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A framework genetic map of *Muscadinia rotundifolia*

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Abstract This study presents a framework linkage map based on microsatellite markers for *Muscadinia rotundifolia* ($1n = 20$). The mapping population consisted of 206 progeny generated from a cross of two *M. rotundifolia* varieties, 'Fry' and 'Trayshed'. A total of 884 primers were tested for their ability to amplify markers: 686 amplified and 312 simple sequence repeat (SSR) primer pairs generated 322 polymorphic markers for either one or both parents. The map for the female parent 'Fry' consisted of 212 markers and covered 879 cM on 18 chromosomes. The average distance between the markers was 4.1 cM and chromosome 6 was not represented due to a lack of polymorphic markers. The map for the male parent 'Trayshed' consisted of 191 markers and covered 841 cM on 19 chromosomes. The consensus map consisted of 314 markers on 19 chromosomes with a total distance of 1,088 cM, which represented 66 % of the distance covered by the *Vitis vinifera* reference linkage map. Marker density varied greatly among chromosomes from 5 to 35 mapped markers. Relatively good synteny was observed across 19 chromosomes based on markers in common with the *V. vinifera* reference map. Extreme segregation distortion was observed for chromosome 8 and 14 on the female parent map, and 4 on the male parent map. The lack of mapping coverage for the 20th *M. rotundifolia* chromosome is discussed in relation to possible evolutionary events that led to the reduction in chromosome number from 21 to 19 in the ancestral genome.

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Introduction

Muscadinia is recognized as a subgenus within *Vitis* and the only other group that produces edible fruit in Vitaceae (Zecca et al. 2012). Taxonomists are still divided on the sub-generic status of *Vitis* (Small 1913; Munson 1909; Wen et al. 2007; Zecca et al. 2012), the number of identified species (Brizicky 1965; Galet 1988; Wan et al. 2008) and their relationships to each other based on DNA analysis (Di Gaspero et al. 2000; Tröndle et al. 2010). From the evolutionary perspectives, the other controversial point is the disjunction in the relationship of the geographic distribution and phylogeny of American (including *Muscadinia*), Asian, Middle Asian and European wild grape species (This et al. 2006; Ren and Wen 2007; Péros et al. 2011). Differentiation of genera within Vitaceae occurred during the Mesozoic era (65 million years ago) before or during the separation of the continents. Fossil evidence suggests that precursors to *Vitis* and *Muscadinia* were widely distributed over the Northern Hemisphere before the Ice Age (Pliocene era) and numerous fossilized seeds of both have been found in the tertiary sediments of northern Europe (Kirchheimer 1939). *Muscadinia* species have 40 somatic chromosomes ($1n = 20$), which differentiate them from *Vitis* species with 38 chromosomes ($1n = 19$) (Patel and Olmo 1955; Bouquet 1980). Apart from cytogenetic differences, many morphological features (simple tendrils, adherent bark, prominent lenticels and continuous pith) distinguish *Muscadinia* from *Vitis* species (Bouquet 1980; Comeaux et al. 1987). The most common *Muscadinia*

species (*M. rotundifolia*) is found throughout the southeastern USA and is at its greatest abundance in states bordering the Gulf of Mexico (Olmo 1986; Olien 1990a). This species is the foundation of the muscadine grape industry of the southeastern USA, which produces fresh fruit, juices and wine. Efforts to produce European *V. vinifera* grapes, the basis of the vast majority of the world's wine, table and raisin grape industries, fail in this region due to strong disease pressure intensified by the warm humid climate (Goldy 1992).

Most of the pests and diseases that limit the worldwide production of *V. vinifera* cultivars are North American in origin. The North American grape species have evolved resistance to these pests and diseases, and *M. rotundifolia* is considered to have the strongest resistance (Olmo 1986). *Muscadinia* species exhibit strong resistance to root and leaf forms of grape phylloxera (Davidis and Olmo 1964; Firoozabady and Olmo 1982; Grzegorczyk and Walker 1998); *Xylella fastidiosa*, the bacterial causal agent of Pierce's disease (Olmo 1971; Ruel and Walker 2006); root-knot nematodes, *Meloidogyne* spp. (Bloodworth et al. 1980; Firoozabady and Olmo 1982; Walker et al. 1994a); *Xiphinema index*, the nematode vector of grapevine fanleaf virus (Bouquet 1980; Olmo 1986; Esmenjaud et al. 2010); anthracnose (Mortensen 1981); armillaria root rot (Clayton 1975); and the most prevalent grape fungal diseases powdery, *Erysiphe necator* (syn. *Uncinula necator*), and downy, *Plasmopora viticola*, mildew (Bouquet 1980; Olmo 1986; Merdinoglu et al. 2003). Field trials indicated that F1 *V. vinifera* × *M. rotundifolia* (VR) hybrids suppress disease symptoms of fanleaf degeneration (Walker et al. 1991, 1994b). This broad pest and disease resistance has stimulated over 100 years of effort to hybridize *Vitis* and *Muscadinia* (Detjen 1919; Dunstan 1962; Patel and Olmo 1955; Olmo 1971; Bouquet 1980; Olmo 1986; Olien 1990b; Goldy 1992).

Classical breeding with *M. rotundifolia* to combine these resistance traits with the higher quality fruit characters of *V. vinifera* is difficult due to the very different blooming dates and the differences in chromosome number leading to typically sterile hybrids. Rare fertile F1 selections can be produced, but their fertility is highly dependent on the selection of the maternal *V. vinifera* parent (Patel and Olmo 1955). Production of *Vitis* spp. × *M. rotundifolia* rootstocks has been limited by their poor rooting inherited from *M. rotundifolia*, which do not root from woody cuttings (Davidis and Olmo 1964).

The most comprehensive molecular breeding efforts have focused on the introgression of *M. rotundifolia*'s resistance to powdery and downy mildew into *V. vinifera* cultivars (Pauquet et al. 2001; Merdinoglu et al. 2003; Barker et al. 2005; Riaz et al. 2011). Several resistance loci have now been genetically mapped. The first of these was *Run1* (Resistance to *Uncinula necator* 1), which was mapped to chromosome 12 (Pauquet et al. 2001). Physical mapping of this locus was attempted (Barker et al. 2005); however, repressed recombination was observed in the vicinity of the resistance region being introgressed from *M. rotundifolia*. More than 900 seedlings carried co-segregating genetic markers (GLP1–12, sdf3, and MHD145) that were linked to resistance; however, these three markers were in three contigs that spanned over 1 Mb and not physically linked. Riaz et al. (2011) identified a second *M. rotundifolia*-based powdery mildew resistance locus, *Run2*, which mapped to chromosome 18. They observed that molecular markers derived from the *V. vinifera* PN40024 genome sequence (Jaillon et al. 2007) did not amplify in the region of the *M. rotundifolia* parental lines closely associated with the *Run2* locus on chromosome 18. These observations suggest that the *M. rotundifolia* genome has diverged from the *Vitis* genome in and around disease resistance regions while coevolving with pathogens. This process has resulted in blocks of genomic regions with low homology to *Vitis* and hence low rates of recombination; as observed for the *M. rotundifolia*-based *Run1* powdery mildew resistance region (Barker et al. 2005). However, sequence comparisons of both genomes must be done to verify this assumption. DNA sequence comparisons and comparative genetic mapping have been used to examine genome evolution among different species and genera, and to examine differences in collinear genomic regions for overall genome organization (Schranz et al. 2007a).

In terms of comparative genomics, a tremendous amount of *Vitis*-based molecular genetic information has become available to the grape research community, including genetic maps from a wide range of backgrounds (Fischer et al. 2004; Riaz et al. 2006; Hoffmann et al. 2008; Marguerit et al. 2009; Bellin et al. 2009; Blasi et al. 2011), physical maps (Moroldo et al. 2008; Scalabrin et al. 2010), a completed grape genome sequence (Jaillon et al. 2007; Velasco et al. 2007) and ongoing sequencing for a wide range of *Vitis* species aimed at single nucleotide polymorphism (SNP) discovery (http://www.vitaceae.org/index.php/Current_Sequencing_Projects). However, very little sequence information is available for *M. rotundifolia*. A recent NCBI search indicated that only 25 nucleotide sequence records were available for *M. rotundifolia* (http://www.ncbi.nlm.nih.gov/gquery/?term=muscadinia+rotundifolia). There is only one report of a genetic map for *M. rotundifolia*; however, it consisted of only 56 simple sequence repeat (SSR) markers and did not allow thorough map comparisons (Smith 2010). Well-saturated genetic maps of *M. rotundifolia* would greatly facilitate comparative genetic analysis to establish synteny with *V. vinifera* and understand the evolution of the genome. They would also help breeders to exploit the diverse pest and disease

resistance potential of this important species by identifying genomic regions linked to pest and disease resistance, and allow targeted introgression of genomic regions of interest into *V. vinifera*, optimizing molecular breeding, the cloning of disease resistance genes and their functional characterization.

This study reports on the framework genetic map of *M. rotundifolia* based on SSR markers. Comparisons were made to the genetic maps of *V. vinifera* to obtain preliminary information on the degree of synteny between the two genera (Doligez et al. 2006). These results are discussed in reference to the evolution of the grape genome and the degree to which the genetic maps of these two genera co-align or differ. This framework genetic map is the initial step in understanding the genome organization of this important species, and it will facilitate physical mapping of *M. rotundifolia* resistance regions and the cloning of resistance genes.

Materials and methods

Plant material and genomic DNA extractions

The mapping population (07190) consisted of 206 progeny from a cross *M. rotundifolia* cv. Fry × *M. rotundifolia* cv. Trayshed. Crosses were made in three consecutive years (2007–2009) to obtain the desired population size. The maternal parent 'Fry' has pistillate flowers and originated from a cross of Ga. 19–13 × US 19–11 (Fry 1967); its pedigree is presented in Supplementary Fig. 1. It has bronze fruit color with berry size up to 10 g. The paternal parent, 'Trayshed', has staminate flowers and was used extensively in the University of California grape breeding program to develop VR (*vinifera* × *rotundifolia*) hybrids. It has excellent resistance to powdery mildew, downy mildew, Pierce's disease, both dagger and root-knot nematodes, fanleaf tolerance and phylloxera (Olmo 1986). The parentage of 'Trayshed' is unknown. The 07190 population and its parents are maintained at the Department of Viticulture and Enology, University of California, Davis, CA.

DNA was extracted from young leaves using a modified CTAB procedure as described by Lodhi et al. (1994) with the exclusion of the RNase step. DNA precipitations were carried out after one cycle of chloroform–isoamyl alcohol and stock solutions were kept in 1× TE buffer at −20 °C for further use.

SSR markers

A total of 811 nuclear and EST-derived SSR primer sequences were tested. These sequences were developed from different *Vitis* species and reported in the following studies or resources: the VMC and VMCNg series was developed by the *Vitis* Microsatellite Consortium (coordinated by Agrogene, Moissy Cramayel, France); the VVI series by Merdinoglu et al. (2005); the UDV primer series by Di Gaspero et al. (2005), and the sequences of the VMC, VMCNg, VVI and UDV primers are available in the NCBI database uniSTS (http://www.ncbi.nlm.nih.gov/); the VChr series by Cipriani et al. (2008); VVMS by Thomas and Scott (1993); VVMD by Bowers et al. (1996, 1999); VrZAG by Sefc et al. (1999); FAM series by Huang et al. (2010); SCU by Scott et al. (2000); VVC by Decroocq et al. (2003); and the CTG primer sequences were obtained from the EST-SSR database developed at the University of California, Davis (http://www.cgf.ucdavis.edu/).

A total of 56 new primer sequences were developed utilizing the grapevine genome sequence (Supplementary Table 1a). Simple sequence repeats were searched for in clone sequences generated by the VMC consortium (coordinated by Agrogene, Moissy Cramayel, France). Functional primers had not been designed from these sequences because the repeat motif was located too near the ends of the clones. The BLAT search engine (http://www.genoscope.cns.fr/blat-server/cgi-bin/vitis/webBlat) was used to identify the position of these sequences on the 12X genome of PN40024 (Jaillon et al. 2007) and to obtain additional sequence around the repeat region to design primers. The original clone name designated by Agrogene was maintained. Primers were designed with the Web-based software Primer3 with the following criteria: 35–60 % GC content, 22 base pair length and optimum melting temperature of 60 °C (Rozen and Skaletsky 2000). A total of 15 primers were re-designed from VMC clones whose initial primers had failed to produce any fragments or had generated multiple bands (Supplementary Table 1b). The re-designed primers were distinguished with the suffix "N-" to distinguish them from initial primers. The primer sequences and their map location on the grape genome are presented in Supplementary Table 1a and 1b. Two unpublished primers that were also designed from the sequence of PN40024 from another study brought the total to 884 SSR primers tested for amplification and polymorphism in the 07190 population (Table 1).

Genotyping

Genomic DNA amplifications for all SSR primers were carried out based on the protocols described earlier in Riaz et al. (2004). All primers were first tested with a small set of progeny, the parents and 'Thompson Seedless' (a *V. vinifera* cultivar used as a positive control for amplification). Only informative markers that segregated with the parents were used on the entire population. The PCR

Table 1 Characteristics of each tested primer series and segregation types of the polymorphic markers for the 07190 population

Marker series	Tested	Amplified	Polymorphic	Fry (ab × aa)	Tray shed (aa × ab)	Both parents (ab × ab)	Both parents (ab × ac)	Both parents (ab × cd)
a								
VMC, VMCNg	245	163	72	29	17	1	12	14
VVI	177	135	53	15	15	3	12	9
UDV	108	84	53	11	19	5	12	8
VChr	44	35	10	4	5	2	0	0
VVMS	6	5	2	1	0	0	0	1
VVMD	12	12	8	1	2	2	2	1
VrZAG	15	13	7	2	1	0	4	0
New and redesigned VMC and VMCNG	71	57	28	11	5	0	7	8
Unpublished	2	2	2	1	1	0	0	0
b								
ctg	83	78	42	20	10	2	5	6
SCU	12	10	5	1	3	1	0	0
VVC	6	5	4	1	0	1	0	2
FAM	103	87	26	13	8	1	4	1
Total	884	686 (78 %)	312 (45 %)	110	86	18	58	50

All primers in category "a" are nuclear SSR, and category "b" consists of SSR primers derived from expressed sequence tags (EST)

amplifications were performed in 10 µl reactions consisting of 10 ng template DNA, 5 pmol of each primer, 2.5 mM of each NTP, 1 µl 10× gold PCR buffer (Perkin Elmer), 0.05 unit AmpliTaq Gold DNA polymerase (Perkin Elmer) and 2 mM $MgCl_2$ solution. All SSR markers were amplified at an annealing temperature of either 56 or 52 °C, keeping all other conditions of the protocol constant: 10 min at 95 °C; 35 cycles of 45 s at 92 °C, 45 s at 56 or 52 °C, 1 min at 72 °C; with a final extension of 10 min at 72 °C. Amplification products were separated on denaturing 5 % polyacrylamide sequencing gels and visualized by silver staining with a commercial kit (Promega, Madison, Wisconsin, USA). Gels were scanned and stored in a digital archive. Scoring for each marker was double checked, and any ambiguous genotypes were rerun or scored as missing data.

Map construction

Polymorphic markers for each parent were scored separately to generate two data sets that contained the meiotic segregation information from each parent (Table 1). A third consensus data set was obtained by merging the data of both parents using the appropriate segregation codes for each marker (aa × ab; ab × aa; ab × ab; ab × ac; and ab × cd). Linkage analysis was performed using JoinMap 4.0 and parental and consensus linkage maps were obtained (van Ooijen 2006). All markers were evaluated for goodness of fit for the observed and expected Mendelian ratios 1:1 ($P \leq 0.05$) using a χ^2 method to detect gametic segregation distortion. A recombination frequency parameter (start 0.22, end 0.05) was used for grouping the markers. Grouping of markers was also confirmed with the test for independence with a minimum LOD score of 6.0 and maximum 9.0 with one-step intervals. Marker order within each group was calculated with the regression-mapping algorithm and verified with the maximum likelihood module using the default parameters of the program. Marker order was retained from the first or second round only. Map units in centimorgans (cM) were derived from the Kosambi's mapping function (Kosambi 1944). Markers with significant segregation distortion were included in the analysis if the order of the surrounding markers was not greatly disturbed. Chromosome numbers and their orientations were determined by comparisons to the grape reference genetic map (Doligez et al. 2006) as well as using information from the grape genome browser (http://www.genoscope.cns.fr/externe/GenomeBrowser/Vitis/). All chromosomes maps were drawn with MapChart 2.1 (Voorips 2002).

Results

Marker amplification and segregation distortion

The 884 SSR primers were tested on the parents and a small set of the 07190 progeny; 686 amplified (78 %) and

produced clear bands (Table 1). Among the 198 primer pairs that did not amplify for the *M. rotundifolia* parents, 122 (62 %) produced a clear banding pattern for 'Thompson Seedless' (the *V. vinifera* control sample) indicating that amplifications of the *M. rotundifolia* parental and progeny DNA failed because they lacked priming sites. We were able to identify positions of 108 primers that amplified 'Thompson Seedless' from other published studies, and by comparing the primer and clone sequences (when available) to the PN40024 genome sequence. They were distributed across different chromosomes and no specific patterns related to genomic blocks that may be different between the two genera were detected (Table 2, Supplementary Fig. 2). Chromosomes 8, 9, 11, 13 and 18 had seven or more markers that did not amplify for *M. rotundifolia*. Eleven primers (VMC8c2, VMCNg1d12, VVIn57, UDV016, UDV019, UDV048, UDV086, VMC3h7, VMC5a6, FAM6 and FAM74) generated multiple bands. Only markers with clear segregation patterns were scored for either one or both parents. The level of polymorphism was relatively low for the male parent 'Trayshed' (30 %) as compared to the female parent 'Fry' (34 %). A total of 312 primers (45 %) were polymorphic for either one or both parents resulting in 322 useful loci that segregated in the progeny. A total of 110 markers segregated for 'Fry' (ab × aa), 86 segregated for 'Trayshed' (aa × ab), 18 segregated for both parents (ab × ab), and 108 markers were fully informative—58 (ab × ac) and 50 (ab × cd) (Table 1). The ratio of polymorphic loci for nuclear and EST-derived SSR primers was 46 and 42 %, respectively. Both 'Fry' and 'Trayshed' had 4 % missing data points for different markers or genotypes due to failed amplification or ambiguous banding patterns that were scored as missing data. Chi-square analysis indicated that 24 (11 %), 26 (13 %) and 48 (14.9 %) markers were significantly distorted ($P = 0.05$ or above) for 'Fry', 'Trayshed' and the consensus data set, respectively (Table 2).

Table 2 Features of the parental and consensus maps for 19 chromosomes in the 07190 population

Chromosome	Fry		Trayshed		Consensus		Number of primers not amplifying in *M. rotundifolia*
	Mapped markers	Length (cM)	Mapped markers	Length (cM)	Mapped markers	Length (cM)	
1	27	74.9	17	80.0	31	79.1	5
2	6	56.2	7	23.6	11	54.3	3
3	4	34.6	7	28.5	8	33.0	3
4	5	1.8	17	54.8	22	58.5	6
5	28	69.7	25	49.3	35	64.7	6
6	–	–	5	45.2	5	45.2	5
7	10	51.0	8	46.9	13	51.0	4
8	11	59.8	5	48.5	13	65.4	8
9	8	57.3	10	39.1	15	57.7	10
10	4	7.5	6	39.3	10	50.2	2
11	7	44.0	6	46.9	9	58.1	7
12	17	47.1	11	42.7	20	51.2	5
13	8	31.0	12	48.1	15	42.4	7
14	13	87.5	7	38.6	16	90.6	6
15	6	34.8	4	29.6	7	35.5	4
16	17	70.7	8	24.7	19	58.2	4
17	14	54.3	11	34.8	22	59.6	6
18	20	75.0	17	76.9	30	88.9	12
19	7	21.8	8	44.3	13	44.4	5
Total mapped markers	212	879.0	191	841.8	314	1,088.0	108
Total SSR markers	218		194		322		
Ave marker distance (cM)	4.1		4.3		3.5		
Number of distorted loci at $P = 0.05$ (% distorted)	24 (11.0)		26 (13.4)		48 (14.9)		
Number of gaps > 20 cM	3		2		0		
Chromosomes containing two or more distorted loci	8, 11, 14		2, 3, 4, 12, 16		2, 3, 4, 8, 9, 11, 12, 14, 16, 17		

Parental and consensus genetic maps and comparison to the reference *V. vinifera* map

A total of 218 markers were used to develop the 'Fry' linkage map: 214 markers were ordered in 19 groups; 212 markers were mapped; two markers (VMC2b3, and ctg1007611) were linked, but their results were not included because of weak linkage to neighboring markers (Table 2; Fig. 1). Four markers (VVIm43, VMC2f10, VMC8d11 and VVIr21) were not linked to any group. Chromosome numbers were assigned to all groups after common markers were compared to the *V. vinifera* reference map. Chromosome 6 was not represented due to the lack of polymorphic markers and chromosome 4 was fragmented into two pieces due to poor coverage, which brought the total to 18 chromosomes (Fig. 1). Total map length using all mapped markers was 879 cM with an average marker distance of 4.1 cM. There were only three gaps larger than 20 cM (Table 2). The size of chromosomes varied from 21.8 cM (chromosome 19) to 87.5 cM (chromosome 14). Both chromosomes 4 and 10 were poorly covered (1.8 and 7.5 cM respectively).

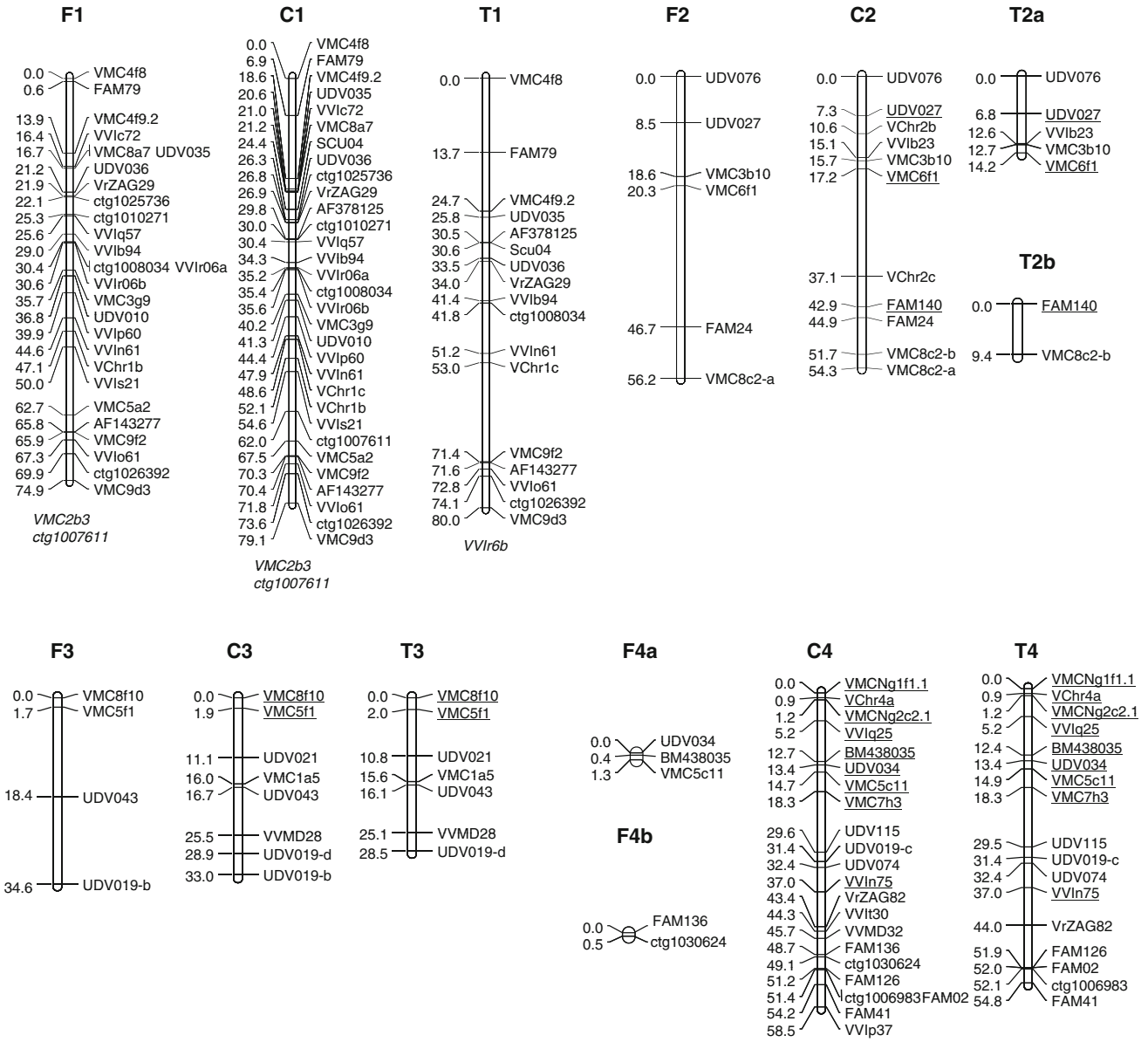

Fig. 1 SSR marker-based genetic map of *M. rotundifolia*. The maternal parent 'Fry' (F) map is on the *left*, the consensus (C) map of both parents is in the *middle*, and the paternal parent 'Trayshed' (T) map is on the *right*. Chromosomes 2 and 4 were fragmented in 'Trayshed' and 'Fry', respectively. No markers from chromosome 6 were polymorphic for 'Fry'. Distances are in cM. *Markers* that showed segregation distortion at $P \leq 0.05$ are *underlined*. Chromosome numbers were assigned based on the *V. vinifera* reference framework map

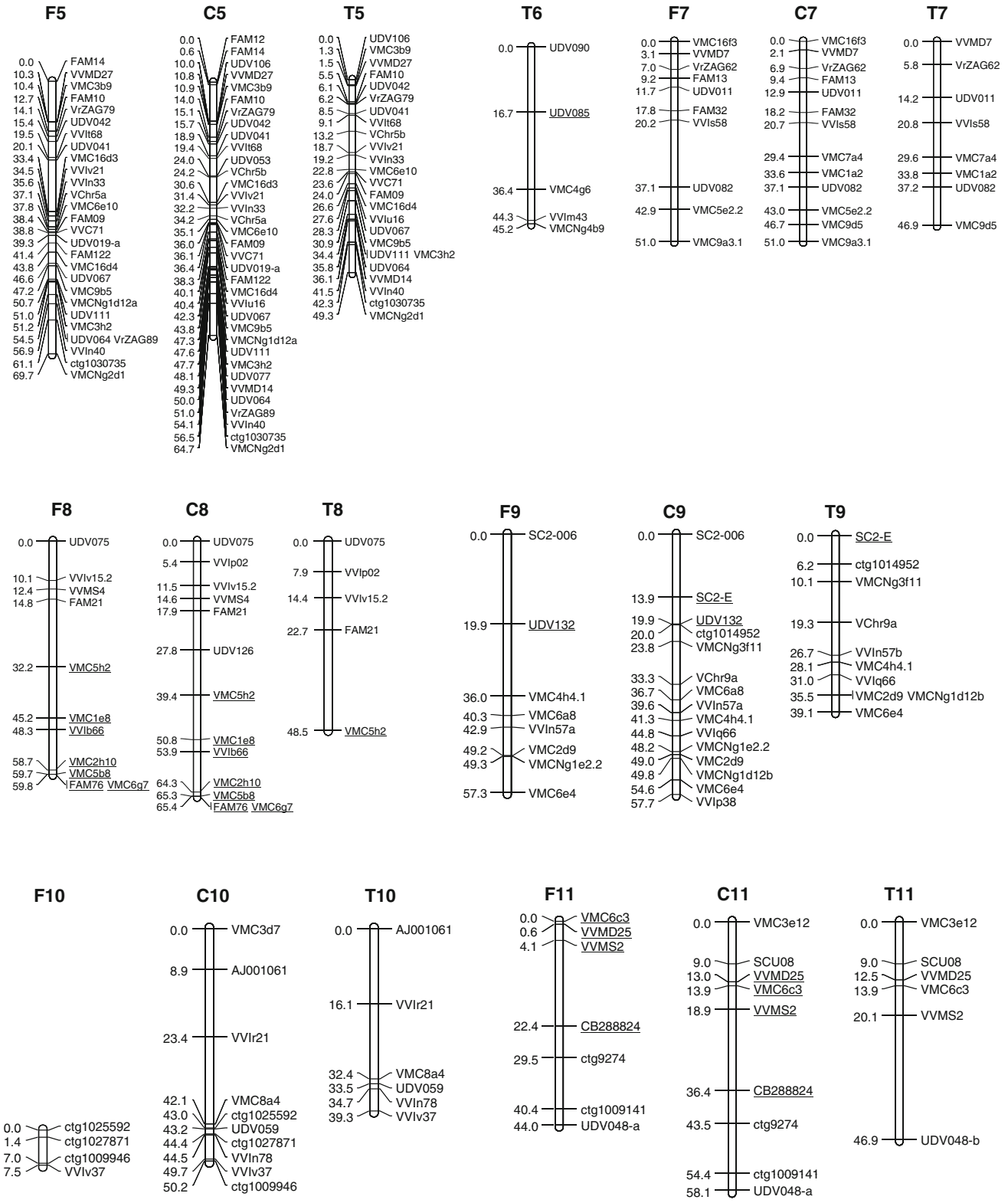

Fig. 1 continued

A total of 194 markers were used to develop the 'Trayshed' linkage map: 191 were mapped into 20 groups; one marker (FAM93) was unlinked; two other markers were grouped, but were weakly linked to the other markers. Chromosome 2 was fragmented into two groups, which brought the total to 19 chromosomes (Fig. 1). The total

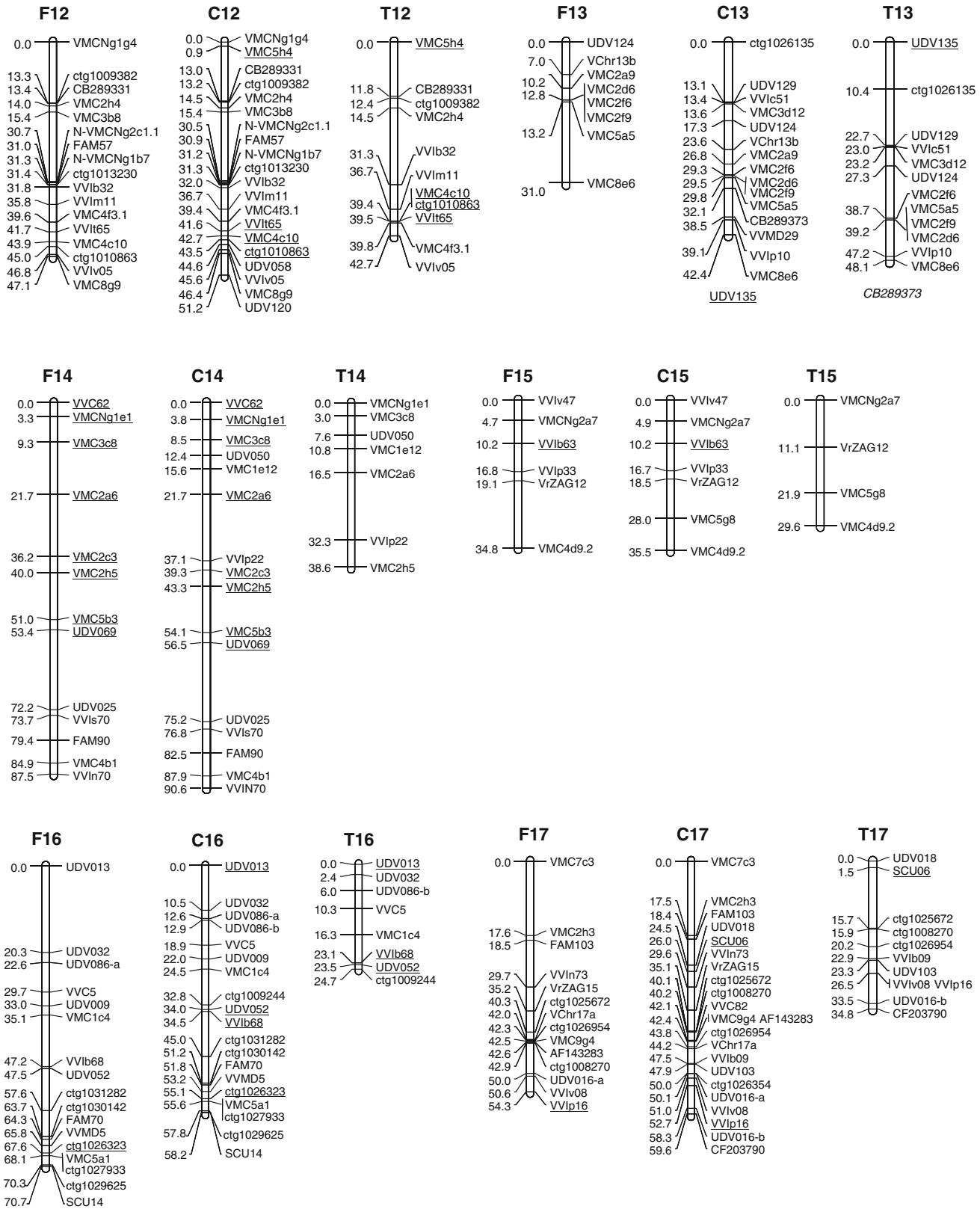

Fig. 1 continued

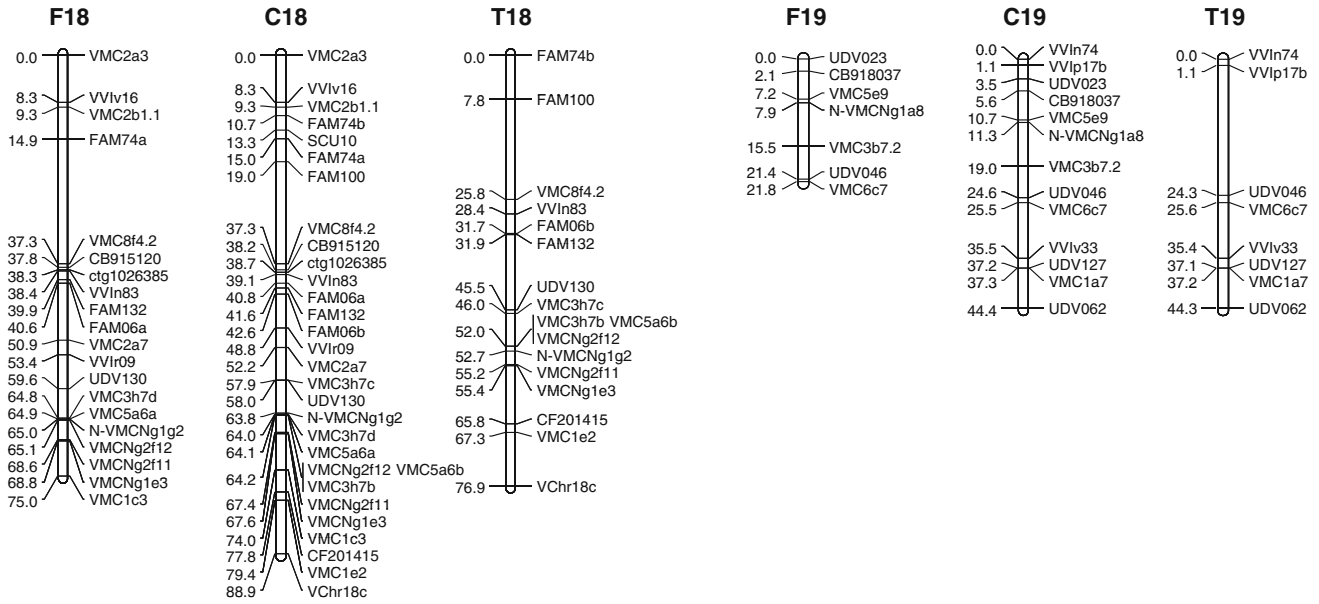

Fig. 1 continued

map length was 842 cM with an average distance of 4.3 cM between markers (Table 2). There were only two gaps larger than 20 cM. The chromosome size varied from 23.6 to 80.0 cM (Table 2; Fig. 1).

The consensus map spanned 1,088 cM with 314 markers mapped to 19 chromosomes (Table 2; Fig. 1). Five markers were not linked to any chromosomes and three markers were linked, but did not map. The average distance between markers was 3.5 cM with no gap larger than 20 cM. In general, marker order was consistent between the parental and consensus maps, except on chromosomes 4, 5, 12 and 13 where the inversions were observed for markers that were either too close (within 1-cM interval) or did not segregate normally in *M. rotundifolia*. Marker order was also compared with an integrated map of five *V. vinifera*-based populations (Doligez et al. 2006) and to a composite map of *V. vinifera* cultivars and interspecific hybrids (Di Gaspero et al. 2007) (Supplementary Fig. 2). In general, there was consensus in marker order between the *M. rotundifolia* and the two *V. vinifera*-based maps, except for chromosomes 4, 5 and 17. On these chromosomes, there were more than one instance of marker order difference compared to the integrated map (Doligez et al. 2006); however, marker order was consistent with the composite map published by Di Gaspero et al. (2007). Markers that showed segregation distortion were observed in blocks for both parental maps. Chromosomes 8, 11 and 14 had blocks of markers that were distorted for 'Fry', and chromosome 4 had markers that were distorted for 'Trayshed' (Fig. 1). Most of the markers that amplified multiple sites were located on chromosome 18.

Genetic distance and map coverage comparisons based on common distal markers and the distance between them revealed that the *M. rotundifolia* consensus map covered 66 % of the genome (Table 3). Only four chromosomes (1, 14, 15 and 17) had a genetic distance ratio of ≥90 %. The total length of these chromosomes covered by markers was also comparable to the integrated map (Table 3, Supplementary Fig. 2). Other chromosomes had reduced genetic distance ratios due either to low rates of recombination (chromosomes 6, 9, 10 and 16), or the presence of blocks of genomic regions that were not represented in the *M. rotundifolia* genetic map because of the lack of polymorphic markers or primers that did not amplify (chromosomes 8, 13 and 18) (Supplementary Fig. 2).

Discussion

In this study, we are report on the first reference genetic map of *M. rotundifolia*, a species that has captured the attention of grape geneticists and breeders due to its strong resistance to multiple pests and diseases (Bouquet 1980; Olmo 1986). The *Muscadina* species are currently found only in North America, although reports of fossilized seed in tertiary sediments of northern Europe indicate that *Muscadinia* species were once present in other continents (Kirchheimer 1939). This genus is also important with regard to its evolutionary relationship to *Vitis* and other genera in Vitaceae and the role it could play in understanding the evolutionary pathway from the ancestral grape genome. Comparative genetic mapping is a useful tool to

Table 3 Genetic distance and genome coverage comparisons between the *M. rotundifolia* and *V. vinifera* reference (Doligez et al. 2006) genetic maps

Chromosome	Markers common between maps		Distance between common markers			Maximum distance			Ratio of genetic distance (maximum distance vs. common markers distance)
	Start marker	End marker	Genetic distance on *V. vinifera* reference map	Genetic distance on *M. rotundifolia* map	Genetic distance ratio	Genetic distance on *V. vinifera* reference map	Genetic distance on *M. rotundifolia* map	Genetic distance ratio	
1	VMC4f8	VMC9d3	87.5	79.1	0.90	87.5	79.1	0.90	1.00
2	UDV076	VMC8c2	62.2	54.3	0.87	79.7	54.3	0.68	0.78
3	VMC8f10	VVMD28	48.4	25.5	0.53	70.3	33.0	0.47	0.89
4	VMCNg1f1.1	VVIp37	68.0	58.5	0.86	90.9	58.5	0.64	0.75
5	VVMD27	VVIn40	65.9	43.3	0.66	83.4	64.7	0.78	1.18
6	UDV090	VMCNg4b9	82.5	45.2	0.55	82.5	45.2	0.55	1.00
7	VMC16f3	VMC9a3.1	53.1	51.0	0.96	102.7	51.0	0.50	0.52
8	UDV075	VMC2h10	80.4	64.3	0.80	112.7	65.4	0.58	0.73
9	UDV132	VMC6e4	84.3	34.7	0.41	104.1	57.7	0.55	1.35
10	VMC3d7	VVIv37	74.2	49.7	0.67	83.7	50.2	0.60	0.90
11	VMC3e12	UDV048	61.1	46.9	0.77	75.1	58.1	0.77	1.01
12	VMC2h4	VMC8g9	46.5	31.9	0.69	81.9	51.2	0.63	0.91
13	UDV129	VMC8e6	53.3	29.3	0.55	101.1	42.4	0.42	0.76
14	VVC62	VVIn70	94.0	90.6	0.96	94.8	90.6	0.96	0.99
15	VVIb63	VMC4d9.2	32.4	25.3	0.78	37.9	35.5	0.94	1.20
16	UDV013	SCU14	87.2	58.2	0.67	92.4	58.2	0.63	0.94
17	VMC2h3	VVIp16	40.7	35.2	0.86	58.0	59.6	1.03	1.19
18	VMC2a3	VMCNg2f12	83.9	64.1	0.76	131.5	88.9	0.68	0.88
19	VVIn74	UDV062	69.0	44.4	0.64	76.9	44.4	0.58	0.90
Total			1,274.6	931.5	0.73	1,647.1	1,088.0	0.66	0.90

reconstruct the evolutionary events that led to present-day karyotypes by comparing chromosome colinearity among different species and genera. This tool has been used successfully in the Poaceae where a large degree of colinearity was found among the corn, sorghum, oat, fox millet and sugarcane genomes (Devos 2005). The last decade has witnessed the generation of a vast amount of genetic information for *Vitis*, including genetic mapping in a wide range of *V. vinifera* intraspecific crosses (Adam-Blondon et al. 2004; Riaz et al. 2004; Doligez et al. 2006; Troggio et al. 2007; Hoffmann et al. 2008; Blasi et al. 2011), interspecific breeding populations (Dalbó et al. 2000; Fischer et al. 2004; Riaz et al. 2006; Lowe and Walker 2006; Di Gaspero et al. 2007; Bellin et al. 2009; Marguerit et al. 2009; Zhang et al. 2009) and the sequencing of two *Vitis vinifera* genomes (Jaillon et al. 2007; Velasco et al. 2007). However, very little genetic mapping or sequencing information has been generated for *Muscadinia*, which limits evolutionary understanding of the events that led to the present-day cultivated grape. The framework genetic map presented here is a first step in that direction. Only SSR markers were used so that this genetic map could be compared to those constructed from *Vitis* species in order to identify colinearity and differences between the two genera based on the use of common markers.

Marker amplification and segregation distortion

The 884 SSR primers that were used in this study were developed from either repeat rich libraries or cDNA sequences of different *Vitis* species, or were developed using the PN40024 genome sequence (Table 1). The successful amplification of 686 primers (78 %) with clear bands in *M. rotundifolia* indicates that there is a high level of sequence similarity with *Vitis*, which validates the usefulness of SSR markers as robust genomic tools for comparative mapping studies on genome organization and synteny (Thomas and Scott 1993; Blasi et al. 2011). Sixty-two percent of the primers that failed to generate amplified products in *Muscadinia* did amplify in the *V. vinifera* cv. Thompson Seedless indicating that regions of sequence dissimilarity exist between the two genera. The positions of

108 such primer pairs were distributed across different chromosomes and no specific patterns were observed to help identify genomic blocks that may be different between the two genera (Supplementary Fig. 2). However, chromosome 8, 9, 11, 13 and 18 had seven or more markers that did not amplify *M. rotundifolia* genomic DNA, indicating a lack of priming sites due to sequence divergence (Supplementary Fig. 2). On chromosome 18, two primers (VMC3h7 and VMC5a6) amplified multiple sites, and markers mapped adjacent to each other indicated that the genomic region was duplicated (Fig. 1). Chromosomes 9 and 18 also carry large blocks of resistance gene analog (RGA) regions (Di Gaspero et al. 2007), on which resistance to downy and powdery mildew from different genetic backgrounds exists (Merdinoglu et al. 2003; Marguerit et al. 2009; Bellin et al. 2009; Zyprian et al. 2009; Riaz et al. 2011). *Muscadinia rotundifolia* developed strong resistance to both of these fungal diseases while co-evolving with these pathogens, which may have contributed to the genetic sequence divergence seen here. However, genomic sequence comparisons will be necessary to fully study the basis of differences in *Muscadinia*'s cytogenetics, and pest and disease resistance.

A low level of polymorphism was observed for both cultivars (34 % for 'Fry' and 30 % for 'Trayshed') in comparison to the *Vitis* species. Riaz et al. (2004, 2006) observed that 50 % of the tested markers were polymorphic for the *V. vinifera* cvs. Riesling and Cabernet Sauvignon and 56 % were polymorphic for the parents of the 9,621 population, which were complex hybrids of multiple Native American *Vitis* species. Blasi et al. (2011) reported that 52 % of the tested markers were polymorphic for a hermaphrodite accession of *V. amurensis*. One possible explanation for the observed low level of polymorphism in *M. rotundifolia* is a narrow genetic base as a result of its domestication history. In the early sixteenth century, European settlers cultivated *M. rotundifolia* grapes. The older varieties were direct selections of high-quality pistillate vines from their native ranges (southeastern and south central USA). These varieties were later used in breeding programs focused on the development of hermaphroditic cultivars with improved fruit characteristics (Husmann and Dearing 1913; Reimer and Detjen 1914). Riaz et al. (2008) utilized SSR markers to assess the level of genetic diversity among different *M. rotundifolia* cultivars and noted that although muscadine cultivars had unique allelic profiles in comparison to *V. vinifera* cultivars, the majority of the alleles were shared among them indicating a narrow genetic base. This study also observed that sections of chromosome 2, 3, 4, 7, 8, 9, 11, 13, 18 and 19 when compared to *V. vinifera* were not represented due to a lack of polymorphic markers (Supplementary Fig. 2). The level of polymorphism/heterozygosity could vary from chromosome to chromosome, with lower levels for those chromosomes where regions were fixed during the selection process. Costantini et al. (2007) observed varying levels of heterozygosity for different chromosomes with 86 highly polymorphic SSR primers using 13 *V. vinifera* table grape varieties. They observed complete homozygosity for chromosome 6 in the cultivar 'Autumn Seedless' for 14 tested markers. In the study presented here, lower sections of chromosome 14 and 16 were not represented in the 'Trayshed' map compared to the 'Fry' map due to a lack of polymorphic markers. 'Trayshed' was discovered as a seedling growing near a raisin-drying shed at the University of California, Davis in the 1940s (hence the name 'Trayshed'). The parentage is unknown, but it is likely the progeny of two muscadine varieties in the Davis breeding collection, perhaps with a shared pedigree.

The proportion of markers with segregation distortion (14.9 %) on the *M. rotundifolia* consensus map was lower than other reported studies of intra- and interspecific grape crosses. Grando et al. (2003) reported a segregation distortion of 22.4 % in a *V. vinifera* × *V. riparia* population; Riaz et al. (2006) found 17 % distortion in a *V. rupestris* × *V. arizonica/candicans* population; Lowe and Walker (2006) found 16 % distortion in a *V. champinii* × *V. riparia* population; Zhang et al. (2009) found 34 % distortion in a *V. vinifera* hybrid × (*V. cinerea* × *V. riparia*) population; and Troggio et al. (2007) found 20 % distortion in a *V. vinifera* population. The level of segregation distortion was higher than that observed in an integrated map of five different *V. vinifera*-based population of 9 % (Doligez et al. 2006) and in a map of *V. amurensis* of 11 % (Blasi et al. 2011). The low levels of observed segregation distortion detected in the *M. rotundifolia* consensus map could be due to the intraspecific nature of the cross resulting in less disruption of chromosomal regions as compared to the complex or wide interspecific crosses as reported in the studies above.

The presence of blocks of distorted markers for both parental maps was an interesting discovery. The 'Fry' map had blocks of distorted markers on the lower end of chromosome 8 and the upper end of chromosomes 11 and 14. The 'Trayshed' map had a block of distorted markers on the upper end of chromosome 4 and in a small section of chromosome 12. Segregation distortion can occur due to selection at either the gametic or genotypic level. Gametic selection can result from incompatibility at some stage of fertilization or the loss of potential progeny due to exposure of deleterious alleles; it is considered the major reason for markers that deviate from Mendelian 1:1 ratios (Schranz et al. 2007b). In previous studies, Riaz et al. (2006) observed a segregation distortion region on the upper arm of chromosome 14 that is common with this study. In another study, Zhang et al. (2009) reported that a

block of distorted markers existed on chromosome 4 and 11, also common with this study.

The presence of transposable elements (TE) also generates variation in genome organization and can cause highly distorted regions. The grape genome sequence analysis revealed that 41.4 % of the genome was repetitive or contained TE, and that the distribution of these repeats and TE along chromosomes was uneven (Jaillon et al. 2007). Moisy et al. (2008) found that chromosome 12, 14 and 18 of *Vitis* carried the highest number of TE. It is possible that TE on these chromosomes have caused organizational changes. However, it is difficult to interpret the reasons for marker segregation distortion in the *M. rotundifolia* genome in the absence of any sequence information. This genetic map provides the first glimpse into synteny between *Vitis* and *Muscadinia*. Future genetic maps with greater marker saturation will greatly improve the understanding of these differences.

Chromosome number and genome size comparisons

Nineteen chromosomes were detected for the 'Trayshfed' male parent and the consensus map, and 18 chromosomes were detected for the 'Fry' female parent in contrast to the expected 20 chromosomes ($1n = 20$). Comparisons to the *V. vinifera* reference map (Doligez et al. 2006) indicated that chromosome 6 was missing from the 'Fry' map due to a lack of polymorphic markers. 'Trayshed' has been successfully crossed with *V. vinifera* to produce F1 selections with 39 chromosomes (Patel and Olmo 1955; Bouquet 1980). No cytogenetic studies have been conducted using 'Fry', but its morphology (glossy leaves, absence of diaphragm, presence of lenticels, size of the flower and fruit) and its history (Ga. 19–13 × US 19–11) indicate that it is a pure *M. rotundifolia* cultivar (Fry 1967; Olmo 1986). Therefore, it is unlikely that this *M. rotundifolia* mapping population has parents with 19 chromosomes.

One plausible explanation for the missing 20th chromosome in the *M. rotundifolia* map could be a lack of polymorphic markers. Both parents showed remarkably low levels of polymorphism as compared to other *Vitis* species (Riaz et al. 2004, 2006; Blasi et al. 2011). There was great variation in the marker density for different chromosomes, ranging from 5 to 35 mapped markers (Table 2; Fig. 1). The primers utilized in this study were developed from *Vitis*-based sequences, and it is possible that development and utilization of primers from *M. rotundifolia*-based sequence might resolve the missing chromosome in future mapping studies. This study and population provide the foundation for future dense mapping using SNP markers developed from both *Vitis* and *Muscadinia*-based sequences.

A second possible explanation is that the 20th chromosome of *M. rotundifolia* has merged in the map with another chromosome due to pseudo-linkage between markers. The comparisons of paralogous regions of grape genome sequence has revealed that the present-day haploid genome originated from the contribution of three ancestors via either successive genome duplications or by hexaploidization, as most *Vitis* genetic regions have two different paralogous regions (Jaillon et al. 2007). If the ancient ancestor consisted of 21 chromosomes, then there are two hypotheses that could have led to the reduced chromosome number found in modern-day grape. In the first, two events of fusion could have given rise to the present-day *Vitis* ($1n = 19$), while *Muscadinia* had just one fusion ($1n = 20$) of ancestral chromosomes, suggesting that *M. rotundifolia* was closer to the ancestral genome. The other scenario proposes that there were two fusion events. The first was normal, but the second fusion event was coupled with fission to give rise to *Vitis* ($1n = 19$) and *Muscadinia* ($1n = 20$), respectively, from the ancestral genome. However, in this scenario, *Vitis* would have lost a fragment of the genome that is only present in *Muscadinia*. Analysis of the grape genome sequence (Jaillon et al. 2007) indicates that the *Vitis* genome carries all copies of the ancestral genome. This conclusion is based on the observation that all of the chromosome arms are represented three times, and therefore there was less of a chance that *Vitis* lost important fragments of the genome via fission or genome redundancy. It is possible to lose a segment of a chromosome in a recently polyploid individual due to redundancy, but it is rare and has only been observed in polyploids with high numbers of chromosomes like sugarcane and sorghum (Wang et al. 2010).

The mechanisms of chromosome number reduction have been elucidated in vertebrates (Murphy et al. 2004) and the Brassicacea (Lysak et al. 2006; Schranz et al. 2006) through the use of comparative chromosome painting (CCP). The CCP technique was used to detect the inversion, translocation and fusion events that contributed to the evolution of *Arabidopsis thaliana* (Lysak et al. 2006). Lysak et al. (2006) suggested that a reduction in chromosome number was the result of pericentric inversions leading to acrocentric chromosomes and reciprocal translocations between these chromosomes. Reciprocal translocation involves exchange of two terminal segments between two non-homologous chromosomes and is well documented in barley (Farré et al. 2011). These translocations are induced spontaneously or by transposons. In many cases, recombination between loci in the region involved in the translocation is greatly suppressed, and consequently markers tend to show pseudo-linkage. Extreme segregation distortion is also observed around the translocation breakpoints (Farré et al. 2011).

Chromosome 4 of the 'Fry' genetic map was fragmented due to a lack of polymorphic markers, and the markers on the upper arm (18 cM) of the same chromosome in the 'Trayshed' map showed segregation distortion (Fig. 1). There were also minor inversions in marker order on the lower end of the 'Fry' chromosome 4 map in comparison to the *V. vinifera* reference map (Supplementary Fig. 2). On chromosome 14, there was an 18-cM gap between markers UDV069 and UDV025, all of the markers covering the upper arm of this chromosome (53 cM) on the 'Fry' map were extremely distorted from normal Mendelian ratios (Fig. 1), and a large genomic region (47 cM) on this chromosome was not represented in the 'Trayshed' map. When marker order and genetic distance between markers were compared across all of the *Vitis* and *Muscadinia* chromosomes, chromosome 14 was the most similar (Table 3). Chromosome 14 also has a high density of retrotransposable elements (Moisy et al. 2008). Based on these observations, one can speculate that chromosome 4 or 14 has markers that are pseudo-linked, thus reducing the chromosome number to 19 instead of 20. The supplementary data of grape genome sequence published by Jaillon et al. (2007) provided a schematic representation of the paralogous pairs of genes in Fig. S5, which suggested that there was also fusion between two chromosomes of the hypothetical 21-chromosome ancestor for chromosome 4 in PN40024. This demonstrates that *M. rotundifolia* may be closer to the ancestral karyotype following the ancient hexaploidization event, and lacks one of the chromosomal fusions that may have led to the modern *V. vinifera* karyotype. On the 'Trayshed' map, the fact that chromosome 4 is a single LG could be a result of pseudo-linkage between the markers. However, this speculation needs to be verified with cytogenetic techniques such as comparative aberrant chromosome banding morphology, the CCP technique or by linkage analysis with fine-scale marker-saturated genetic maps (Jáuregui et al. 2001).

On comparing the *M. rotundifolia* consensus map with the reference *V. vinifera* genetic map (Doligez et al. 2006), it was found that the *M. rotundifolia* map provides up to 66 % coverage based on common markers. The ratio between maximum genetic distance and the distance between common markers was up to 90 %, indicating that *M. rotundifolia* genome had a much reduced rate of recombination, which was obvious on chromosomes 6, 9, 10 and 16. Overall, the *M. rotundifolia* genetic map was collinear to the *V. vinifera* reference map except for large blocks of genomic regions that were not represented on chromosomes 7, 13 and 18 in *M. rotundifolia* due to the lack of polymorphic markers. These unrepresented areas could be resolved with the development of more markers utilizing the PN40024 (Jaillon et al. 2007) sequence or by sequencing the *M. rotundifolia* genome to develop markers covering these regions.

This study provides a glimpse into the genetic differences between *Vitis* and *Muscadinia* and the results will lead to a more focused analysis of these differences. It is noteworthy that no estimation of nuclear DNA content information is available for *Muscadinia*. There is only one report that compared nuclear DNA content of different *Vitis* species (Lodhi and Reisch 1995). This study also included two other genera, *Ampelopsis* and *Parthenocissus*, which have a nuclear DNA content of 666 and 516 Mbp/C, respectively. Species belonging to *Ampelopsis* and *Parthenocissus* have $1n = 20$ chromosomes like *Muscadinia* spp. (Karkamkar et al. 2010). One can assume that *Muscadinia* has comparable DNA content to other Vitaceae genera with the same number of chromosomes, but this assumption needs verification. It is common to have large differences in genome size among species in a genus as has been reported for the rice species (http://www.data.kew.org/cvalues/).

To expand the knowledge of *M. rotundifolia* genetics, the genome must be sequenced with enough resolution to answer evolutionary questions and allow better utilization of *Muscadinia*'s remarkable pest and disease resistance. Sequencing efforts would also provide *Muscadinia*-specific SSR markers, which will be essential for fine-scale genetic maps and map-based positional cloning of resistance genes. The genome sequence will also provide necessary probes for use in studying the evolution of cytogenetic differences between these genera and others in Vitaceae.

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