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ORIGINAL PAPER

Refined mapping of the Pierce's disease resistance locus, *PdR1*, and *Sex* on an extended genetic map of *Vitis rupestris* × *V. arizonica*

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Abstract A framework genetic map based on genomic DNA-derived SSR, EST-derived SSR, EST-STS and EST-RFLP markers was developed using 181 genotypes generated from D8909-15 (female) × F8909-17 (male), the '9621' population. Both parents are half siblings with a common female parent, *Vitis rupestris* 'A. de Serres', and different male parents (forms of *V. arizonica*). A total of 542 markers were tested, and 237 of them were polymorphic for the female and male parents. The female map was developed with 159 mapped markers covering 865.0 cM with an average marker distance of 5.4 cM in 18 linkage groups. The male map was constructed with 158 mapped molecular markers covering 1055.0 cM with an average distance of 6.7 cM in 19 linkage groups. The consensus '9621' map covered 1154.0 cM with 210 mapped molecular markers in 19 linkage groups, with average distance of 5.5 cM. Ninety-four of the 210 markers on the consensus map were new. The '*Sex*' expression locus segregated as single major gene was mapped to linkage group 2 on the consensus and the male map. *PdR1*, a major gene for resistance to Pierce's disease, caused by the bacterium *Xylella fastidiosa*, was mapped to the linkage group 14 between markers VMCNg3h8 and VVIN64, located 4.3 and 2.7 cM away from *PdR1*, respectively. Differences in segregation distortion of markers were also compared between parents, and three clusters of skewed markers were observed on linkage groups 6, 7 and 14.

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Introduction

Genetic linkage maps developed with molecular markers are valuable tools for localizing disease resistance genes and quantitative trait loci (QTLs) for important agronomic and morphological traits for map-based positional cloning (Lukowitz et al. 2000; Yan et al. 2003). Molecular markers that are tightly linked to disease resistance and QTLs can also be used in marker-assisted selection (MAS). This technique may be most useful for disease and pest resistance breeding efforts where resistance is the critical first step prior to the inclusion of agronomic or horticultural traits. MAS also allows breeders to rapidly screen and select plants at the seedling stage, thus, reducing the cost of maintaining large numbers of undesirable plants in the greenhouse and field.

In grapes, genetic linkage maps have been created from populations targeting disease resistance genes (Pauquet et al. 2001; Doucleff et al. 2004; Fischer et al. 2004) and important agronomic quantitative and qualitative traits (Dalbó et al. 2000; Doligez et al. 2002). The majority of markers on these maps were dominant (AFLP, RAPD, ISSR) in nature and difficult to transfer among different mapping populations. Recently, two reference genetic linkage maps from a *Vitis vinifera* background were created with co-dominant, simple sequence repeat (SSR) markers (Adam-Blondon et al. 2004; Riaz et al. 2004). These maps have greatly improved the availability, and facilitated the selection, of useful markers to saturate linkage maps in different *Vitis* species backgrounds.

A collection of more than 150,000 expressed sequence tags (EST) derived from randomly selected grape cDNAs is now available (http://www.tigr.org/tdb/tgi/vvgi/). A proportion of these expressed genes also contain SSR arrays, which may be located in the 5′- or 3′-untranslated regions as well as in the coding sequence (Scott et al. 2000; Cordeiro et al. 2001; Decroocq et al. 2003). These so-called EST-derived SSR markers have the potential advantage of tight linkage with significant gene variants. EST-SSR markers are also less expensive to develop as compared to SSRs derived from genomic DNA sequences, are more conserved (Decroocq et al. 2003), and are easy to transfer among species for comparative analysis (Scott et al. 2000; Eujayl et al. 2002; Gupta et al. 2003). EST-SSR markers have been used to generate linkage maps in a variety of crops such as rice (Kurata et al. 1994), maize (Chao et al. 1994), and wheat (Yu et al. 2004). The genome facility at the University of California, Davis, developed eight cDNA libraries from different *Vitis* species. They searched all contig and singleton ESTs for the presence of SSRs, and made a set of 1,000 EST-derived SSR markers available to the public (http://www.cgf.ucdavis.edu/).

Previously, Doucleff et al. (2004) presented an AFLP molecular marker based genetic map of the '9621' population using 116 genotypes. In this study, we report on an extended genetic map of the '9621' population based on 181 genotypes using genomic DNA derived SSR, EST-SSR and functionally associated EST-STS and EST-RFLP markers. This mapping population segregates for resistance to *Xylella fastidiosa*, the bacterial causal agent of Pierce's disease (PD), on which Krivanek et al. (2006) mapped a primary resistance gene (*PdR1*) on the linkage group (LG) 14 with AFLP and three genomic SSR markers. The study presented here reports more detailed mapping of the *PdR1* resistance gene and *Sex* expression (as a single major gene) with SSR markers. A genetic map of the '9621' population would provide a source of markers for MAS, and facilitate efforts in map-based positional cloning of PD resistance genes.

Materials and methods

Plant material and DNA extraction

The mapping population, '9621', consisted of 181 progeny plants from the cross of two half-sib genotypes, D8909-15 (*V. rupestris* 'A. de Serres' × *V. arizonica* b42-26) × F8909-17 (*V. rupestris* 'A. de Serres' × *V. arizonica/candicans* b43-17). Only 86 plants of this study were common to population set used by Doucleff et al. (2004). All plants are maintained in the vineyards of the Department of Viticulture and Enology, University of California, Davis. DNA was extracted from young leaves and shoot tips using CTAB procedure described by Lodhi et al. (1994).

Molecular markers

Nuclear and EST-derived SSR markers

Information on the SSR markers used is provided in Table 1. The majority of SSR markers were from two large series: VMC (*Vitis* Microsatellite Consortium, Agrogene SA, Moissy Cramayel, France) and VVI (INRA Génoplante). Laboratories interested in unpublished VMC SSR primer sequences should contact Agrogene SA, Moissy Cramayel, France. All markers developed within the platform of "INRA Génoplante" are available as NCBI uni-STS sequences (http://www.ncbi.nlm.nih.gov/). SSR markers from previously published studies are cited in Table 1.

Most of the EST-SSR markers were developed by Dr. Douglas Cook (Department of Plant Pathology, University of California, Davis, USA) from an EST database of *Vitis* species (http://www.cgf.ucdavis.edu/index.cfm). The NCBI non-redundant database was used to establish the function of EST-SSR markers. Ten EST-SSR markers developed by Scott et al. (2000) and Decroocq et al. (2003) were also used (Table 1).

EST-RFLP markers

Sequences for the development of EST-RFLP markers were acquired from two sources: 18 cDNA sequences of cloned grape genes were acquired from GenBank (coded with suffix 'WEST' or accession numbers); 48 cDNA sequences were obtained from Dr. Douglas Adams, Department of Viticulture and Enology, University of California Davis (coded with suffix 'VEST'). Primers for all cDNA clones were designed with PRIMER 0.5 (http://www.broad.mit.edu/ftp/distribution/software/). Primers with similar properties were selected to standardize conditions for PCR reactions; primers were 20–23 nucleotides long with 50–60% GC content and melting temperatures ranged from 59–64°C. Primer sequences of polymorphic EST-RFLP for the '9621' population are provided in Table 2.

Amplification

A total of 354 SSR, 122 EST-SSR and 66 EST-RFLP markers were tested on a small set of eight genotypes that included both parents. PCR conditions used were described by Riaz et al. (2004). The amplified products

Table 1 Sources of grape SSR, ESTs and EST-SSR markers used to construct a genetic map of the '9621' mapping population

Marker symbol	Sources	No. of informative markers for this cross	Reference
VVS	CSIRO, Australia	2	Thomas and Scott (1993)
VVMD	University of California, Davis, USA	10	Bowers et al. (1996, 1999)
VrZAG	University of Agriculture, Vienna, Austria	12	Sefc et al. (1999)
VMC	University of Udine, Italy	7	Di Gaspero et al. (2000)
SCU	Southern Cross University, Australia	4	Scott et al. (2000)
VVC	INRA, France	7	Decroocq et al. (2003)
VMC	University of Madrid, Spain	2	Arroyo-Garcia and Martinez-Zapater (2004)
VMC	INRA, France	1	Adam-Blondon et al. (2004)
VMC, VMCNg	Vitis Microsatellite Consortium	97	Agrogene SA (Moissy Cramayel, France), now Eurofins (http://www.eurofins.com/)
VVI	NCBI uni-STS	34	Merdinoglu et al. (2005)
ctg, CF, AF, BM	University of California, Davis	44	http://www.cgf.ucdavis.edu/
WEST	NCBI EST sequences	8	http://www.ncbi.nlm.nih.gov/
VEST	Doug Adams, unpublished	9	University of California, Davis
	Morphological marker (Sex)	1	
	PdR1	1	
	Total no. of markers	239	

of SSR and EST-SSR markers were separated on denaturing 6% polyacrylamide sequencing gels and visualized by silver staining with a commercial kit (Promega, Madison, Wisconsin, USA). Up to three markers differing in allele sizes were combined after amplification and run on the same gel. Only informative markers polymorphic for the parents were used on the entire mapping population of 181 genotypes.

The EST-RFLP markers that were not polymorphic on 6% polyacrylamide gels in test run were further subjected to restriction enzyme digestions to obtain restriction site based polymorphism. No prior sequence divergence information on the parental lines (D8909-15 and F8909-17) was available. A total of ten restriction enzymes with different restriction sites were selected to test on a small set including parents and progeny samples (*Ava*II, *Hin*dIII, *Eco*RI, *Hin*f1, *Msp*I, *Bst*NI, *Eco*RV, *Rsa*I, *Dra*I, and *Hae*III). Restriction enzyme digestions were carried out in a 1:1 (v/v) ratio of restriction mixture (1-μl 10× restriction buffer, 0.5-μl restriction enzyme (2–10 units), and 3.5 μl water) and amplified DNA. Digest products were run on 1% agarose gels to determine possible restriction site based length polymorphism. Only polymorphic EST-RFLP markers were used on the entire progeny.

Scoring, data organization and linkage analysis

All marker types were scored visually for the presence or absence of bands without any prior knowledge of phase information for each parent. Two independent data sets (one for each parent) were generated and they were combined into a single consensus set containing each individual's complete genotype based on parental segregation data. Segregation patterns for each marker were assigned according to the JoinMap data entry notation (<abxcd>, <abxac>, <abxab>, <abxaa>, and <aaxab>). Some SSR primer pairs detected the amplification of more than one locus that were on different locations on the chromosome; they were designated with small letters. To detect the possible deviation ($P \leq 0.05$) of gametic segregation from Mendelian ratios (1:1), all markers were evaluated by Chi square analysis. Distorted markers were kept in the linkage analysis unless they were of low quality and affected the marker order within a linkage group. All five possible segregation types were present in the data set: a marker segregating in one or the other parent with two alleles (ab × aa or aa × ab), a marker segregating in both parents with two alleles (ab × ab), a marker segregating in both parents with three alleles (ab × ac), and a marker segregating in both parents with four alleles (ab × cd).

The '9621' individuals not previously screened for PD resistance were evaluated using the procedures described by Krivanek et al. (2006). The details of the screening process and the data analysis are presented in Krivanek et al. (2005) and Krivanek and Walker (2005). The '9621' population was also scored for the '*Sex*' expression locus. Clusters and flowers of all genotypes were examined over two consecutive years; all plants were either staminate or pistillate. *Sex* was scored as a male segregating marker.

Linkage analysis was performed with JoinMap 3.0, with linkage parameters set at LOD 5.0 and a recombination frequency of 0.45 to construct the parental and

Table 2 Primer sequences of EST-STS and EST-RFLP markers polymorphic for the '9621' population

Coded marker name	Accession number	Function	Primer sequences	Mapping enzyme	Map location
CGF1000660a	CGF1000660b	Unknown	F-TCAAATCTCATTAGGATTAAATGGA R-CAAAGGAACTTCAACAAAACCA	No RE	19
VINST1	AB046375.1 B	*Vitis vinifera* VINST1 gene for stilbene synthase	F-TGAGGCTCACCTCCAAGC R-TGGGGTGATCTTGGACTTTGG	No RE	16
WEST-4	AB015872	Chalcone synthase	F-AAATGTCACCTTGGGACTGGG R-CAAGAGAGAATGCCGATGCC	No RE	19
TC14826	TC14826	Cinnamyl-alcohol dehydrogenase	F-CAAGGTGGTGTGCGTGAC R-GAAAACTGGATCTGAAAACCAA	*Ava*II	Unlinked
WEST-1	AJ001062	Hexose transporter promoter	F-TGGAGATTGAGCTTGGGTGG R-GTGAGGTGTGGCCTGTACTTCC	*Hin*dIII	18
WEST-9	U97522	Class IV endochitinase (VvChi4B)	F-GTAGCGGCAGTGGTAGCTCG R-ACCGGAGACACATGGATATTGG	*Hin*dIII	Linked to 17
WEST-14	X75963	Chalcone isomerase	F-GGTCGCCAGTATTCAGACAAGG R-TCAATTTTCTCATCCCCAGCC	*Bst*NI	13
WEST-18	L34836	Malate dehydrogenase	F-GAGGATTCTGGGACTTGGGG R-AAGCGAGATGAGTTGTGCCG	*Rsa*I	11
VEST-17	University of California, Davis	Initiation factor	F- TTCGATAACCAGTCCAAGCC R- CACACCACATATTTCATCAGCC	*Ava*II	5
VEST-83	University of California, Davis	Latex abundant protein	F-CATGCTCTGAACCATCGGC R-GATCCGTACTCCGAACTGGG	*Rsa*I	14
VEST-195	University of California, Davis	Superoxide dismutase	F-AAAAGGGCGATTCATCTACGG R- ATCCTGATTTGCAGAGTTTCACC	*Ava*II	13
VEST-235	University of California, Davis	Function unknown	F-CGATCTTTCCCACAATTCCC R-AGGTTCTCAAACCCAGAAGGC	*Msp*I	Linked to10
VEST-238	University of California, Davis	Malate dehydrogenase	F-TCTAAGGAGGGTCTCATCCCC R-CTTTTCTTTGGAACACCGGC	*Hinf*I	10
VEST-390	University of California, Davis	Lipid transfer protein	F-AGCTTGTGTGATGGTGATATGC R-GTGAAGGGCTGATCTTGTAAGG	*Msp*I	8
VEST-401	University of California, Davis	Glutaredoxin-like protein	F-ACGGAGGAGTTCTGGAAATGG R-GTAGAAACTTCAGCAATGGCACC	*Hae*III	17
VEST-525	University of California, Davis	Argenine/serine rich splicing factor	F-TGTATTCATCCCCAGAGATCG R-TCTGCNTCTACTTCTGCTGCG	*Hinf*I	18
VEST-533	University of California, Davis	Small heat shock protein	F-AATCTCTGACACCCAACATGG R-TTGACGCCTTCNTTCTCTTCC	*Eco*RI	4

Sequences coded with a VEST suffice were obtained from Dr. Doug Adams, University of California, Davis. All other sequences with accession numbers and code WEST were obtained from GenBank web site

consensus maps (Stam 1993; Stam and Van Ooijen 1995). Marker order was determined relative to the established order obtained from the JoinMap 3.0 analysis for each parent. Map units in centimorgans (cM) were derived from the Kosambi (K) mapping function (Kosambi 1944). Linkage groups were drawn using the Mapchart 2.1 software (Voorips 2002).

Estimation of genome length and map coverage

The genome length [$E(G)^a$] estimation was determined by a method of moments estimator (Hulbert et al. 1988). The 95% confidence interval for G was calculated according to Gerber and Rodolphe (1994). The expected genome map coverage [$E(C_n)$] was calculated according to Bishop et al. (1983). Two estimates of observed genome coverage were calculated for each parent: the total length of the framework map (G_{obs}) and the genome length estimates (G_{on}) obtained by the formula described by Nelson et al. (1994). For a thorough description of these methods, see Riaz et al. 2004.

Results

Analysis of molecular markers

Out of the 354 SSR markers tested on D8909-15 and F8909-17, 80 did not amplify, 109 produced an unclear banding pattern or lacked polymorphism for parents and were thus discarded. A total of 163 SSR markers detected 165 (61%) useful loci in at least one parent (Table 3). Of the 122 tested EST-SSR primer pairs, 88 amplified successfully and 55 (63%) of them were

Table 3 Segregation types obtained with SSR, EST-SSR and EST-RFLP markers in the '9621' population

Marker type	Primers tested	Primers amplified	D8909-15 (female parent)	F8909-17 (male parent)	D8909-15 and F8909-17			Useful loci	Polymorphism percentage
			ab × aa 1:1	aa × ab 1:1	ab × ac 1:1:1:1	ab × cd 1:1:1:1	ab × ab 1:2:1		
SSR	354	274	33	30	64	28	11	165	61
EST-SSR	122	88	14	10	18	7	6	55	63
EST-RFLP	66	59	5	8	1	1	2	17	29
Total	542	421	52	47	83	36	19	237	51

polymorphic for this cross. Among the 66 tested EST-RFLP primer pairs, 59 amplified successfully, 14 were polymorphic after restriction enzyme digestions, and only 3 markers, CGF1000660a, VINST 1 and WEST-4, were polymorphic without restriction enzyme digestion (Table 2). EST-RFLP markers that were polymorphic without restriction enzyme digestion will be called sequence tagged site (EST-STS) markers later in this manuscript.

Of the 237 molecular markers tested in the progeny, 36 were fully informative (ab × cd), 83 had three alleles (ab × ac) and 118 had two alleles [47 (aa × ab), 52 (ab × aa) and 19 (ab × ab)] (Table 3). A total of 72% of the markers were useful for the female parent D8909-15 and 70% were useful for the male parent F8909-17. Chi square analysis indicated segregation distortion for 25 markers (14%) for D8909-15 and 12 markers (7%) for F8909-17. A total of 41 markers (17%) showed segregation distortion for the '9621' population consensus data set: 28 markers were SSR, 11 were EST-SSR, and one each were EST-STS and EST-RFLP markers.

Construction of parental and consensus maps

A total of 171 markers were used to develop the D8909-15 female parent linkage map. Nine markers were unlinked, 159 markers were mapped into 20 linkage groups (LG), and three markers were linked but unmapped (Table 4). LG 15 was missing from the female map because 75% of its markers were male. When compared to the consensus map, we observed that LG 8 was fragmented into three groups with two markers in each group (Table 4). With the missing and fragmented LGs taken into consideration, there were 18 LGs on the female map. The total length of the map was 865.0 cM with an average distance of 5.5 cM between markers. The largest LG consisted of 23 mapped markers. There were only five gaps larger than 20 cM. The biggest gap was on LG 16 where the distance between marker VVMD37 and VVC5 was 34.6 cM. Marker order on the female map was consistent with the consensus and the male maps (Fig. 1 consensus map, female map not shown), except in two cases where marker order was flipped in comparison to consensus and male map [(LG 1, markers ctg1011774 and ctg1012992) (LG 13, markers VMCNg4e10.1 and VMC9h4.2)].

A total of 167 molecular markers and the *PdR1* locus were used for the F8909-17 male parent map. One hundred fifty-eight molecular markers were mapped into 21 LGs (Table 4). Nine markers remained unlinked. We observed that LG 9 and 18 were fragmented into two groups each when compared to the consensus map. These fragments were consolidated resulting in a total of 19 LGs for F8909-17 (Table 4). Marker order on the male map was consistent with the consensus map (Fig. 1 consensus map, male map not shown). The map length was 1,055.0 cM with an average distance of 6.7 cM between markers. There were 11 gaps on the male map with distances larger than 20 cM. The largest gap was on LG 8, which had a 35 cM distance between marker VMC5g6.1 and VMC3c9.

The '9621' consensus map was developed with 237 molecular markers, the *PdR1* locus and the morphological marker, *Sex* expression. The consensus map consisted of a total of 235 linked molecular markers ordered into 19 LGs (depicting the 19 *Vitis* chromosomes) with 210 mapped molecular markers (Fig. 1, Table 4). Only one SSR marker, VMC6g1 segregating for female parent, and one EST-RFLP marker TC14826 segregating for male parent were unlinked. Among 25 unmapped markers, nine segregated in the female parent, seven segregated in the male parent, and nine segregated in both parents. Unmapped markers are shown at the bottom of the LGs (Fig. 1). Placement of unmapped markers on their respective LGs affected the order of neighboring markers; therefore, they were excluded from the final map. Only three unmapped markers showed segregation distortion.

The *PdR1* locus was mapped as a single major gene on LG 14 between markers VMCNg3h8 and VVIN64, for both the male and consensus maps (Fig. 1). On the male map and the consensus map, the *PdR1* locus was 4.34 and 2.78 cM away from markers VMCNg3h8 and VVIN64, respectively. These results were in agreement

Table 4 Features of the D8909-15, F8909-17, and '9621' consensus genetic maps

Linkage group	D8909-15 (female)			F8909-17 (male)			Consensus '9621' map					Integrated map[a]
	Linked markers	Mapped	Size	Linked markers	Mapped	Size	No. of linked markers	No. of mapped markers	Distorted markers	Size	No. of new markers	Size
1	13	13	52.7	14	14	95.3	18	16	3	91.2	8	87.5
2	8	8	53.6	8	8	38.9	10	9	0	51.0	0	79.7
3	6	6	39.2	6	6	72.1	8	8	2	65.9	4	70.3
4	11	11	58.1	9	9	92.1	15	14	0	80.0	4	90.9
5	12	11	62.4	9	9	54.8	17	11	2	46.8	4	83.4
6	8	8	37.5	11	11	72.0	17	11	11	75.8	3	82.5
7	6	5	51.2	4	4	50.3	9	8	5	71.4	1	102.7
8	2	2	6.1	6	6	58.9	9	7	0	56.3	2	112.7
8	2	2	2.8									
8	2	2	15.7									
9	6	6	62.6	4	4	7.5	10	10	0	71.1	2	104.1
9				2	2	30.6						
10	6	6	34.7	5	5	33.4	9	7	0	30.9	3	83.7
11	5	5	45.8	4	4	30.8	8	8	2	48.9	4	75.1
12	10	10	33.1	10	10	32.8	13	12	1	33.2	4	81.9
13	5	5	16.9	7	7	66.3	8	8	0	57.3	3	101.1
14	24	23	60.9	21	21	81.3	30	29	8	76.8	5	94.8
15				4	4	17.8	4	4	1	17.8	0	37.9
16	4	4	47.0	4	4	8.6	9	9	1	51.5	2	92.4
17	7	7	42.4	7	7	51.9	9	9	3	61.1	4	58.0
18	12	12	96.8	5	5	34.3	15	15	0	105.7	4	131.5
18				5	5	63.2						
19	13	13	45.3	13	13	62.2	17	15	2	61.3	3	76.6
Total	162	159	865.0	158	158	1055.0	235	210	41	1154.0	60	1647.0
Avg. distance in cM		5.5			6.7				5.5		3.3	

[a] International integrated map developed with SSR markers based on five different *V. vinifera* mapping populations (Doligez et al. 2006)

with Krivanek et al. (2006) who also placed *PdR1* locus on LG 14 between markers VMC5b3 and VMC6e1.

Multiple loci were detected with the SSR marker VMC9f4; the locus mapped to LG 9 on the Riaz et al. (2004) reference map of Riesling × Cabernet Sauvignon and to LG 14 on the '9621' consensus map. Table 4 presents the main features of all three maps. Only the consensus map is presented in Fig. 1 because there were only minor changes in marker order between the male and female maps and no new information was gained from inclusion of the parental maps. The LGs on the '9621' consensus map were numbered from 1 to 19 according to the order found on the two international reference maps (Adam-Blondon et al. 2004; Riaz et al. 2004). The '9621' consensus map covers 1154.0 cM distance with an average distance of 5.5 cM between markers (Table 4).

Mapping of *Sex* expression locus

Segregation of the *Sex* expression locus fit the single major gene model of Antcliff (1980). Among the 181 plants of the '9621' population, 92 were female, 83 male and 6 did not flower. The *Sex* locus was placed on LG 2 between markers VVIB23 and VVMD34, for both the male and consensus maps (Fig. 1). On the male map, the sex locus was 1.59 and 5.95 cM away from markers VVIB23 and VVMD34, respectively. The marker VVIB23 segregated only for the male parent (aa × ab), while marker VVMD34 segregated for both parents with one common allele (ab × ac), and allele 'c' was linked to the male allele. On the consensus map, the *Sex* locus was 1.62 and 6.88 cM away from VVIB23 and VVMD34 markers, respectively. Dalbó et al. (2000) mapped the *Sex* expression locus on LG 14 of the Illinois 547-1 map, which has the SSR marker "VVMD34" common to this study. The marker VVMD 34 mapped to LG 2, Therefore, we concluded that both studies are reporting placement of *Sex* expression locus on the same LG in two different genetic backgrounds.

Genome length and coverage

The expected and observed genome lengths were calculated for both the parental and consensus maps

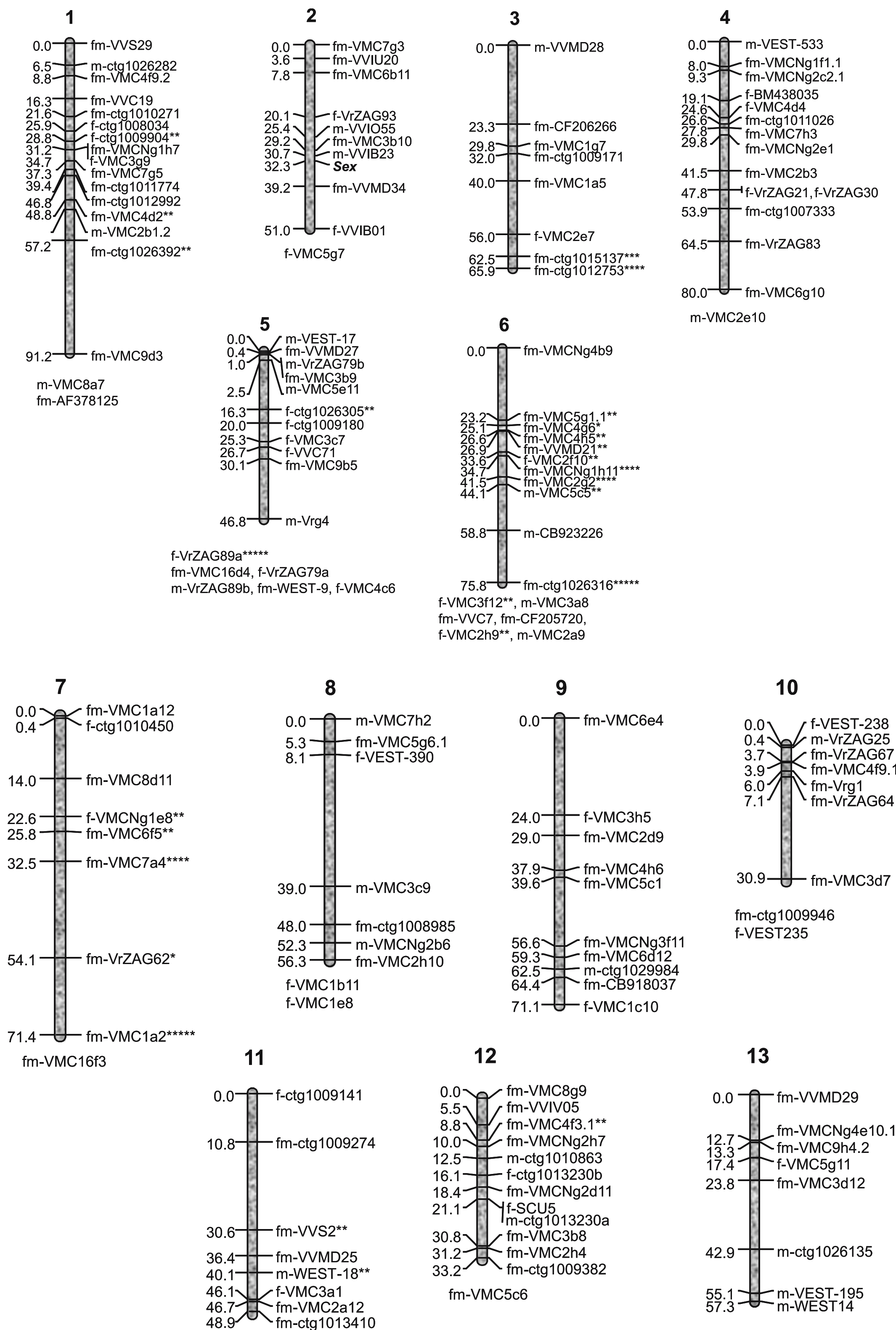

Fig. 1 Consensus linkage map of the '9621' mapping population based on SSR, EST-SSR and RFLP markers with a LOD 5.0 and a recombination frequency of 0.45. Linked but unmapped markers are listed at the bottom of each linkage group. The letter '*f*' at the beginning of markers denotes that it segregates for the female parent only, the letter '*m*' denotes that it segregates for the male parent only, and the letters '*fm*' denotes that marker segregates for both the female and male parent. Markers with an asterisk indicate the level of segregation distortion (*$P < 0.05$; **$P < 0.02$; ***$P < 0.01$; ****$P < 0.001$; *****$P < 0.0001$)

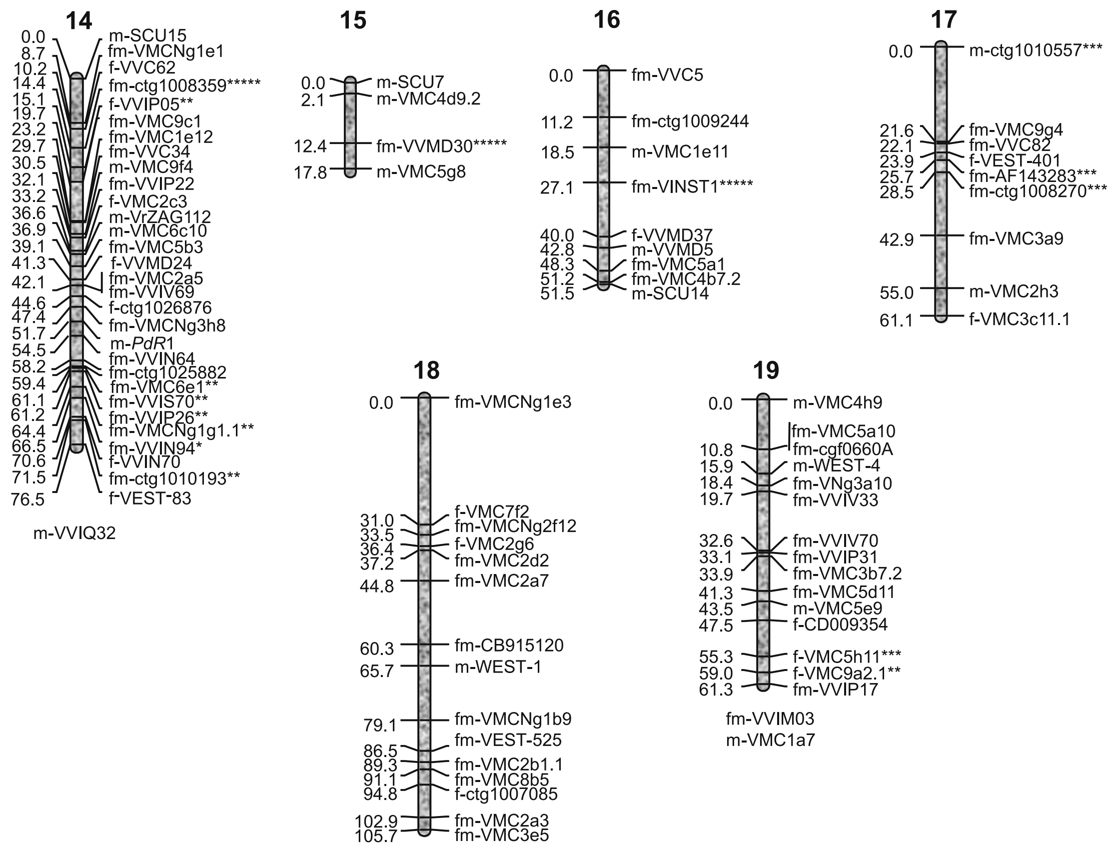

Fig. 1 continued

using JoinMap (Table 5). The estimated genome size of the female map was 1,647 cM with a confidence interval of 1,520–1,797 cM, and expected genome coverage of 94%. The estimated genome size for the male map was 2,040 cM with a confidence interval of 1,863–2,254 cM, and expected genome coverage of 91%. The calculations for genome size and expected coverage for the consensus map were carried out with 211 markers, resulting in an expected genome size of 1,706 cM with confidence interval of 1,600–1,827 cM and an estimated genome coverage of 97%. The observed genome coverage for all three maps was also calculated by the method of Nelson et al. (1994). This method takes into account the unlinked, unmapped markers, doublets, triplets and the total number of linkage groups. The observed genome coverage calculated from the Nelson et al. method was very different than the actual map lengths obtained with JoinMap, and were 380, 385 and 1,046 cM greater for the female, male and consensus maps, respectively. The Nelson et al. method estimates for genome coverage resulted in lower values for the female, 75%, and male, 70%, maps but a higher value for the consensus map, 100% (Table 5).

Discussion

Molecular markers

Four categories of molecular markers (genomic SSR, EST-derived SSR, EST-STS and EST-RFLP) were used to develop parental genetic maps of D8909-15, F8909-17, and the '9621' population consensus map. Most of the SSR markers used in *V. vinifera* genetic linkage maps have been in the VMC and VVI series (Adam-Blondon et al. 2004; Riaz et al. 2004). Of the polymorphic SSR loci used for this study, 116 were common to the Riesling × Cabernet Sauvignon map (Riaz et al. 2004), 94 were common to Syrah × Grenache map, and 42 were common to selfed Riesling map (Adam-Blondon et al. 2004). In this study, there were 34 SSR markers that have not been reported in previously published maps (Adam-Blondon et al. 2004; Riaz et al. 2004). The high level of reproducibility and polymorphism of *V. vinifera* based SSR markers in non-*vinifera* species emphasizes the efficiency of the SSR marker system as a valuable genomic tool that provides a broader selection of markers for other non-*V. vinifera* mapping projects.

Table 5 Expected and observed genome map coverage for D8909-15, F8909-17 and the '9621' consensus map

	D8909-15	F8909-17	Consensus map	Mean
No. of loci	162	158	210	
E(G)[a]	1,647	2,040	1,706	1,798
Confidence interval (cM)	1,520–1,797	1,863–2,254	1,600–1,827	1,661–2,000
C_{obs}[b]	865	1,055	1,154	1,025
X[c]	34.66	35.05	33.99	
L[d]	30	30	47	
R[e]	19	19	19	
(Nelson et al. 1994) Gon	1,245	1,440	2,105	1,597
C_{obs} (%)[f]	75	70	>100	89
E(Cn) (%)[g]	94	91	97	94

[a] Estimated genome length in cM using Kosambi (K) mapping function

[b] Observed genome coverage calculated from framework maps of female, male and consensus maps

[c] Maximum distance between two markers

[d] Total number of unlinked and unmapped markers, doublets, triplets and linkage groups

[e] No. of haploid chromosomes

[f] Percentage of observed genome coverage by the method of Nelson et al. (1994)

[g] Percentage of expected genome coverage with Kosambi mapping function

(Thomas and Scott 1993; Thomas et al. 1994; Di Gaspero et al. 2000).

EST-derived SSR markers were developed from *V. vinifera* cDNA libraries, as well as from non-*vinifera* species. The use of EST-derived SSR markers represents a shift towards the mapping of functionally annotated markers, which relate to transcribed regions of the grape genome and are more likely to be conserved, and easily transferred, among species than anonymous sequence-derived markers (Scott et al. 2000; Eujayl et al. 2002; Decroocq et al. 2003; Faville et al. 2004). A total of 55 EST-SSR markers (63%) were polymorphic in at least one parent for this study (Table 3), a level that was almost equal to the genomic SSR markers utilized in this study. EST-SSR markers are also less expensive to develop. On the other hand, EST-RFLP markers are more time consuming to develop and less polymorphic in comparison to EST-SSR markers. Nonetheless, genetic mapping with EST-SSR, EST-STS and EST-RFLP markers have great potential for the identification of gene(s) responsible for traits of interest. In this study, we are reporting mapping of 60 EST-SSR, EST-STS and EST-RFLP markers that are not reported in previous grape maps. These markers would be ideal for identification of functionally defined genes for agronomic traits on genetic linkage maps (Faville et al. 2004), as well as for PCR anchoring of grape physical maps to existing genetic linkage maps (Bernole et al. (2004).

Segregation distortion difference among parents

A genetic map developed with markers is a valuable tool to identify genomic regions associated with segregation distortion (SD). In this study, the female and male parents differ greatly in the number of markers showing SD; 25 (14%) markers in the female and 12 (7%) in the male parent. Forty-one markers (17%) on the consensus map showed SD. The number of distorted markers was higher than for previously reported pure *V. vinifera* and *V. vinifera* hybrids maps (Doligez et al. 2002; Grando et al. 2003; Adam-Blondon et al. 2004; Riaz et al. 2004). Doucleff et al. (2004) observed that only 9% of AFLP markers showed SD in a map from a subset of 116 genotypes from '9621' population. Both genomic SSR (15%) and EST-SSR (20%) markers showed SD. Although the distorted markers were distributed across linkage groups, the majority of the distorted markers were on LG 6, 7 and 14 (Fig. 1). These results indicate that chromosomal segments on LG 6, 7 and 14 might have regions with SD (i.e., *segregation distortion regions* or SDR). If a gene that causes SD is segregating in the population, then markers close to it would also tend to show SD. SDR have been reported in other crop species including tomato (Paterson et al. 1988), rice (Xu et al. 1997), coffee (Ky et al. 2000) and maize (Lu et al. (2002). Most commonly, skewed segregation appears to arise from male gametophytic selection, through selective influences of the gynoecium, and termed a gametophytic factor (Ottaviano et al. 1982). It is possible that LG 6, and segments of LG 7 and 14 might carry gametophytic factors that contribute to the higher SD. The female parent D8909-15 produces pistillate flowers, and the male parent F8909-17 produces staminate flowers. Both parents are half sibs with a common female grandparent (that produces only pistillate flowers) and different male grandparents (both producing

only staminate flowers). Therefore, it is possible that gametophytic factors might be present in the genetic background of the '9621' population. The implications of SDR for linkage mapping depend on the number of gametophytic factors that cause SD in a given SDR (Lorieux et al. 1995). Very little is known about the role of gametophytic factors, including genetic incompatibility, environmental effects and the genetic control of pollen morphology in grapes. This is the first report where three major regions of SD have been observed. Markers that show SD in this study were also compared to other grape maps (et al. 2004, Riaz et al. 2004) to determine whether there is a common SDR. Only one distorted marker (VVIS70, LG 14) was common to the Syrah × Grenache map, where 22 out of 220 markers showed SD (et al. 2004). Two markers showing SD [VMC4f3.1 (LG 12) and VMV9a2.1 (LG 19)] were common to the Riesling × Cabernet Sauvignon map (Riaz et al. 2004). However, more information is needed to conclude that there are common SDR in these different populations. The development of genetic maps in different genetic backgrounds would shed more light on the underlying genetic factors that cause SD.

Genetic maps and fine scale mapping of *PdR1* locus

The usefulness of genetic maps depends largely on the correct locus order (Plomion et al. 1995). The parental and consensus maps were developed with the JoinMap 3.0 program. This program uses the goodness-of-fit statistic to assess the quality of map order and contributions of individual loci (Stam 1993; Van Oojen and Voorips 2001). Doucleff et al. (2004) observed no difference in the order of framework markers with JoinMap and the PGRI (Plant Genome Research Initiative) mapping program. Marker order on the parental maps was consistent with each other and with the consensus map (Fig. 1); with the exception of two cases where the order between two markers on the male map was different from the female map [LG 1 (marker ctg1011774 and ctg1012992) and LG 13 (marker VMCNg4e10.1 and VMC9h4.2)]. The marker order of the female map was consistent with the consensus map for these two linkage groups. In both instances of marker order differences, the markers were tightly linked with only 2.5 and 0.5 cM distance on LG 1 and LG 13, respectively. Statistical power is limited when determining the correct order of closely linked markers. Researchers can at best order markers within a linkage group and compare the results to other published maps, in the absence of a physical map. Overall, marker order on the '9621' consensus map was in agreement with common SSR markers on the recently completed integrated framework map from five different *V. vinifera* populations (Doligez et al. 2006).

The Pierce's disease resistance locus, *PdR1*, was mapped to LG 14 between marker VMCNg3h8 and VVIN64 that are 4.34 and 2.78 cM away, respectively, on both the male and the consensus map. Krivanek et al. (2006) placed a major QTL for the *PdR1* locus on LG 14 between marker VMC5b3 and VMC6e1, which were 13.0 and 8.0 cM away from *PdR1* locus. The addition of markers and recombinants reduced the distance between markers linked to *PdR1* locus. Markers that are tightly linked to PD resistance are of critical importance for MAS now underway in a breeding program to develop resistant wine and table grape cultivars.

The female map was 865 cM long and it was relatively smaller, 190 and 289 cM, than the male and the consensus maps, respectively. The male map was 1,055 cM long and approximately 100 cM smaller than the '9621' consensus map. The size of '9621' consensus map was only 1,154 cM in contrast to other published *V. vinifera*-based maps developed with SSR markers in grape. The relatively smaller size of the '9621' genetic map might be due to lower rates of recombination, and greater genome coverage could be obtained by increasing the number of recombinants, as well as the number of molecular markers. There are 60 new markers (EST-SSR, EST-STS and EST-RFLP) on the '9621' consensus map that were not used in the integrated grape map of Doligez et al. (2006). The '9621' consensus map is 493 cM smaller in comparison to the integrated grape map of Doligez et al. (Table 4), which has 305 additional markers. Given this distance, one might conclude that there would be a 0.61 cM increase in map coverage with the addition of each new marker. However, the genetic background and size of the population also play a critical role in obtaining a high resolution genetic map (Lukowitz et al. 2000). A population size of 150–200 genotypes is adequate to develop a framework map, but it is not enough for map-based positional cloning of genes where a high resolution map is required around the area of interest. In addition, the ratio of genetic and physical distance is by no means constant, and it varies with respect to the position on the chromosome and background of the mapping population (Lukowitz et al. 1996).

Placement of the *Sex* expression locus

The morphological trait *Sex* was mapped as a single major gene on LG 2. Dalbó et al. (2000) also reported

mapping of the *Sex* locus as a single major gene, on LG 14 of Illinois 547-1 map. Their mapping population was a cross of a perfect flowered genotype (Horizon) by a staminate flowered genotype (Illinois 547-1). The SSR marker VVMD34 was common to both studies and allowed us to infer that LG 14 of Illinois 547-1 is LG 2 of the grape reference maps (et al. 2004; Riaz et al. 2004). Flower sex has also been mapped on LG 2 with a common linkage to the VVMD34 and VVIB23 marker in a grape rootstock–based map (Lowe and Walker 2006). The Lowe and Walker map and the current study are both crosses of a pistillate female parent by a staminate male parent. These studies in three different genetic backgrounds placed the *Sex* locus on LG 2, and the inheritance data support the single gene, three alleles model of Antcliff (1980). Markers tightly linked to the *Sex* locus would be very important in grape breeding programs when disease or pest resistance genes are introgressed from wild dioecious *Vitis* species into *V. vinifera* backgrounds. Tightly linked markers could be used in MAS at the post-germination stage to identify undesirable male plants thus saving time and resources to grow these plants in the field.

Genome length and coverage

The expected coverage for both the parental and consensus maps was well within the 95% confidence interval (Table 5). However, the observed genome coverage of the consensus map (2,105 cM) calculated by the method of Nelson et al. (1994) was larger than the 95% confidence interval limit for expected genome length (G_e). This discrepancy was due to a large '*L*' value, which takes into account all unmapped and unlinked markers, doublets, triplets and total number of linkage groups. There were a total of 27 unmapped and unlinked markers that inflated the value of *L*. Fishman et al. (2001) concluded that the calculation of expected genome length and the percentage of coverage based on mapped markers, maximum distance between markers, number of linkage groups, and unlinked and un-mapped markers should be considered only as a qualitative measure, because many factors can cause over- or underestimation of recombination frequencies. The average genome coverage of both parental and consensus maps with the Nelson et al. method was less than the expected genome coverage and within the 95% confidence interval limit (Table 5).

The expected genome sizes reported by Doucleff et al. (2004) for the AFLP marker-based map of '9621' population were different than presented in current SSR-based map from an expanded '9621' population. The difference might be due to a larger number of triplets and duplets from the AFLP markers (Doucleff et al. 2004). The difference in estimated genome and observed genome size from the same population with different marker sets further supports the qualitative nature of these estimations.

Future use of the map

The '9621' population segregates for resistance to Pierce's disease (caused by bacterium *X. fastidiosa*) as well as the dagger nematode (*Xiphinema index*) vector of grapevine fanleaf virus. A primary resistance locus to *X. fastidiosa*, *PdR1*, has been placed on LG 14 in a previous study (Krivanek et al. 2006). The results reported in this study are a step forward in efforts to localize the resistant gene(s) with molecular markers, which can be used easily across different genetic backgrounds. Markers tightly linked to *PdR1* are currently being used for MAS in a grape-breeding project to develop Pierce's disease resistant wine, table and raisin grapes. This genetic map will be used as a resource to assist in map-based positional cloning of Pierce's disease resistant genes.

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