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ORIGINAL PAPER

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Genetic linkage map of the interspecific grape rootstock cross Ramsey (*Vitis champinii*) × Riparia Gloire (*Vitis riparia*)

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Abstract The first genetic linkage map of grape derived from rootstock parents was constructed using 188 progeny from a cross of Ramsey (*Vitis champinii*) × Riparia Gloire (*V. riparia*). Of 354 simple sequence repeat markers tested, 205 were polymorphic for at least one parent, and 57.6% were fully informative. Maps of Ramsey, Riparia Gloire, and the F1 population were created using JoinMap software, following a pseudotestcross strategy. The set of 205 SSRs allowed for the identification of all 19 *Vitis* linkage groups ($2n=38$), with a total combined map length of 1,304.7 cM, averaging 6.8 cM between markers. The maternal map consists of 172 markers aligned into 19 linkage groups (1,244.9 cM) while 126 markers on the paternal map cover 18 linkage groups (1,095.5 cM). The expected genome coverage is over 92%. Segregation distortion occurred in the Ramsey, Riparia Gloire, and consensus maps for 10, 13, and 16% of the markers, respectively. These distorted markers clustered primarily on the linkage groups 3, 5, 14 and 17. No genome-wide difference in recombination rate was observed between Ramsey and Riparia Gloire based on 315 common marker intervals. Fifty-four new *Vitis*-EST-derived SSR markers were mapped, and were distributed evenly across the genome on 16 of the 19 linkage groups. These dense linkage maps of two phenotypically diverse North American *Vitis* species are valuable tools for studying the genetics of many rootstock traits including nematode resistance, lime and salt tolerance, and ability to induce vigor.

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Introduction

Grapevines have been cultivated for centuries as a source of fresh fruit, raisins, wine, and distilled beverages. As the spread of viticulture followed the travels of European explorers, North American grape species and their pests and diseases were brought back to Europe. The mid-nineteenth century importation of the North American insect grape phylloxera (*Daktulosphaira vitifoliae*) was the most damaging importation, and it wreaked havoc on European vineyards forever changing the manner in which grapevines were grown. To protect vineyards from grape phylloxera, growers began to graft susceptible *Vitis vinifera* fruiting cultivars onto rootstocks bred from resistant North American *Vitis* species. Today, grafting on resistant rootstocks remains the only reliable means of growing *V. vinifera* grapes where grape phylloxera is present. Rootstocks are also used for protection against other soil-borne problems including nematodes, saline soils, and calcareous soils (Pongrácz 1983).

Grape rootstocks were bred from a very diverse group of wild *Vitis* species that vary in pest resistance, adaptation to soil chemistry, structure and water content, and in the depth and extent of their root systems. Given these variables, choosing a rootstock with appropriate characteristics for a given vineyard site can be difficult. Yet, the correct matching of a rootstock with a vineyard site is one of the most important decisions to be made when establishing a vineyard. Detailed information exists on the viticultural characteristics of grape rootstocks (Pongrácz 1983). However, few studies have investigated the mechanisms of resistance and tolerance to biotic and abiotic soil-borne problems, and the genetic mapping of important rootstock traits has not been reported.

To date, grape mapping efforts have focused on two main areas. First, researchers have developed dense linkage maps of *V. vinifera* as tools to study fruit traits in the historically important winegrape varieties (Doligez et al. 2002; Adam-Blondon et al. 2004; Riaz et al. 2004). Second, maps exist of populations created to locate pathogen resistance genes and quantitative trait loci

(QTL) from wild *Vitis* species, leading to their future incorporation into fruiting varieties (Dalbó et al. 2000; Grando et al. 2003; Fischer et al. 2004). In this paper, we expand the breadth of *Vitis* mapping studies and present the first map created from two important commercially used rootstocks, Ramsey and Riparia Gloire.

Ramsey (incorrectly known as Salt Creek in California) is a selection of *V. champinii*. *Vitis champinii* is considered to be a naturally occurring hybrid of *V. candicans* and *V. rupestris*, which occurs in Texas where the range of these two species overlaps (Pongrácz 1983). As a rootstock, Ramsey promotes highly vigorous growth, is resistant to phylloxera, has strong resistance to nematodes, and is tolerant of lime and saline soils. It roots and grafts relatively poorly, but is nonetheless used in vineyards and in rootstock breeding programs for regions with high nematode populations or saline soils, such as California's Central Valley and Australia.

Riparia Gloire (synonym Riparia Gloire de Montpellier) is one of the oldest rootstocks (Pongrácz 1983). It is pure *V. riparia*, a species native to fertile alluvial soils east of the Rocky Mountains in North America. It exhibits high resistance to phylloxera, promotes low vigor in scions, and is an easily propagated rootstock. Unlike Ramsey, Riparia Gloire is sensitive to lime-induced chlorosis, and does not tolerate drought. Although Pongrácz (1983) describes Riparia Gloire as moderately resistant to nematodes, Cousins and Walker (2002) concluded that it was susceptible to *Meloidogyne incognita* in their study on the inheritance of root-knot nematode resistance in rootstocks.

Because Ramsey and Riparia Gloire vary in the way they adapt to soil-borne problems, the population derived from a cross between them was expected to segregate for multiple important rootstock traits. We sought to create a framework map of Ramsey × Riparia Gloire, using a large set of simple-sequence repeat (SSR) markers. SSR markers are codominant, highly reproducible, and useful across *Vitis* (Lin and Walker 1998; Sefc et al. 1999). In addition to genomic SSR markers from previous studies (see Table 1 for references), the Vitis Microsatellite Consortium, and the NCBI website, 108 EST-derived SSR markers were tested from the *Vitis*-EST database, which do not yet appear on any published grape linkage map. This mapping population of 188 individuals is the largest population size used to date for a *Vitis* map. This map is the first of *V. champinii*, and the first *V. riparia* map utilizing a large number of SSR markers. It will be an important tool for identifying major genes or QTLs for traits including nematode resistance, lime and drought tolerance, and the ability to promote vigor. Because of its unique parentage, this Ramsey × Riparia Gloire map will allow the grape genetics community to further compare marker placement and recombination frequency among commonly used SSR markers and across *Vitis* species. We also report a closely linked SSR marker to the sex locus, previously reported by Dalbó et al. (2000).

Materials and methods

Plant material

Rootstocks Ramsey and Riparia Gloire were crossed as part of a factorial mating design study on the inheritance of root-knot nematode (*Meloidogyne incognita*) resistance in grape rootstock varieties (Cousins and Walker 2002). The F1 population (cross '9715') was planted in a University of California vineyard in 1998. Based on the diverse nature of its parents, the 9715 population was made to study the genetics of many important rootstock traits.

DNA extraction

High quality DNA was extracted from the young grape leaves and shoot tips of Ramsey, Riparia Gloire, and all 188 genotypes of the 9715 population following a previously published modified CTAB protocol (Lodhi et al. 1994). To improve the DNA quality, samples were then processed through an additional RNAse step. DNA pellets were resuspended in 100 µl of Tris–EDTA buffer at 65°C, and 5 µl of RNAse A (20 mg/ml) was added. After two 24:1 chloroform: isoamyl alcohol extractions, DNA was precipitated with 7.5 M ammonium acetate and cold (4°C) isopropanol. The resulting pellets were suspended in 50 µl of 1× Tris–EDTA buffer.

Table 1 Simple-sequence repeat markers used in construction of the Ramsey, Riparia Gloire and 9715 linkage maps

Source	Marker prefix	Marker type	Sequence information
Agrogene, SA, Moissy Cramayel, France	VMC, VMCNG	SSR	Agrogene, SA, Di Gaspero et al. (2000), Adam-Blondon et al. (2004)
University of Agriculture, Vienna, Austria	VrZAG	SSR	Sefc et al. (1999)
University of California, Davis, USA	VVMD	SSR	Bowers et al. (1996, 1999)
Southern Cross University, Australia	SCU	EST-SSR	Scott et al. (2000)
INRA Centre de Bordeaux, France	VVI	SSR	NCBI UniSTS http://www.ncbi.nlm.nih.gov/
INRA Centre de Bordeaux, France	VVC	EST-SSR	Decroocq et al. (2003)
University of California, Davis, USA	CTG, CB, CF, AF	EST-SSR	*Vitis*-EST database http://www.cgf.ucdavis.edu/
CSIRO, Australia	VVS	SSR	Thomas and Scott (1993)
University of Udine, Udine, Italy	UDV	SSR	NCBI UniSTS http://www.ncbi.nlm.nih.gov/, Di Gaspero et al. (2005)

Microsatellite markers

Microsatellite marker primer sequences were obtained from multiple sources including previously published primers, the Vitis Microsatellite Consortium (VMC) coordinated by AgroGene S. A. (Moissy Cramayel, France), and publicly available EST-derived microsatellites (see Table 1 for references). Primers were synthesized by Operon Technologies (Alameda, CA, USA). Microsatellite markers were first tested on the parents and six individuals in the population. Polymorphic markers were then run on the entire mapping population.

Marker amplification and visualization

PCR amplifications were performed in 20 µl reactions of 10 ng genomic DNA, 10 pmol of forward and reverse primer, 2.5 mM of each NTP (Applied Biosystems, Foster City, CA, USA), 2 µl 10× gold PCR buffer (Perkin Elmer, Wellesley, MA, USA), 2 mM $MgCl_2$ (Perkin Elmer) and 0.5 units AmpliTaq Gold DNA polymerase (Perkin Elmer). All reactions were run on a Peltier Thermal Cycler-200 (MJ Research, Waltham, MA, USA). Annealing temperatures of 50, 52 or 56°C were used based on previous amplification optimization. Amplification conditions were the same for all primer pairs (2 min at 94°C to activate AmpliTaq Gold, 35 cycles of 1 min denaturation at 94°C, 1 min annealing at 50, 52, or 56°C, and 2 min extension at 72°C, followed by 10 min of additional extension at 72°C). Four microliters of reaction product was visualized on 2% agarose gels prepared with 1× TBE buffer to estimate amplification strength. To separate amplification products, PCR reactions were mixed with denaturing dye (98% formamide, 10 mM EDTA, 0.05% bromophenol blue and xylenecyanol) and heated at 94°C for 2 min before loading on a 6% polyacrylamide sequencing gel. Gels were run at a constant 70 W for 2–3 h depending on allele sizes. Samples were visualized by silver staining with a commercial kit (Promega, Madison, WI, USA).

Segregation analysis and map construction

Markers were scored visually for each parent, and compiled into a single Excel file containing each individual's complete genotype based on parental segregation data. Segregation patterns were assigned to each marker following JoinMap data entry notation (<abxcd>, <abxac>, <abxaa>, <aaxab>, <abxab>). Linkage analysis was performed with JoinMap 3.0 (Van Ooijen and Voorrips 2001), which analyzes cross-pollinated populations derived from heterozygous parents to create a consensus linkage map. The "locus genotype frequency" function calculated chi-square values for each marker to test for expected 1:1 or 3:1 segregation. Markers were placed into linkage groups with the "LOD groupings" command using the Kosambi map function (Kosambi 1944). Calculation parameters were set for a minimum LOD threshold of 4.0, and recombination fraction of 0.450. Markers showing segregation distortion were included in the final map if their presence did not alter surrounding marker order on the linkage group.

Individual parental maps were created with JoinMap 3.0 as above, with the following modification: for each marker, alleles were scored separately for the male and female parent, and segregating loci were paired with a dummy locus. As a result, all female marker data was entered as <abxaa> type markers, and male data entered as <aaxab> type markers, following a double pseudo-testcross strategy (Grattapaglia and Sederoff 1994).

Final marker order was established by comparing female, male, and consensus maps for each linkage group. Where large differences in marker order were present between maps, the "fixed order" command was used to determine which marker order resulted in the lowest chi-square value (estimating goodness of fit) for that linkage group, and the lowest mean square contributions for all loci. When appropriate, markers with segregation distortion or high mean square contributions to the final marker order were removed from the linkage group, and listed as grouped but unmapped markers. Final female, male, and consensus maps were aligned using the free software MapChart (Voorrips 2002).

Comparison of male and female recombination rates

To compare recombination rates between Ramsey and Riparia Gloire, new parental maps were constructed with 104 common markers (315 marker pairs) following Groover et al. (1995). Marker orders were aligned according to the original parental maps. JoinMap allows users to compare two maps under the "Join-combine groups for map integration" function. Here, the "Heterogeneity test" window lists all pairwise groups of common markers, their recombination frequencies, and LOD values, and calculates which pairs show significant differences based on a chi-square test. To compare genome-wide recombination differences, mean recombination frequencies with their error values were calculated for each parent in Excel. For each marker pair, we calculated two point estimates of recombination, LOD scores indicating likelihood of linkage, and chi-square tests for significant differences between maternal and paternal recombination frequencies using 2×2 contingency tables. A genome-wide test for differences in mean maternal and paternal recombination rates was performed using a Z test for comparisons between two population means.

Genome length and map coverage

Estimated genome length (G_e) was calculated following a method-of-moments type estimator, method 3, in Chakravarti et al. (1991). The 95% confidence intervals were determined for the G_e value according to Gerber and Rodolphe (1994). Expected genome map coverage was calculated following Bishop et al. (1983), as a function of chromosome number, total number of mapped markers

(*N*), and maximum observed map distance above a chosen LOD threshold (*X*). All calculations used a LOD threshold of 4.0 and Kosambi map distances in cM. For a comprehensive review of the equations used, see Riaz et al. (2004).

Scoring plants for sex

Each individual in the mapping population was scored for its sex phenotype during flowering in 2004 and 2005. All plants were either purely female (pistillate) or male (staminate). Pistillate flowers have a single pistil, with a stigma that glistens white when receptive, and have short, reflexed anthers. Staminate flowers lack a pistil, but have five erect filaments bearing anthers. The 9715 population was scored for sex over 2 years by visual inspection of inflorescences during bloom, followed by verification of fruit set in the fall. To place this morphological trait on the 9715 map, sex was entered as a male segregating marker (<aaxab>) with the b allele referring to the dominant male allele.

Results

Polymorphism of microsatellite markers

Of the 354 SSR markers tested, 205 segregated in the 9715 population for at least one parent (Table 2). Some marker sets were more polymorphic than others when used in this population. Seventy-seven percent of the VMC markers were useful for the population, while only 33% of VMCNg markers were polymorphic with strong amplification. Many primer pairs from both the VMCNg and *Vitis*-EST database-derived SSRs (prefixes CTG, CB, CF, and AF) frequently showed poor amplification and nonspecific binding despite testing with three different annealing temperatures. The linkage map was coNSnstructed with 205 markers; 118 of these segregated for two alleles in both parents, representing the most informative segregation types <abxcd> and <abxac>. Of the 84 markers segregating for one parent only (<abxaa> or <aaxab>), 63 segregated for Ramsey, which accounts for the smaller number of markers placed on the Riparia Gloire map.

The VMC6f11, VMC6f5, VMCNg3h8, and VMCNg1b2.2 markers exhibited amplification of multiple genomic regions. Three sets of distinct segregating loci pairs were scored for VMC6f11, given the suffixes "a", "b", and "c" which all mapped to linkage group 18 (Fig. 1). The other three markers could each be scored as two separate segregating loci, noted with "a" and "b" as well. These loci assorted independently, however, and were placed on different linkage groups in male and female maps. All other markers used amplified alleles of a single locus.

Segregation analysis

Markers were tested for deviation from expected Mendelian segregation with chi-square tests in JoinMap under the "locus genotype frequency" command. Markers were sorted based on their chi-square values, and placed into three categories (0–5.0, 5.0–10.0, and >10.0) for easy visual denotation (see Fig. 1). For markers on the Ramsey, Riparia Gloire, and 9715 maps, 10, 13, and 16% showed segregation distortion, respectively (Table 3). Interestingly, most of the distorted markers appear in clusters on linkage groups 3, 5, 14, and 17 (Fig. 1), suggesting that differences in recombination rates may account for the apparent distortion in these regions.

Parental map construction

All maps were constructed at LOD 4.0, although 14 of the linkage groups on each map showed no change in marker order and distances when tested at LOD 8.0. For the Ramsey map, 172 markers were placed into 19 linkage groups with an average of 9.1 markers per group. Seven VMC markers were unlinked, and four markers linked but did not map due to high mean square contributions, or weak linkages to other markers within the group. Linkage group sizes ranged from 32.1 cM (group 15) to 124.3 cM (group 18), with an average size of 65.5 cM.

Table 2 Polymorphism and segregation pattern for each SSR marker category tested on the 9715 mapping population

Marker category	No. tested	No. polymorphic	Segregation type				
			<abxaa>	<aaxab>	<abxac>	<abxab>	<abxcd>
VMC	98	75	23	10	14	–	28
VMCNG	51	17	3	2	2	–	10
VrZAG	12	8	1	1	2	2	2
VVC	8	6	–	–	1	–	5
VVS	3	1	–	–	–	–	1
VVMD	16	11	5	–	2	–	4
SCU	8	7	3	–	1	1	2
VVI	44	20	7	3	2	–	8
UDV	5	4	–	1	–	–	3
CTG, CB, CF, AF	108	55	21	3	10	–	21
Morphological	1	1	–	1	–	–	–
Total	354	205	63	21	34	3	84

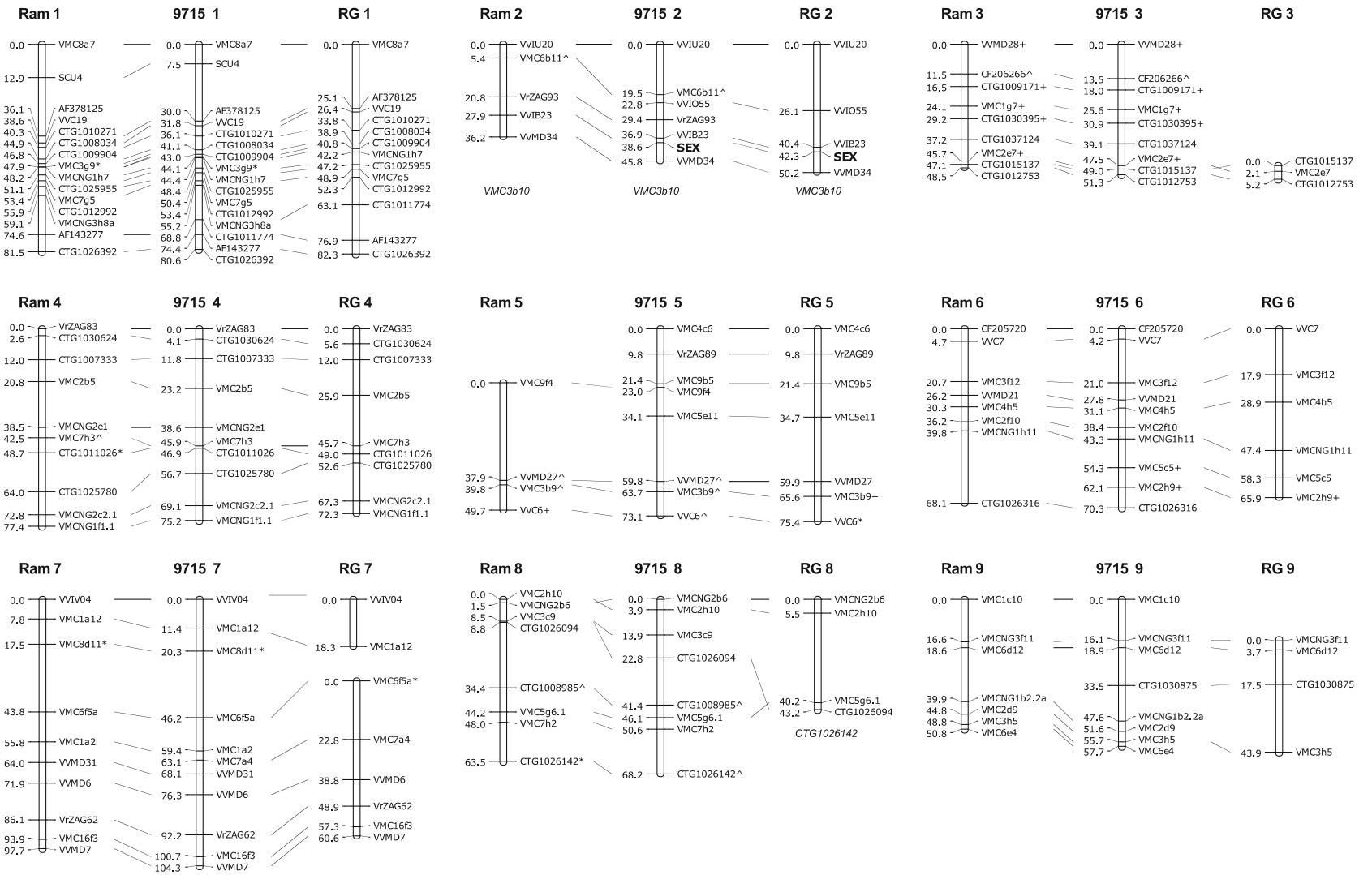

Fig. 1 Linkage maps of Ramsey (*V. champinii*), Riparia Gloire (*V. riparia*), and F1 population (9715). Linkage groups are named according to international consensus map. For each linkage group, the Ramsey map is on the left (*RAM*), the Riparia Gloire map is on the right (*RG*) and the consensus map is in the center (9715). Markers showing seggregation distortion are depicted with: *asterisk* χ^2 value of 0–5, *plus* χ^2 value of 5–10, *hat* χ^2 value of > 10. *Italicized markers* represent markers that are linked to the group but unmapped

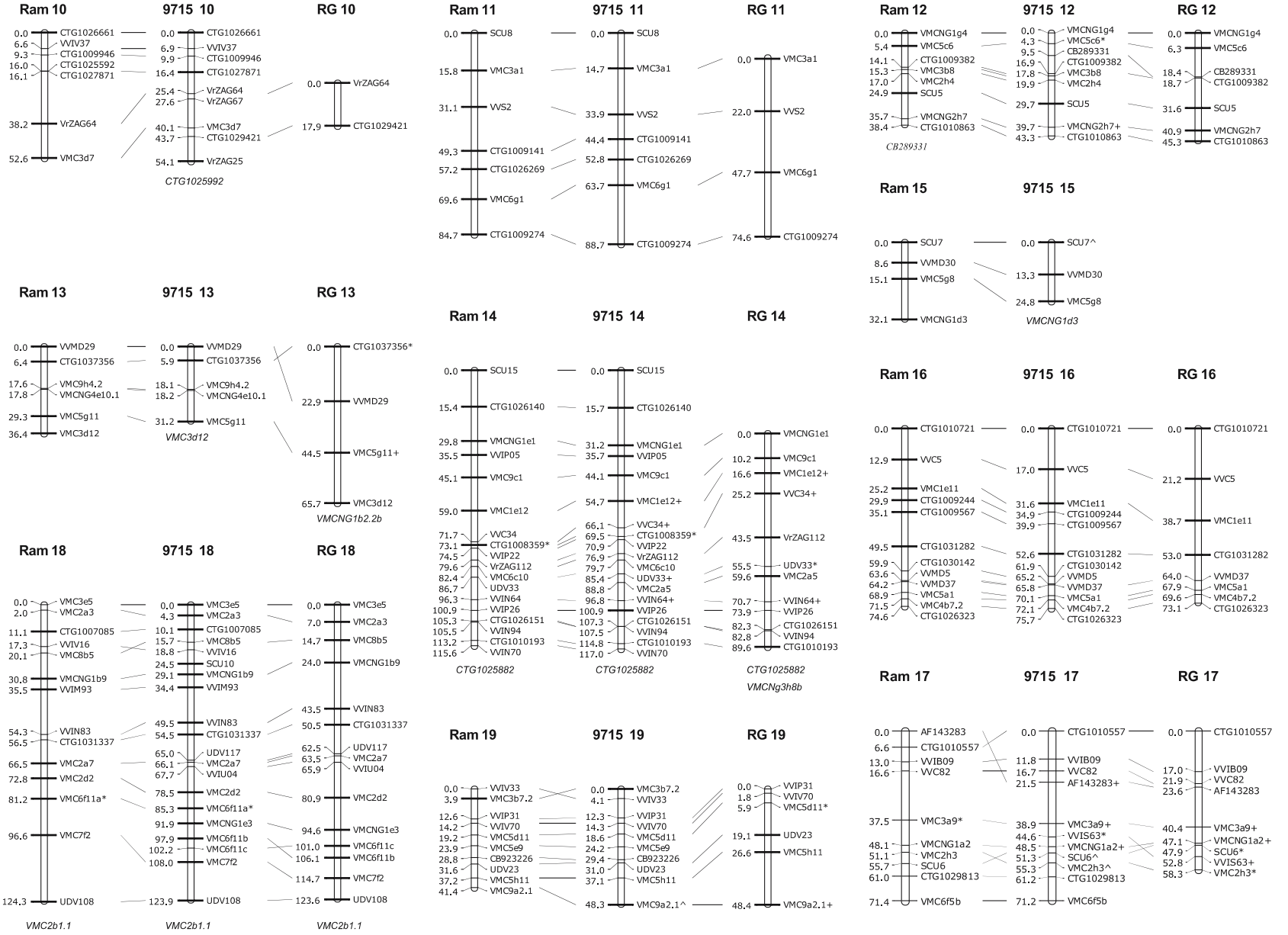
Ram 10
9715 10
RG 10
0.0 CTG1026661
6.6 VVIV37
9.3 CTG1009946
16.0 CTG1025592
16.1 CTG1027871
38.2 VrZAG64
52.6 VMC3d7
0.0 CTG1026661
6.9 VVIV37
9.9 CTG1009946
16.4 CTG1027871
25.4 VrZAG64
27.6 VrZAG67
40.1 VMC3d7
43.7 CTG1029421
54.1 VrZAG25
CTG1025992
0.0 VrZAG64
17.9 CTG1029421
Ram 11
9715 11
RG 11
0.0 SCU8
15.8 VMC3a1
31.1 VVS2
49.3 CTG1009141
57.2 CTG1026269
69.6 VMC6g1
84.7 CTG1009274
0.0 SCU8
14.7 VMC3a1
33.9 VVS2
44.4 CTG1009141
52.8 CTG1026269
63.7 VMC6g1
88.7 CTG1009274
0.0 VMC3a1
22.0 VVS2
47.7 VMC6g1
74.6 CTG1009274
Ram 12
9715 12
RG 12
0.0 VMCNG1g4
5.4 VMC5c6
14.1 CTG1009382
15.3 VMC3b8
17.0 VMC2h4
24.9 SCU5
35.7 VMCNG2h7
38.4 CTG1010863
CB289331
0.0 VMCNG1g4
4.3 VMC5c6*
9.5 CB289331
16.9 CTG1009382
17.8 VMC3b8
19.9 VMC2h4
29.7 SCU5
39.7 VMCNG2h7+
43.3 CTG1010863
0.0 VMCNG1g4
6.3 VMC5c6
18.4 CB289331
18.7 CTG1009382
31.6 SCU5
40.9 VMCNG2h7
45.3 CTG1010863
Ram 13
9715 13
RG 13
0.0 VVMD29
6.4 CTG1037356
17.6 VMC9h4.2
17.8 VMCNG4e10.1
29.3 VMC5g11
36.4 VMC3d12
0.0 VVMD29
5.9 CTG1037356
18.1 VMC9h4.2
18.2 VMCNG4e10.1
31.2 VMC5g11
VMC3d12
0.0 CTG1037356*
22.9 VVMD29
44.5 VMC5g11+
65.7 VMC3d12
VMCNG1b2.2b
Ram 14
9715 14
RG 14
0.0 SCU15
15.4 CTG1026140
29.8 VMCNG1e1
35.5 VVIP05
45.1 VMC9c1
59.0 VMC1e12
71.7 VVC34
73.1 CTG1008359*
74.5 VVIP22
79.6 VrZAG112
82.4 VMC6c10
86.7 UDV33
96.3 VVIN64
100.9 VVIP26
105.3 CTG1026151
105.5 VVIN94
113.2 CTG1010193
115.6 VVIN70
0.0 SCU15
15.7 CTG1026140
31.2 VMCNG1e1
35.7 VVIP05
44.1 VMC9c1
54.7 VMC1e12+
66.1 VVC34+
69.5 CTG1008359*
70.9 VVIP22
76.9 VrZAG112
79.7 VMC6c10
85.4 UDV33+
88.8 VMC2a5
96.8 VVIN64+
100.9 VVIP26
107.3 CTG1026151
107.5 VVIN94
114.8 CTG1010193
117.0 VVIN70
0.0 VMCNG1e1
10.2 VMC9c1
16.6 VMC1e12+
25.2 VVC34+
43.5 VrZAG112
55.5 UDV33*
59.6 VMC2a5
70.7 VVIN64+
73.9 VVIP26
82.3 CTG1026151
82.8 VVIN94
89.6 CTG1010193
CTG1025882
CTG1025882
CTG1025882
VMCNg3h8b
Ram 15
9715 15
0.0 SCU7
8.6 VVMD30
15.1 VMC5g8
32.1 VMCNG1d3
0.0 SCU7^
13.3 VVMD30
24.8 VMC5g8
VMCNG1d3
Ram 16
9715 16
RG 16
0.0 CTG1010721
12.9 VVC5
25.2 VMC1e11
29.9 CTG1009244
35.1 CTG1009567
49.5 CTG1031282
59.9 CTG1030142
63.6 VVMD5
64.2 VVMD37
68.9 VMC5a1
71.5 VMC4b7.2
74.6 CTG1026323
0.0 CTG1010721
17.0 VVC5
31.6 VMC1e11
34.9 CTG1009244
39.9 CTG1009567
52.6 CTG1031282
61.9 CTG1030142
65.2 VVMD5
65.8 VVMD37
70.1 VMC5a1
72.1 VMC4b7.2
75.7 CTG1026323
0.0 CTG1010721
21.2 VVC5
38.7 VMC1e11
53.0 CTG1031282
64.0 VVMD37
67.9 VMC5a1
69.6 VMC4b7.2
73.1 CTG1026323
Ram 17
9715 17
RG 17
0.0 AF143283
6.6 CTG1010557
13.0 VVIB09
16.6 VVC82
37.5 VMC3a9*
48.1 VMCNG1a2
51.1 VMC2h3
55.7 SCU6
61.0 CTG1029813
71.4 VMC6f5b
0.0 CTG1010557
11.8 VVIB09
16.7 VVC82
21.5 AF143283+
38.9 VMC3a9+
44.6 VVIS63*
48.5 VMCNG1a2+
51.3 SCU6^
55.3 VMC2h3^
61.2 CTG1029813
71.2 VMC6f5b
0.0 CTG1010557
17.0 VVIB09
21.9 VVC82
23.6 AF143283
40.4 VMC3a9+
47.1 VMCNG1a2+
47.9 SCU6*
52.8 VVIS63+
58.3 VMC2h3*
Ram 18
9715 18
RG 18
0.0 VMC3e5
2.0 VMC2a3
11.1 CTG1007085
17.3 VVIV16
20.1 VMC8b5
30.8 VMCNG1b9
35.5 VVIM93
54.3 VVIN83
56.5 CTG1031337
66.5 VMC2a7
72.8 VMC2d2
81.2 VMC6f11a*
96.6 VMC7f2
124.3 UDV108
0.0 VMC3e5
4.3 VMC2a3
10.1 CTG1007085
15.7 VMC8b5
18.8 VVIV16
24.5 SCU10
29.1 VMCNG1b9
34.4 VVIM93
49.5 VVIN83
54.5 CTG1031337
65.0 UDV117
66.1 VMC2a7
67.7 VVIU04
78.5 VMC2d2
85.3 VMC6f11a*
91.9 VMCNG1e3
97.9 VMC6f11b
102.2 VMC6f11c
108.0 VMC7f2
123.9 UDV108
0.0 VMC3e5
7.0 VMC2a3
14.7 VMC8b5
24.0 VMCNG1b9
43.5 VVIN83
50.5 CTG1031337
62.5 UDV117
63.5 VMC2a7
65.9 VVIU04
80.9 VMC2d2
94.6 VMCNG1e3
101.0 VMC6f11c
106.1 VMC6f11b
114.7 VMC7f2
123.6 UDV108
VMC2b1.1
VMC2b1.1
VMC2b1.1
Ram 19
9715 19
RG 19
0.0 VVIV33
3.9 VMC3b7.2
12.6 VVIP31
14.2 VVIV70
19.2 VMC5d11
23.9 VMC5e9
28.8 CB923226
31.6 UDV23
37.2 VMC5h11
41.4 VMC9a2.1
0.0 VMC3b7.2
4.1 VVIV33
12.3 VVIP31
14.3 VVIV70
18.6 VMC5d11
24.2 VMC5e9
29.4 CB923226
31.0 UDV23
37.1 VMC5h11
48.3 VMC9a2.1^
0.0 VVIP31
1.8 VVIV70
5.9 VMC5d11*
19.1 UDV23
26.6 VMC5h11
48.4 VMC9a2.1+

Fig. 1 (Contd.)

Table 3 Characteristics of the Ramsey, 9715, and Riparia Gloire linkage maps

	Ramsey				9715				Riparia Gloire			
	Size (cM)	Markers	Distorted markers	Gaps (>20 cM)	Size (cM)	Markers	Distorted markers	Gaps (>20 cM)	Size (cM)	Markers	Distorted markers	Gaps (>20 cM)
1	81.5	15	1	1	80.6	16	1	1	82.3	13	0	1
2	36.2	5	1	0	45.8	7	1	0	50.2	5	0	1
3	48.5	9	6	0	51.3	9	6	0	5.2	3	0	0
4	77.4	10	2	0	75.2	10	0	0	72.3	9	0	0
5	49.7	4	2	1	73.1	8	2	1	75.4	7	2	0
6	68.1	8	0	1	70.3	10	2	0	65.9	6	1	0
7	97.7	10	1	1	104.3	11	1	1	60.6	2/6	1	1
8	63.5	8	2	1	68.2	8	2	0	43.2	4	0	1
9	50.8	7	0	1	57.7	8	0	0	43.9	4	0	1
10	52.6	7	0	1	54.1	9	0	0	17.9	2	0	0
11	84.7	7	0	0	88.7	7	0	1	74.6	4	0	2
12	38.4	8	0	0	43.3	9	2	0	45.3	7	0	–
13	36.4	6	0	0	31.2	5	1	0	65.7	4	2	1
14	115.6	18	1	0	117	19	5	0	89.6	12	4	0
15	32.1	4	0	0	24.8	3	1	0	–	–	–	–
16	74.6	12	0	0	75.7	12	0	0	73.1	8	0	1
17	71.4	10	1	1	71.2	11	6	0	58.3	9	5	0
18	124.3	14	1	1	123.9	20	1	0	123.6	15	0	0
19	41.4	10	0	0	48.3	10	0	0	48.4	6	2	11
Average	65.5	9.1	0.9	0.5	68.7	10.1	1.6	0.2	60.9	6.9	0.9	0.6
Total	1,244.9	172	18	9	1,304.7	192	31	2	1,095.5	126	17	10
Average distance between markers	7.2				6.8				8.7			

Only nine gaps of over 20 cM were present, and the average distance between markers was 7.2 cM (Table 3).

The Riparia Gloire map consisted of 126 markers on 19 groups with an average of 6.9 markers per group. Based on linkage group numbering established by the international consensus map (Adam-Blondon et al. 2004; Riaz et al. 2004), linkage group 7 split into two groups on the Riparia Gloire map, and group 15 was absent. Linkage group sizes ranged from 5.2 cM (group 3) to 123.6 cM (group 18) with an average length of 60.9 cM. There were ten gaps greater than 20 cM, and the average distance between markers was 8.7 cM. There were nine unlinked loci, and six unmapped loci.

Marker order was highly conserved between Ramsey and Riparia Gloire, with only six rearrangements present on linkage groups 3, 8, 13, and 17. Differences in linkage group size and coverage were most likely due to the different number of markers used to construct the maps.

9715 Map construction

There were 19 linkage groups on the 9715 consensus map, matching the number of *Vitis* chromosomes ($2n = 38$), with 192 mapped markers, six unlinked and six unmapped markers, and an average of 10.1 markers per linkage group. Linkage group sizes ranged from 24.8 cM (group 15) to 123.9 cM (group 18) with an average size of 68.7 cM. The 9715 map covered 1,304.7 cM, with 6.8 cM on average between markers. Marker order was generally consistent between homologs from the parental and the consensus map, with small inversions present on linkage groups 8, 13, 17, 18, and 19. When compared to the two other published maps with high numbers of SSRs (Adam-Blondon et al. 2004; Riaz et al. 2004) marker order in the 9715 population map was similar. There are small discrepancies (two marker inversions) in marker order between the 9715 consensus map and the Riesling × Cabernet Sauvignon map for linkage groups 3, 5, 9, and 13. Larger marker order differences are apparent on groups 4 and 7. Compared to the Syrah × Grenache consensus map, marker order in the 9715 map differed only on groups 6, 7, and 10, with small terminal inversions.

Placement of sex locus

The current model for sex inheritance in grape involves a single major locus with three alleles, M (male), H (hermaphrodite), and F (female), with dominance descending in that order (M > H > F) (Carbonneau 1983). The parents of the 9715 population have pure pistillate (Ramsey) and staminate (Riparia Gloire) flowers. There were 93 males, 73 females, and 20 individuals that lacked inflorescences and thus could not be scored. Since no hermaphrodites were identified in the population, parental genotypes of FF (Ramsey) and MF (Riparia Gloire) were inferred. The resulting chi-square value of 2.4 supports that sex is segregating 1:1 as a single gene.

The sex locus was placed between markers VVIB23 and VVMD34 on linkage group 2 in both the Riparia Gloire and 9715 maps. This locus maps 1.5 and 1.9 cM from VVIB23 on the 9715 and Riparia Gloire maps,

respectively. The VVIB23 marker is of the <abxcd> segregation type. Close inspection of inheritance of alleles from Ramsey ('a' or 'b') and Riparia Gloire ('c' or 'd') showed that sex segregation follows that of the 'c' and 'd' alleles, as expected in the parental genotype model FF × MF. Only four recombinants were identified which inherited the 'c' allele of VVIB23 from Riparia Gloire, but exhibited a female phenotype.

Comparison of female and male recombination rates

Parental recombination rates were compared at 315 intervals between common markers. Recombination was slightly higher in Riparia Gloire (0.2512 vs. 0.2428), although not statistically significant at the $\alpha = 0.05$ level based on a Z test (0.7622). Performing the same comparison using only those marker pairs exhibiting statistically different recombination rates yielded similar results (Table 4).

While no differences in global recombination were calculated, marker pairs showing different rates of recombination in the parents were not evenly distributed. Thirty percent of the marker intervals on groups 4, 5, 13, 14 and 18, and 42.9% of those on groups 7 and 17 had statistically different recombination rates, suggesting that there may be hot spots for recombination across the genome. These groups also had many more distorted markers and rearrangements on average than other linkage groups, indicating that local recombination rate differences between parents may account for problems with segregation distortion and marker order discrepancies.

Genome length and coverage

Estimated genome lengths (cM) for the female, male, and combined maps were 1,468.7, 1,588.3, and 1,296.15, respectively. Only two gaps over 20 cM were present, indicating even coverage. Following Bishop's method (1983), expected coverage based on these lengths was over 90% for all maps (Table 5). Observed coverage was lower in the Ramsey and Riparia Gloire maps (84.8 and 69.0%, respectively), but 100% in the combined map. The estimated genome length based on Hubbert's equation (1988) was smaller than the sum of all linkage group lengths, likely because of the large number of mapped markers (M) and strong linkages (K).

Discussion

Comparison of the 9715 map with other Vitis linkage maps

Only two of the published grape maps were created with large numbers of SSR markers (Adam-Blondon et al. 2004; Riaz et al. 2004). Prior to these *V. vinifera* maps, researchers focused on RAPD and AFLP markers in efforts to quickly create framework linkage maps useful for identifying major genes and QTLs for fungal and bacterial resistance (Lodhi et al. 1995; Dalbó et al. 2000; Grando et al. 2003; Doucleff et al. 2004; Fischer et al. 2004), sex (Dalbó et al. 2000), seedlessness (Doligez et al. 2002), berry color (Doligez et al. 2002; Fischer et al. 2004), and veraison (Fischer et al. 2004). RAPD and AFLP markers generate large numbers of polymorphisms per primer pair; however, as they are primarily dominant, tend to cluster, and have a relatively low degree of repeatability across research groups, they are not ideal. Through the efforts of the Vitis Microsatellite Consortium, there are now hundreds of SSRs available for grape through Agrogene, NCBI, the *Vitis*-EST database, and individual published primers. These markers have allowed the grape genetics community to establish an international consensus map and system for naming the 19 linkage groups of *Vitis* (Adam-Blondon et al. 2004; Riaz et al. 2004).

Grando et al. (2003) published a map of *V. vinifera* Moscato bianco × *Vitis riparia* as a tool to study downy mildew resistance. The 9715 map shares 22 of 42 SSRs that appear on their map, identifying homology with 12 of the 19 linkage groups. Seventy-five SSRs appear on both the 9715 and the Riaz et al. Cabernet Sauvignon × Riesling map, and 73 are common between the 9715 and the Adam-Blandon et al. Syrah × Grenache map. These markers fall on the same linkage groups in all three maps, providing evidence for the reproducibility of SSR locations across *Vitis*. This comparison is not meant to suggest the redundancy of these maps, however, since only the 9715 map has used markers from the *Vitis*-EST database, and only 44 of 170 available VVI markers were tested on the 9715 population. The results suggest that researchers beginning grape mapping efforts de novo need not test the available SSRs randomly. An efficient approach would be to consult previously published maps,

Table 4 Estimation of meiotic recombination rate frequencies between the Ramsey and Riparia Gloire linkage maps

Parent	Marker intervals	Mean recombination frequency	Standard deviation	Standard error	*Z* test	*P* value
Ramsey	315	0.2428	0.1411	0.007902	0.7622	0.2236
Riparia Gloire	315	0.2512	0.1373	0.007687	NS	
Ramsey[a]	77	0.2152	0.1261	0.014371	0.6091	0.2743
Riparia Gloire[a]	77	0.2272	0.1191	0.013578	NS	

[a] Marker intervals with statistically different recombination rate differences between Ramsey and Riparia Gloire

Table 5 Estimated genome length and expected and observed genome coverage calculated with map distances based on Kosambi's mapping function

	Ramsey	Riparia Gloire	9715
Number of markers (M)	172	126	192
Number of strong linkages (K)[a]	633	380	726
Maximum observed map distance (X)	31.61	38.32	25.66
Estimated genome length (G_e, cM)	1,468.7	1,588.3	1,296.15
95% Confidence interval	1,362.6–1,592.8	1,443.2–1,765.8	1,208.3–1,397.8
Expected genome map coverage (%)	95.7	92.4	96.0
Observed genome map coverage (G_o, cM)[b]	1,244.9	1,095.5	1,304.7
Observed genome map coverage[c] (%)	84.8	69.0	100.7

[a] LOD threshold of 4.0
[b] Based on the sum length of all linkage groups for each linkage map
[c] Determined as G_o/G_e

and identify five to ten primer pairs per linkage group to test for polymorphism in order to quickly develop framework maps representing all 19 chromosomes.

The estimated genome sizes of Ramsey (1,468.7 cM) and Riparia Gloire (1,588.3 cM) are slightly smaller than those estimated in Syrah (1,708 cM) and Grenache (1,778 cM) (Adam-Blondon et al. 2004), and Cabernet Sauvignon (2,374 cM) and Riesling (2,385 cM) (Riaz et al. 2004). This discrepancy may be due to the size of the largest marker gap on each of the maps, as genome size estimations based on Hulbert's equation (1988) will inflate with higher maximum observed map distances (X). Riaz et al. (2004) reported maximum distances between markers of 49.0 and 44.7 cM, while X values for the 9715 map were 31.6 and 38.2 for female and male maps, respectively.

Considerations in linkage map construction and marker order for grape

The statistical strength of a given linkage map depends on a number of factors, including population size and structure, marker number and type (dominant or fully informative), mapping function, and stringency of the parameters in mapping programs. Developing a genetic map of grape at a high LOD value is challenging for all of these reasons. Large population sizes require space and management time, and long generation times and high levels of heterozygosity greatly inhibit the creation of inbred parents and introgressed lines. Before the availability of SSRs, mapping in grape was done with dominant markers, and homologs were aligned with the few codominant markers segregating 3:1, following a double pseudotestcross strategy (Grattapaglia et al. 1994). Clustering of RAPDs and AFLPs made even coverage of the grape genome, and identification of the appropriate number of *Vitis* chromosomes, difficult. Placement of markers showing high levels of segregation distortion, such as AFLPs, is an added challenge. Researchers have circumvented this by separating markers into those placed on a framework map with higher LOD scores, and those added as secondary markers (Doucleff et al. 2004).

Marker order and genetic distances within linkage groups will be affected by population size, differences in recombination rates between parents, and loci exhibiting segregation distortion (Maliepaard et al. 1997). Where markers are closely linked and population sizes are small, there is limited statistical power to determine the correct order of loci. Without a published physical map of grape, the presence of real chromosomal rearrangements is uncertain, and researchers can at best order loci within a linkage group statistically, and compare the results to other maps. Marker order is established in the program JoinMap through a sequential addition of markers. Highly informative marker pairs are defined as starting points for each linkage group. As new markers are added, markers within a group are reshuffled, and the effect of the new marker on the statistical goodness-of-fit of the group is determined, as expressed with a chi-square value (Stam 1993). The parameters affecting this process are user-controlled, such that markers can be removed if they result in a "jump" in the goodness-of-fit value that exceeds a given threshold. Users can view the resulting mean square contributions, and decide whether to remove "hard to order" loci to achieve a lower chi-square value overall for the linkage group. This logic was used to resolve marker order in the few linkage groups showing discrepancies between Ramsey, Riparia Gloire, and the 9715 maps. Where small inversions are still shown (linkage groups 3, 8, 13, 17, 18, 19), these marker orders represent the lowest chi-square values for the group, and may or may not be true rearrangements. Many of these small inversions may be accounted for by segregation distortion, as a number of markers involved in these inversions are distorted. A simple correlation between distorted markers and rearrangements cannot be made, however, as other linkage groups (14 for example) have many distorted markers, and no rearrangements. Some inversions on groups 3, 8, 17 also occur between closely linked markers, which present another challenge in determining marker order.

Ramsey and Riparia Gloire recombination rates

Statistically significant differences between genome-wide maternal and paternal recombination rates in published grape maps have yet to be reported. However, others have found differences in recombination rates in particular regions of the genome (Lodhi et al. 1995; Adam-Blondon et al. 2004; Riaz et al. 2004). The 9715 population also had uneven recombination rates and these regions were correlated with linkage groups containing multiple distorted markers or rearrangements (groups 5, 7, 13, 14,

18). Other crops exhibit sex-related meiotic recombination differences, including maize, (Robertson 1984), apple (Hemmat et al. 1994), and pine (Moran et al. 1983), in which male recombination rates are higher. Suppressed recombination rates, caused by sex or intergeneric differences, impede efforts in fine mapping disease resistant gene locations, as high numbers of individuals are needed to break linkages. This finding has been reported in a number of crops (Stirling et al. 2001; Neu et al. 2002), and may be limiting efforts to clone the *Run1* locus controlling resistance to powdery mildew in grape (Pauquet et al. 2001; Barker et al. 2005). The identification of such locations across *Vitis* species will be important for future resistance gene mapping studies.

Placement of the sex locus

The 9715 population is composed of purely male and female plants, segregating in a 1:1 ratio. The current model for sex inheritance in grape involves a single major locus with three alleles, M (male), H (hermaphrodite), and F (female), with dominance descending in that order (M > H > F) (Carbonneau 1983). Carbonneau also described an additional locus with epistatic effects on the expression of the staminate phenotype, after he observed male plants in populations derived from hermaphroditic parents. Antcliff (1980) proposed a single gene model with a dominant staminate allele (M) and recessive pistillate allele (F), with hermaphroditic flowers resulting from the heterozygote (MF). Scoring this morphological marker as <aaxab>, representing FF × MF phenotypically, placed the gene controlling sex at a distance of 1.5 cM from VVIB23, and 7.2 cM from VVMD34. This placement confirms the original map position for sex as reported by Dalbó et al. (2000). In the population 'Horizon' × 'Illinois 547-1', sex cosegregated with VVMD34 on Illinois 547-1 additional group 14, although map distance was not reported. We can now report that this group is linkage group 2 according to the international consensus notation.

Grapevines typically do not flower until their second or third year in the field, although precocious flowering can be achieved by the application of exogenous cytokinins (Gerrath 1992). Marker-assisted selection for sex would be of great use in breeding programs incorporating disease resistant genes from native, dioecious *Vitis* into *V. vinifera* backgrounds for new fruiting varieties, when male plants are not needed. Such selection would greatly decrease the number of vines needed to be screened and sent to field trials, saving time and space. Further testing of VVIB23 across a number of wild *Vitis* individuals and segregating populations will reveal the general usefulness of this primer as a close marker for sex.

Applications for the map

This Ramsey × Riparia Gloire map represents the first map created from two commercially used rootstocks, and the first SSR-based maps of pure *V. riparia* and *V. champinii*. These maps are valuable tools with which to examine rootstock traits not only in Ramsey and Riparia Gloire, but in other important rootstock cultivars that share their species heritage including Harmony, Freedom, 101-14 Mgt, Kober 5BB, Teleki 5C, and SO4, among many others (Pongrácz 1983). *Vitis riparia* is also an important source of powdery mildew, downy mildew, botrytis, and cold tolerance genes for hybrid grape cultivars used in the U.S. and Canada (Grando et al. 2003). The 9715 population is expected to segregate for root-knot nematode resistance, drought tolerance, lime tolerance, and vigor. The phenotypic characterization and subsequent mapping and QTL studies for these traits is underway.

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