons

To combine the two parental maps, markers heterozygous in both parents were added using JoinMap (these 3:1 markers are prefaced by a "3" in Fig. 1). Of the 181 3:1 markers not showing significant segregation distortion, 136 and 155 were successfully positioned on the paternal and maternal framework maps, respectively. The markers concurrent to both maps were used to join the linkage groups. Seven of these 3:1 markers grouped with a doublet (dE32M12N8, dE7M14N10), forming an additional linkage in the female map, group 18 (Fig. 1). All seven of these markers were also present on linkage group 18 of the male map. Another six 3:1 markers grouped together at LOD 10. These six markers corresponded to the six 3:1 markers ordered on group 19 of the male map. Two 3:1 markers grouped at LOD=3 but could not be mapped.

The 3:1 markers were distributed evenly throughout the linkage groups on both maps, with an average of 7.7 markers per group. These markers merged all 19 linkage groups from the parental maps, with an average of six bridging markers per group (Fig. 1).

Genome length and coverage

G_e and G_{on} were calculated for each parental framework map (Table 3). Using only the framework markers, the G_es for the female and male parents were 1,370 and 2,478 cM, respectively. Using all 1:1 markers, the G_es were slightly lower (1,284 cM for the female parent and 2,328 cM for the male parent), but the G_es were still within the 95%

confidence interval calculated using only the framework markers (Table 3).

G_{on}s for the female and male framework maps, calculated by the formula of Nelson et al. (1994), were 844 cM and 1,205 cM, respectively. C_{of} (G_{on}/G_e) was 62% for the female map and 49% for the male map (Table 3). C_es for the framework maps were calculated by the equation of Lange and Boehnke et al. (1982). Their estimates have been shown to give similar results to the equation of Bishop et al. (1983, cited by Cervera et al. 2001). C_es for the female and male framework maps were 84% and 77%, respectively (Table 3).

Discussion

Generation of markers

As reported for many plant species (Marques et al. 1998; Debener and Mattiesch 1999; Cervera et al. 2001), AFLP analysis was an efficient method for generating molecular markers in this mapping population. Every selective primer combination (E+3/M+3) gave at least three reliable markers, with an average of eight per primer combination. This is comparable to the average obtained in other grape and fruit-tree mapping projects (Dalbo et al. 2000; Dirlewanger et al. 1998), but considerably lower than the average reported for mapping projects using interspecific crosses (Marques et al. 1998; Cervera et al. 2001; Testolin et al. 2001).

Although ISSR markers have not been applied to grape mapping projects, they have been used successfully in mapping projects of other plant species (Barcaccia et al. 2000; Winter et al. 2000; Casasoli et al. 2001). The ISSR procedure was faster and easier than the AFLP procedure, but less efficient with an average of four markers per ISSR primer. Like SSR, ISSR markers are highly polymorphic, robust, and tend to be evenly distributed throughout plant genomes (Casasoli et al. 2001). Moreno et al. (1998) found the reproducibility of grape ISSR markers, after discarding faint bands, to be 92% versus 86% for RAPD markers. Our study had similar results—three researchers in our laboratory could reproduce the ISSR patterns, but had less consistent results with RAPD markers (data not shown).

SSR markers are the most useful type for comparing and combining linkage maps from different populations. As in other plant species, grape SSR markers are typically codominant and multiallelic (Thomas et al. 1994; Bowers et al. 1999). They are also highly reproducible across laboratories. Further, many microsatellite markers are transferable across related *Vitis* species (Lin and Walker 1998). The map presented here contains nine microsatellite markers. Additional microsatellite markers are currently being added to better bridge this map with existing and future *Vitis* maps such as the Riaz et al. (2004) SSR marker-based map.

Marker segregation

More than 38% of all the polymorphic markers generated were present in both parents (Table 2). This is considerably higher than the fraction obtained in mapping projects based on F_1 progeny of interspecific crosses (Hemmat et al. 1994; Marques et al. 1998; Arcade et al. 2000; Casasoli et al. 2001). The 9,621 mapping population used in this study was derived from a cross of the two half sibs, D8909-15 and F8909-17. The resulting increase in shared

Table 3 Genome length and map coverage for the 9621 (D8909-15 × F8909-17) hybrid population

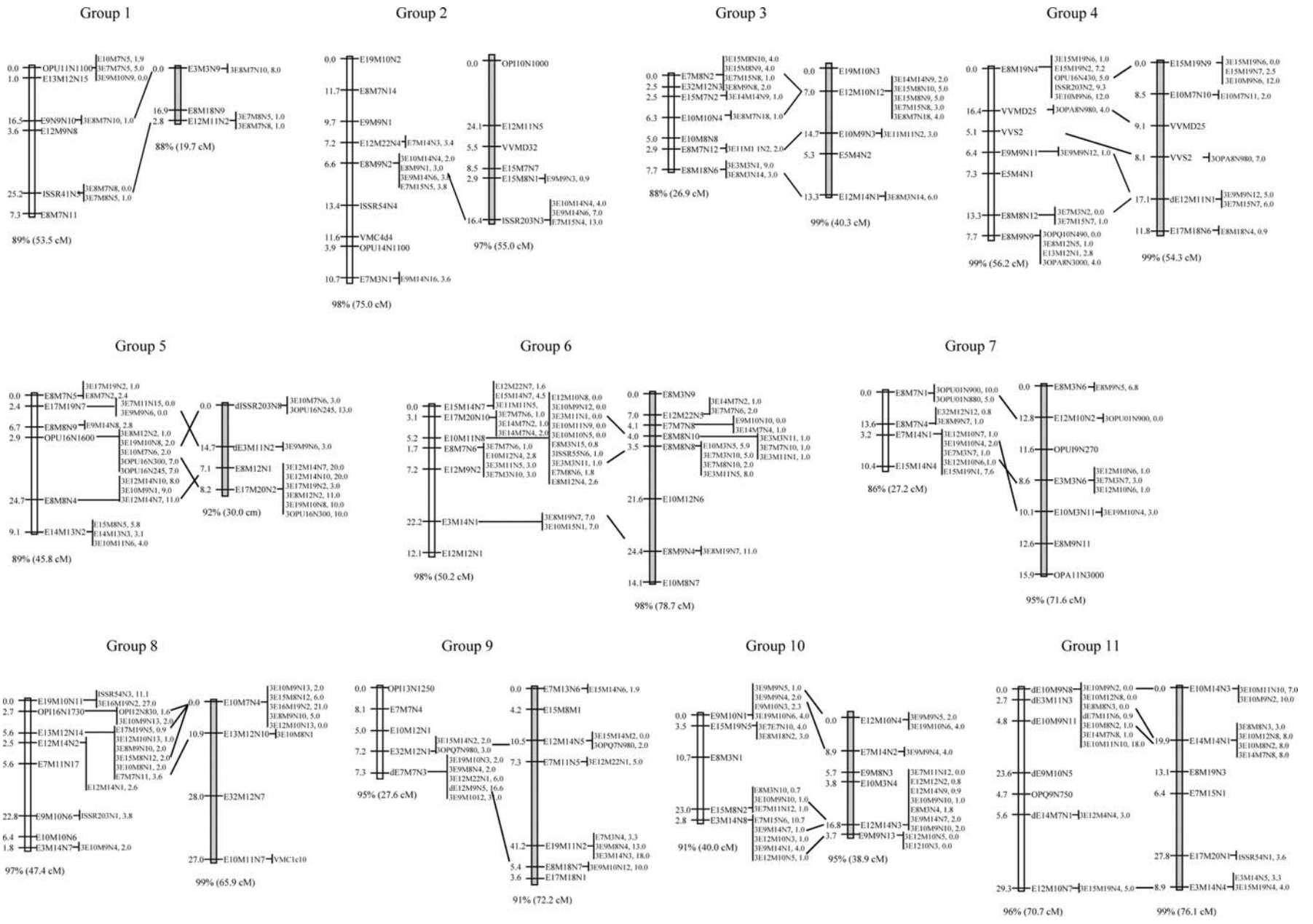
Genome length	Female	Male
Estimated[a]		
n	105	111
X (cM)	29.3	41.2
K	238	203
G_e (cM)	1,370	2,478
95% confidence interval	1,216, 1,569	2,142, 2,814
Observed[b]		
G_{of}	756	1,082
X (cM)	29.3	41.2
L	22	22
R	19	19
G_{on}	844	1,205
Observed and estimated map coverage[c]		
C_{on} (%)	62	49
C_e (%)	84	77

[a] n Total number of framework markers, X observed maximum distance between two marker pairs at LOD=3.5, K number of locus pairs with minimum LOD=3.5, G_e estimated genome length by formula $N(N-1)X/K$

[b] G_{of} Total length of the map based on framework markers; X observed maximum distance between two marker pairs at LOD=3.5; L number of linkage groups, doublets, triplets, and unlinked markers; R haploid number of chromosomes in the hybrid parents; $G_{observed:nelson}$ estimation of observed genome length based on the formula $G_{observed:framework}+X(L-R)$

[c] C_{of} Observed map coverage based G_{on}/G_e, C_e estimated map coverage estimated by $C_e=1-e^{-XN/1.25Ge}$

Fig. 1 Genetic linkage maps of D8909-15 and F8909-17 hybrids ▶ using amplified fragment length polymorphism, random amplified polymorphic DNA, inter-simple sequence repeat, and microsatellite markers. Framework maps with an average confidence level >90% were created for each parent (*f* female, *m* male) with 323 markers heterozygous in only one parent. A minimum LOD value of 3 and recombination frequency of ~0.3 were used. The female map (*white linkage groups on the left*) contained 105 framework markers covering 756 cM. The male map (*gray chromosomes on the right*) contained 111 framework markers covering 1,082 cM. Markers with significant (P=0.05) segregation distortion (denoted with a *d*) were added to the framework map when they increased the overall confidence in locus ordering, and/or they clustered together on the linkage group. Accessory markers, including markers heterozygous in both parents (denoted with a *3*), were positioned next to their closest framework marker. (Some of the markers linking homologous chromosomes are connected with *bolded lines*.) Seven markers segregating in a 1:1 ratio (four in the female map and three in the male map) remained unlinked after the framework construction (listed at the *end* of the map)


Group 1
0.0 OPU11N1100
1.0 E13M12N15
16.5 E9N9N10
3.6 E12M9N8
25.2 ISSR41N5
7.3 E8M7N11
E10M7N5, 1.9
3E7M7N5, 5.0
3E9M10N9, 0.0
3E8M7N10, 1.0
3E8M7N8, 0.0
3E7M8N5, 1.0
0.0 E3M3N9
3E8M7N10, 8.0
16.9
2.8 E8M18N9
E12M11N2
3E7M8N5, 1.0
3E8M7N8, 1.0
88% (19.7 cM)
89% (53.5 cM)
Group 2
0.0 E19M10N2
11.7 E8M7N14
9.7 E9M9N1
7.2 E12M22N4
6.6 E8M9N2
13.4 ISSR54N4
11.6 VMC4d4
3.9 OPU14N1100
10.7 E7M3N1
E7M14N3, 3.4
3E10M14N4, 2.0
E8M9N1, 3.0
3E9M14N6, 3.8
E7M15N5, 3.8
E9M14N16, 3.6
0.0 OPI10N1000
24.1 E12M11N5
5.5 VVMD32
8.5 E15M7N7
2.9 E15M8N1
16.4 ISSR203N3
E9M9N3, 0.9
3E10M14N4, 4.0
3E9M14N6, 7.0
E7M15N4, 13.0
98% (75.0 cM)
97% (55.0 cM)
Group 3
0.0 E7M8N2
2.5 E32M12N3
2.5 E15M7N2
6.3 E10M10N4
5.0 E10M8N8
2.9 E8M7N12
7.7 E8M18N6
3E15M8N10, 4.0
3E15M8N9, 4.0
3E7M15N8, 1.0
3E9M9N8, 2.0
3E14M14N9, 1.0
3E8M7N18, 1.0
3E3M3N1, 9.0
3E8M3N14, 3.0
0.0 E19M10N3
7.0 E12M10N12
14.7 E10M9N3
5.3 E5M4N2
13.3 E12M14N1
3E14M14N9, 2.0
3E15M8N10, 5.0
3E15M8N9, 5.0
3E7M15N8, 3.0
3E8M7N18, 4.0
3E11M1 1N2, 2.0
3E11M11N2, 3.0
3E8M3N14, 6.0
88% (26.9 cM)
99% (40.3 cM)
Group 4
0.0 E8M19N4
16.4 VVMD25
5.1 VVS2
6.4 E9M9N11
7.3 E5M4N1
13.3 E8M8N12
7.7 E8M9N9
3E15M19N6, 1.0
E15M19N2, 7.2
OPU16N430, 5.0
ISSR203N2, 9.3
3E10M9N6, 12.0
3OPA8N980, 4.0
3E9M9N12, 1.0
3E7M3N2, 0.0
3E7M15N7, 1.0
3OPQ10N490, 0.0
3E8M12N5, 1.0
E13M12N1, 2.8
3OPA8N3000, 4.0
0.0 E15M19N9
8.5 E10M7N10
9.1 VVMD25
8.1 VVS2
17.1 dE12M11N1
11.8 E17M18N6
3E15M19N6, 0.0
E15M19N7, 2.5
3E10M9N6, 12.0
E10M7N11, 2.0
3OPA8N980, 7.0
3E9M9N12, 5.0
3E7M15N7, 6.0
E8M18N4, 0.9
99% (56.2 cM)
99% (54.3 cM)
Group 5
0.0 E8M7N5
2.4 E17M19N7
6.7 E8M8N9
2.9 OPU16N1600
24.7 E8M8N4
9.1 E14M13N2
3E17M19N2, 1.0
E8M7N2, 2.4
3E7M11N15, 0.0
3E9M9N6, 0.0
E9M14N8, 2.8
3E8M12N2, 1.0
3E19M10N8, 2.0
3E10M7N6, 2.0
3OPU16N300, 7.0
3OPU16N245, 7.0
3E12M14N10, 8.0
3E10M9N1, 9.0
3E12M14N7, 11.0
E15M8N5, 5.8
E14M13N3, 3.1
3E10M11N6, 4.0
0.0 dISSR203N8
14.7 dE3M11N2
7.1 E8M12N1
8.2 E17M20N2
3E10M7N6, 3.0
3OPU16N245, 13.0
3E9M9N6, 3.0
3E12M14N7, 20.0
3E12M14N10, 20.0
3E17M19N2, 3.0
3E8M12N2, 11.0
3E19M10N8, 10.0
3OPU16N300, 10.0
89% (45.8 cM)
92% (30.0 cm)
Group 6
0.0 E15M14N7
3.1 E17M20N10
5.2 E10M11N8
1.7 E8M7N6
7.2 E12M9N2
22.2 E3M14N1
12.1 E12M12N1
E12M22N7, 1.6
E15M14N7, 4.5
3E11M11N5,
3E7M7N6, 1.0
3E14M7N2, 1.0
3E14M7N4, 2.0
E12M10N8, 0.0
3E10M9N12, 0.0
3E3M11N1, 0.0
3E10M11N9, 0.0
3E10M10N5, 0.0
E8M3N15, 0.8
3ISSR55N6, 1.0
3E3M3N1, 1.0
E7M8N6, 1.8
E8M12N4, 2.6
3E7M7N6, 1.0
E10M12N4, 2.8
3E3M11N5, 3.0
3E7M3N10, 3.0
3E8M19N7, 7.0
3E10M15N1, 7.0
0.0 E8M3N9
7.0 E12M22N3
4.1 E7M7N8
4.0 E8M8N10
3.5 E8M8N8
21.6 E10M12N6
24.4 E8M9N4
14.1 E10M8N7
3E14M7N2, 1.0
3E7M7N6, 2.0
E9M10N10, 0.0
3E14M7N4, 1.0
3E3M3N11, 1.0
3E7M7N10, 1.0
3E3M11N1, 1.0
E10M3N5, 5.9
3E7M3N10, 5.0
3E7M8N10, 2.0
3E3M11N5, 8.0
3E8M19N7, 11.0
98% (50.2 cM)
98% (78.7 cM)
Group 7
0.0 E8M7N1
13.6 E8M7N4
3.2 E7M14N1
10.4 E15M14N4
3OPU01N900, 10.0
3OPU01N880, 5.0
E32M12N12, 0.8
3E8M9N7, 1.0
3E12M10N7, 1.0
3E19M10N4, 2.0
3E7M3N7, 1.0
3E12M10N6,1.0
E15M19N1, 7.6
0.0 E8M3N6
12.8 E12M10N2
11.6 OPUI9N270
8.6 E3M3N6
10.1 E10M3N11
12.6 E8M9N11
15.9 OPA11N3000
E8M9N5, 6.8
3OPU01N900, 0.0
3E12M10N6, 1.0
3E7M3N7, 3.0
3E12M10N6, 1.0
E19M10N4, 3.0
86% (27.2 cM)
95% (71.6 cM)
Group 8
0.0 E19M10N11
2.7 OPI16N1730
5.6 E13M12N14
2.5 E12M14N2
5.6 E7M11N17
22.8 E9M10N6
6.4 E10M10N6
1.8 E3M14N7
ISSR54N3, 11.1
3E16M19N2, 27.0
OPI12N830, 1.6
3E10M9N13, 2.0
E17M19N5, 0.9
3E12M10N13, 1.0
3E8M9N10, 2.0
3E15M8N12, 2.0
3E10M8N1, 2.0
E7M7N11, 3.6
E12M14N1, 2.6
ISSR203N1, 3.8
3E10M9N4, 2.0
0.0 E10M7N4
10.9 E13M12N10
28.0 E32M12N7
27.0 E10M11N7
3E10M9N13, 2.0
3E15M8N12, 6.0
3E16M19N2, 21.0
3E8M9N10, 5.0
3E12M10N13, 0.0
3E10M8N1
VMC1c10
97% (47.4 cM)
99% (65.9 cM)
Group 9
0.0 OPI13N1250
8.1 E7M7N4
5.0 E10M12N1
7.2 E32M12N1
7.3 dE7M7N3
3E15M14N2, 2.0
3OPQ7N980, 3.0
3E9M10N3, 2.0
3E9M8N4, 2.0
3E12M22N1, 6.0
dE12M9N5, 6.6
3E9M1012, 3.0
0.0 E7M13N6
4.2 E15M8M1
10.5 E12M14N5
7.3 E7M11N5
41.2 E19M11N2
5.4 E8M18N7
3.6 E17M18N1
E15M14N6, 1.9
3E15M14M2, 0.0
3OPQ7N980, 2.0
3E12M22N1, 5.0
E7M3N4, 3.3
3E9M8N4, 13.0
3E3M14N3, 18.0
3E9M10N12, 10.0
95% (27.6 cM)
91% (72.2 cM)
Group 10
0.0 E9M10N1
3.5 E15M19N5
10.7 E8M3N1
23.0 E15M8N2
2.8 E3M14N8
3E9M9N5, 1.0
3E9M9N4, 2.0
3E9M10N3, 2.3
3E9M10N6, 4.0
3E7E7N10, 4.0
3E8M18N2, 3.0
E8M3N10, 0.7
3E10M9N10, 1.0
3E7M11N12, 1.0
3E9M14N7, 1.0
3E12M10N3, 1.0
3E9M14N1, 4.0
3E12M10N5, 1.0
0.0 E12M10N4
8.9 E7M14N2
5.7 E9M8N3
3.8 E10M3N4
16.8
3.7 E12M14N3
E9M9N13
3E9M9N5, 2.0
3E19M10N6, 4.0
3E9M9N4, 4.0
3E7M11N12, 0.0
E12M12N2, 0.8
E12M14N9, 0.9
3E10M9N10, 1.0
E8M3N4, 1.8
3E9M14N7, 2.0
3E10M9N10, 2.0
3E12M10N5, 0.0
3E1210N3, 0.0
91% (40.0 cM)
95% (38.9 cM)
Group 11
0.0 dE10M9N8
2.7 dE3M11N3
4.8 dE10M9N11
23.6 dE9M10N5
4.7 OPQ9N750
5.6 dE14M7N1
29.3 E12M10N7
3E10M9N2, 0.0
3E10M12N8, 0.0
3E8M8N3, 0.0
dE7M11N6, 0.9
3E10M8N2, 1.0
3E14M7N8, 1.0
3E10M11N10, 18.0
dE12M4N4, 3.0
3E15M19N4, 5.0
0.0 E10M14N3
19.9 E14M14N1
13.1 E8M19N3
6.4 E7M15N1
27.8 E17M20N1
8.9 E3M14N4
3E10M11N10, 7.0
3E10M9N2, 10.0
3E8M8N3, 3.0
3E10M12N8, 8.0
3E10M8N2, 8.0
3E14M7N8, 8.0
ISSR54N1, 3.6
3E3M14N5, 3.3
3E15M19N4, 4.0
96% (70.7 cM)
99% (76.1 cM)

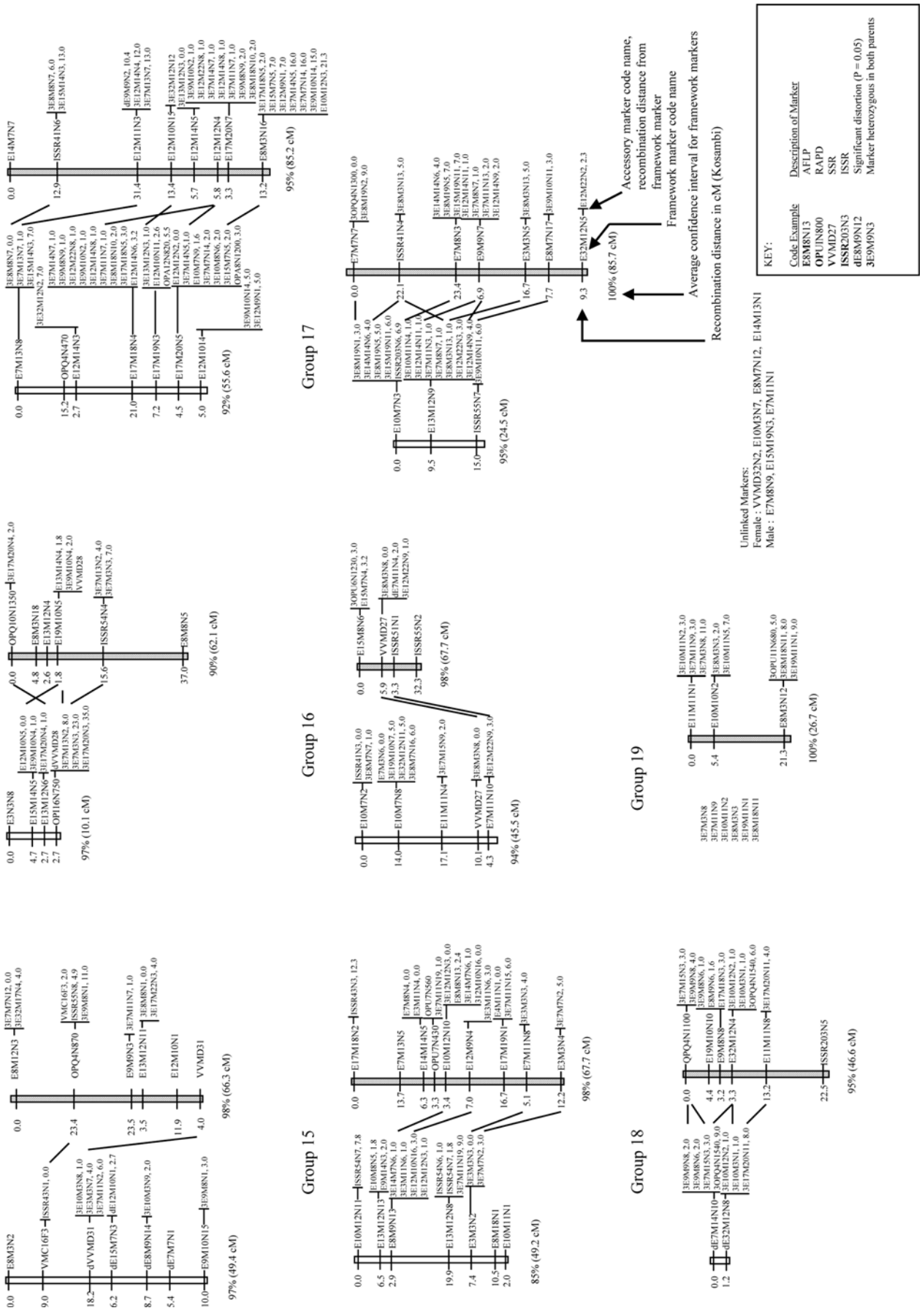


Fig. 1 (continued)

alleles between the two genotypes could explain the larger proportion of 3:1 markers. A higher fraction of 3:1 markers has also been reported in a cross between half sibs in rose (Debener and Mattiesch 1999). The 1:1 markers within the pseudotestcross strategy are more informative and therefore more useful for map construction. It follows that mapping populations derived from full-sib or half-sib crosses would therefore require a larger number of primers to be evaluated to achieve similar genome coverage.

Approximately 9% of all markers scored had significant segregation distortion at P=0.05. This amount of distortion is comparable to the percentages observed in other woody species (Barreneche et al. 1998; Krutovskii et al. 1998; Marques et al. 1998; Cervera et al. 2001), which range from 10% to 18%. A large fraction of markers showing segregation distortion could be accurately ordered onto the framework maps with PGRI. If these markers had been discarded, two linkage groups in the female framework map would have been missed (groups 11 and 12 in Fig. 1). In a high-density map of poplar, Cervera et al. (2001) also found that if distorted markers were rejected, they would have missed a significant part of a linkage group. They proposed including only markers that deviated at the 5% level and not at the 1% level to reduce the chance of type I errors of false linkage. Seven out of 14 framework markers with skewed segregation ratios had a high level of distortion (P<0.01), and without these markers we would still have missed large portions of two linkage groups. Such highly distorted markers have also been useful in other mapping studies (Kuang et al. 1999; Fishman et al. 2001).

The majority of markers that showed segregation distortion clustered together on linkage groups: groups 9, 11, and 12 in the female map and group 5 in the male map. These regions were unidirectional in bias; all markers in the region showed an excess of the parental allele. These patterns suggest a biological mechanism underlying the segregation distortion versus scoring errors or chance (Fishman et al. 2001).

Map construction

One of the most difficult problems of genetic mapping is determining the correct locus ordering because of the large number of possible orders (n!/2 for n loci) (Liu 1998; Marques et al. 1998; Remington et al. 1999). This problem is confounded by scoring and sampling errors that are inevitable in mapping projects. Since the usefulness of a map depends largely on the correct order of the loci (Plomion et al. 1995), we chose to build a framework map with the PGRI mapping program (Liu 1998; Marques et al. 1998; Remington and O'Malley 2000). Using PGRI's features, unreliable markers were removed from the data set. Markers with low bootstrap values and high jackknife values (i.e., low confidence when the marker is present and high confidence when the marker is removed) fell into three categories: markers linked too closely to other markers, markers with a high percentage of missing data (>10%), and markers with a significantly high number of double recombinants.

Overall, the quality of our mapping data was high. Framework maps with an average confidence level greater than 90% were created for both female (93%) and male (96%) parents. Remington et al. (1999) found that a bootstrapping support level of 75–80% for a particular locus order corresponded to a log likelihood of about 3. With an average bootstrap value over 90%, our framework map was built with more stringent criterion than the standard interval support criterion (Keats et al. 1991). The accuracy of the locus ordering was also confirmed with JoinMap (van Ooijen and Voorrips 2001). JoinMap uses a goodness-of-fit statistic (G^2) to assess the quality of a map order and contributions of individual loci (Stam 1993; van Ooijen and Voorrips 2001). The order of the framework markers obtained with JoinMap perfectly matched the order obtained with PGRI. The average contribution of each framework locus was extremely low; no marker contributed an average chi-square value greater than 1.0. These results are strong statistical support for the locus ordering of the framework maps.

Map genome length and coverage

The observed coverage of the two parental framework maps is about 60%. This approximation was calculated using only the framework markers. Several lines of evidence suggest a larger coverage of the maps when the accessory markers are included. After adding the 3:1 markers, the female map acquired two more linkage groups (groups 18 and 19). This addition gave both parents 19 linkage groups, corresponding to the 19 chromosomes found in *Vitis* species. Two of the previously unlinked markers in the female map (E10M3N7 and E8M7N12) and one of the unlinked markers (E7M13N9) in the male map successfully linked with groups after adding the 3:1 markers. The fraction of unlinked markers (<4% if doublets are included) is lower than the number obtained in other grape linkage-mapping studies (Lodhi et al. 1995; Dalbo et al. 2000). Finally, the Hulbert equation, as modified by Chakravarti in method 3, can overestimate the genome length (Chakravarti et al. 1991). Our C_{of}s, 62% and 49%, are probably minimum values. However, as discussed in Fishman et al. (2001), map and genome length should be considered to be qualitative, not quantitative, because many factors can cause over- or underestimation of recombination frequencies.

The total map distances obtained for the 9621 parents (756 cM in the female map and 1,082 cM in the male map) are slightly lower than distances obtained in other grape mapping projects. Lodhi et al. (1995) and Dalbo et al. (2000) both obtained total distances between 1,196 cM and 1,477 cM. The larger number of markers on their maps definitely contributed to larger total G_{on}. The exclusion of "bad" markers from our framework map (including less informative 3:1 markers) helped to minimize artificial lengthening of the map due to errors

in genotyping (Fishman et al. 2001). Additionally, unlike the other grape maps, no markers linked in repulsion were added because recombination fractions for these markers are greatly affected by scoring errors (Liu 1998; Fishman et al. 2001). The addition of more informative markers, like microsatellites, will help to increase the total distance covered and facilitate comparisons with other grape maps (Lodhi et al. 1995; Dalbo et al. 2000; Doligez et al. 2002; Grando et al. 2003; Riaz et al. 2004).

Visual examination of the linkage maps (Fig. 1) shows that the markers were not evenly distributed on the linkage groups. Each linkage group had at least seven markers (framework and accessory) with an average of about 14 markers per group, but the accessory markers often clustered at ends of the linkage groups. Clustering of markers, especially AFLP markers, has been observed in many species, including grape (Tanksley et al. 1992; Lodhi et al. 1995; Marques et al. 1998; Dalbo et al. 2000; Peng et al. 2000). These could be sites of reduced recombination corresponding to telomeres, centromeres, or regions with heterochromatin (Tanksley et al. 1992). Chromosomal rearrangements and noncollinear regions could also result in regions with reduced recombination (Rieseberg et al. 1995; Williams et al. 1995).

Applications of the map

The unique parentage and strong pest resistance of our mapping population makes this map highly valuable. Although the map is only moderately saturated with a minimum genome coverage of ~60%, it is already proving useful for mapping disease-resistance traits. For example, resistance to *X. fastidiosa*, the causal agent of PD, segregates in this mapping population, and efforts are underway to map this trait (Krivanek and Walker 2001). This disease greatly limits the cultivation of *V. vinifera* grapes in the southern US, and breeding efforts to develop resistant cultivars would be greatly accelerated with tightly linked markers.

The mapping population is also segregating for resistance to *X. index*, the dagger nematode vector of GFLV that causes fanleaf degeneration, one of the most serious viral diseases of grape (Walker et al. 1991). Evidence from the mapping population, other crosses, and backcrosses to the parents suggest that resistance to *X. index* feeding is controlled by a single, dominant gene (Walker and Jin 2000).

Once the resistance loci of both of these traits are firmly placed on the map, steps toward saturation of the regions with more markers, increasing the mapping population size, and eventual cloning of the resistance genes will be undertaken.

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References

Arcade A, Anselin F, Rampant PF, Lesage MC, Paques LE, Prat D (2000) Application of AFLP, RAPD, and ISSR markers to genetic mapping of European and Japanese larch. Theor Appl Genet 100:299–307

Barcaccia G, Albertini E, Rosellini D, Tavoletti S, Veronesi F (2000) Inheritance and mapping of 2*n*-egg production in diploid alfalfa. Genome 43:528–537

Barreneche T, Bodenes C, Lexer C, Trontin J-F, Fluch S, Streiff R, Plomion C, Roussel G, Steinkellner H, Burg K, Favre J-M, Glossl J, Kremer A (1998) A genetic linkage map of *Quercus robur* L. (pedunculate oak) based on RAPD, SCAR, microsatellite, minisatellite, isozyme, and 5S rDNA markers. Theor Appl Genet 97:1090–1103

Bowers JE, Dangl GS, Meredith CP (1999) Development and characterization of additional microsatellite DNA markers for grape. Am J Enol Vitic 50:243–246

Casasoli M, Mattioni C, Cherubini M, Villani F (2001) A genetic linkage map of European chestnut (*Castanea sativa* Mill.) based on RAPD, ISSR, and isozyme markers. Theor Appl Genet 102:1190–1199

Cervera MT, Storme V, Ivens B, Gusmao J, Liu BH, Hostyn V, Slycken JV, Montagu MV, Boerjan W (2001) Dense genetic linkage maps of three populus species (*Populus deltoides*, *P. nigra* and *P.trichocarpa*) based on AFLP and microsatellite markers. Genetics 158:787–809

Chakravarti A, Lasher LK, Reefer JE (1991) A maximum likelihood method for estimating genome length using genetic linkage data. Genetics 128:175–182

Cristofani M, Machado MA, Grattapaglia D (1999) Genetic linkage maps of *Citrus sunki* Hort. ex. Tan. and *Poncirus trifoliata* (L.) Raf. and mapping of citrus tristeza virus resistance gene. Euphytica 109:25–32

Dalbo MA, Ye G-N, Weeden NF, Steinkellner H, Sefc KM, Reisch BI (2000) A gene controlling sex in grapevines placed on a molecular marker-based genetic map. Genome 43:333–340

Dalbo MA, Ye G-N, Weeden NF, Wilcox WF, Reisch BI (2001) Marker-assisted selection for powdery mildew resistance in grapes. J Am Soc Hortic Sci 126:83–89

Debener T, Mattiesch L (1999) Construction of a genetic linkage map for roses using RAPD and AFLP markers. Theor Appl Genet 99:891–899

Dirlewanger E, Pronier V, Parvery C, Rothan C, Guye A, Monet R (1998) Genetic linkage map of peach [*Prunus persica* (L.) Batsch] using morphological and molecular markers. Theor Appl Genet 97:888–895

Doligez A, Bouquet A, Danglot Y, Lahogue F, Riaz S, Meredith C, Edwards K, This P (2002) Genetic mapping of grapevine (*Vitis vinifera* L.) applied to the detection of QTLs for seedlessness and berry weight. Theor Appl Genet 105:780–795

Fishman L, Kelly AJ, Morgan E, Willis JH (2001) A genetic map in the *Mimulus guttatus* species complex reveals transmission ratio distortion due to heterospecific interactions. Genetics 159:1701–1716

Grando MS, Bellin D, Edwards KJ, Pozzi C, Stefanini M, Velasco R (2003) Molecular linkage maps of *Vitis vinifera* L. and *Vitis riparia* Mchx. Theor Appl Genet 106:1213–1224

Grattapaglia D, Sederoff R (1994) Genetic linkage maps of *Eucalyptus grandis* and *Eucalyptus urophylla* using a pseudo-testcross mapping strategy and RAPD markers. Genetics 137:1121–1137

Hemmat M, Weeden NF, Manganaris AG, Lawson DM (1994) Molecular marker linkage map for apple. J Hered 85:4–11

Hopkins DL (1989) *Xylella fastidiosa*: a xylem-limited bacterial pathogen of plants. Annu Rev Phytopathol 27:271–290

Hulbert SH, Ilott TW, Legg EJ, Lincoln SE, Lander ES, Michelmore RW (1988) Genetic analysis of the fungus, *Bremia lactucae*, using restriction fragment length polymorphisms. Genetics 120:947–958

Jin Y (1997) Resistance to the dagger nematode, *Xiphinema index*: screening trials and the inheritance of RAPD markers. MS Thesis, University of California

Keats BJS, Sherman SL, Morton NE, Robson EB, Buetow KH, Cann HM, Cartwright PE, Chakravarti A, Franckle U, Green PP, Ott J (1991) Guidelines for human genetic linkage maps: an international system for human linkage maps (ISLM 1990). Genomics 9:557–560

Krivanek, AF, Walker MA (2001) Progress in the genetic analysis of Pierce's disease resistance. In: Technical abstracts of 52nd annual meeting. American Society for Enology and Viticulture, San Diego, p 28

Krutovskii KV, Vollmer SS, Sorensen FC, Adams WT, Knapp SJ, Strauss SH (1998) RAPD genome maps of Douglas fir. J Hered 89:197–205

Kuang H, Richardson T, Carson S, Wilcox P, Bongarten B (1999) Genetic analysis of inbreeding depression in plus tree 850.55 of *Pinus radiata* D. Don I. Genetic map with distorted markers. Theor Appl Genet 98:697–703

Lange K, Boehnke M (1982) How many polymorphic genes will it take to span the human genome? Am J Hum Genet 34:842–845

Lin H, Walker MA (1998) Identifying grape rootstocks with simple sequence repeat (SSR) DNA markers. Am J Enol Vitic 49:403–407

Liu BH (1998) Statistical genomics: linkage, mapping, and QTL analysis. CRC, New York, pp 273–299, 529–544

Lodhi MA, Daly MJ, Ye G-N, Weeden NF, Reisch BI (1995) A molecular marker based linkage map of *Vitis*. Genome 38:786–794

Marques CM, Araujo JA, Ferreira JG, Whetten R, O'Malley DM, Liu B-H, Sederoff R (1998) AFLP genetic maps of *Eucalyptus globulus* and *E. tereticornis*. Theor Appl Genet 96:727–737

Martelli GP, Savino V (1988) Fanleaf degeneration. In: Pearson RC, Goheen AC (eds) Compendium of plant diseases. APS, St. Paul

Moreno S, Martin JP, Ortiz JM (1998) Inter-simple sequence repeats PCR for characterization closely related grapevine germplasm. Euphytica 101:117–125

Nelson CD, Kubisiak TL, Stine M, Nance WL (1994) A genetic linkage map of longleaf pine (*Pinus palustris* Mill.) based on random amplified polymorphic DNAs. J Hered 85:433–439

Ooijen JW van, Voorrips RE (2001) JoinMap 3.0, software for the calculation of genetic linkage maps. Plant Research International, Wageningen

Peng J, Korol AB, Fahima T, Röder MS, Yefirm IR, Ronin YI, Li YC, Nevo E (2000) Molecular genetic maps in wild emmer wheat *Triticum dioccoides*: genome-wide coverage, massive negative interference, and putative quasi-linkage. Genome Res 10:1509–1531

Plomion C, O'Malley DM, Durel CE (1995) Genomic analysis in maritime pine (*Pinus pinaster*). Comparison of two RAPD maps using selfed and open-pollinated seeds of the same individual. Theor Appl Genet 90:1028–1034

Remington DL, O'Malley DM (2000) Whole-genome characterization of embryonic stage inbreeding depression in a selfed loblolly pine family. Genetics 155:337–348

Remington DL, Whetten RW, Liu B-H, O'Malley DM (1999) Construction of a AFLP genetic map with nearly complete genome coverage in *Pinus taeda*. Theor Appl Genet 98:1279–1292

Riaz S, Dangl GS, Edwards KJ, Meredith CP (2004) A microsatellite marker based framework linkage map of *Vitis vinifera* L. Theor Appl Genet 108:864–872

Rieseberg LH (1995) Chromosomal and genetic barriers to introgression in *Helianthus*. Genetics 141:1163–1171

Stam P (1993) Construction of integrated genetic linkage maps by means of a new computer package: JoinMap. Plant J 3:739–744

Tanksley SD, Ganal MW, Prince JP, de Vicente MC, Bonierbale MW, Broun P, Fulton TM, Giovannoni JJ, Grandillo S, Martin GB, Messeguer R, Miller JC, Miller L, Paterson AH, Pineda O, Röder MS, Wing RA, Wu W, Young ND (1992) High density molecular linkage maps of the tomato and potato genomes. Genetics 132:1141–1160

Testolin R, Huang WG, Lain O, Messina R, Vecchione A, Cipriani G (2001) A kiwifruit (*Actinidia* spp.) linkage map based on microsatellites and integrated with AFLP markers. Theor Appl Genet 103:30–36

Thomas MR, Cairn P, Scott NS (1994) DNA typing of grapevines: a universal methodology and database for describing cultivars and evaluating genetic relatedness. Plant Mol Biol 25:939–949

Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M, Zabeau M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414

Walker MA, Jin Y (2000) Breeding *Vitis rupestris* × *Muscadinia rotundifolia* rootstocks to control *Xiphinema index* and fanleaf degeneration. Acta Hortic 528:511–115

Walker MA, Lider LA, Goheen AC, Olmo HP (1991) VR 039-16 grape rootstock. HortScience 26:1224–1225

Williams CG, Goodman MM, Stuber CW (1995) Comparative recombination distances among *Zea mays* L. inbreds, wide crosses and interspecific hybrids. Genetics 141:1573–1581

Winter P, Benko-Iseppon A-M, Huttel B, Ratnaparkhe M, Tullu A, Sonnate G, Pfaff T, Tekeoglu M, Santra D, Sant VJ, Rajesh PN, Kahl G, Muehlbauer FJ (2000) A linkage map of chickpea (*Cicer arietinum* L.) genome based on recombinant inbred lines from a *C. arietinum* × *C. reticulatum* cross: localization of resistance genes for fusarium wilt races 4 and 5. Theor Appl Genet 101:1155–1163

Wu KS, Jones L, Danneberger L, Scolnik PA (1994) Detection of microsatellite polymorphisms without cloning. Nucleic Acids Res 22:3257–3258