Theor Appl Genet (2004) 109: 1017–1027
DOI 10.1007/s00122-004-1704-y

ORIGINAL PAPER

A. -F. Adam-Blondon · C. Roux · D. Claux ·
G. Butterlin · D. Merdinoglu · P. This

Mapping 245 SSR markers on the *Vitis vinifera* genome: a tool for grape genetics

Received: 19 December 2003 / Accepted: 2 April 2004 / Published online: 4 June 2004

Abstract The aim of the present work was to develop a microsatellite marker-based map of the *Vitis vinifera* genome (n=19), useful for genetic studies in this perennial heterozygous species, as SSR markers are highly transferable co-dominant markers. A total of 346 primer pairs were tested on the two parents (Syrah and Grenache) of a full sib population of 96 individuals (S × G population), successfully amplifying 310 markers. Of these, 88.4% markers were heterozygous for at least one of the two parents. A total of 292 primer pairs were then tested on Riesling, the parent of the RS_1 population derived from selfing (96 individuals), successfully amplifying 299 markers among which 207 (62.9%) were heterozygous. Only 6.7% of the markers were homozygous in all three genotypes, stressing the interest of such markers in grape genetics. Four maps were constructed based on the segregation of 245 SSR markers in the two populations. The Syrah map was constructed from the segregations of 177 markers that could be ordered into 19 linkage groups (total length 1,172.2 cM). The Grenache map was constructed with the segregations of 178 markers that could be ordered into 18 linkage groups (total length 1,360.6 cM). The consensus S × G map was constructed with the segregations of 220 markers that were ordered into 19 linkage groups (total length 1,406.1 cM). One hundred and eleven markers were scored on the RS_1 population, among them 27 that were not mapped using the S × G map. Out of these 111 markers, 110 allowed to us to construct a map of a total length of 1,191.7 cM. Using these four maps, the genome length of *V. vinifera* was estimated to be around 2,200 cM. The present work allowed us to map 123 new SSR markers on the *V. vinifera* genome that had not been ordered in a previous SSR-based map (Riaz et al. 2004), representing an average of 6.5 new markers per linkage group. Any new SSR marker mapped is of great potential usefulness for many applications such as the transfer of well-scattered markers to other maps for QTL detection, the use of markers in specific regions for the fine mapping of genes/QTL, or for the choice of markers for MAS.

Communicated by C. Möllers

A. -F.-F. Adam-Blondon (✉)
URGV, INRA,
2 rue Gaston Crémieux,
91058 Evry cedex 5708, France
e-mail: adam@evry.inra.fr
Tel.: +33-160-874534
Fax: +33-160-874549

C. Roux · D. Claux · P. This
UMR1097-vigne, INRA,
2 place P. Viala,
34060 Montpellier cedex 01, France

G. Butterlin · D. Merdinoglu
UMRVV, INRA,
28 rue Herrlisheim,
68 021 Colmar cedex, France

Introduction

The grape is one of the oldest cultivated fruits in the world. It is of great economic importance, its fruit being mostly used for transformation into valuable beverages such as wines and spirits (http://www.onivins.fr/Espace-Pro/Economie/FaitsChiffres.asp). Its breeding has mainly relied on selection through the ages of naturally occurring genotypes, issued from spontaneous crosses that have been recently traced, and to a lesser extent, due to conventional breeding during the last century (Levadoux 1956; Olmo 1976; Bowers et al. 1999a). Overall, both approaches have led to the selection of high-quality worldwide-recognised varieties mainly belonging to the *Vitis vinifera* species.

At the turn of the last century, many diseases were introduced into European vineyards raising the need for breeding new varieties showing resistance to pathogens such as *Uncinula necator* (powdery mildew), *Plasmopara viticola* (downy mildew) or *Phylloxera vastatrix*. All sources of resistance for these pathogens are low-quality wild species. To introduce resistance from wild inferior-quality species, many crosses to *V. vinifera* are required in

order to recover high-quality *vinifera* cultivars. Breeding is further hampered by a long seed-to-seed cycle and a high susceptibility to inbreeding, both of which hamper progress in the knowledge of the genetics of traits of interest (related to quality and resistance). Moreover, most of these traits are quantitatively inherited (Eibach et al. 1989; Doligez et al. 2002; Fischer et al. 2004).

During the past decade, several groups have put their efforts into the development of maps allowing the location of QTLs for agronomic traits, with the goal of using this information for the development of marker-assisted selection in the grape and thus to improve the efficiency of grape breeding (Dalbó et al. 2001; Doligez et al. 2002; Grando et al. 2003; Fischer et al. 2004). These maps were mainly constructed with RAPD or AFLP markers. Such markers allow the rapid construction of maps, but not their comparison. Moreover, bridges between parental maps generated from studies of the same population were made from dominant markers that are ordered with a low statistical power (Lodhi et al. 1995; Doligez et al. 2002; Fischer et al. 2004). Recently, 19 groups involved in the study of grape genetics, coordinated by Agrogène SA, joined their efforts to form the Vitis Microsatellite Consortium (VMC) to develop a set of 371 microsatellite (or SSR for simple sequence repeat) markers, which present the advantage of being PCR-derived, co-dominant, polymorphic and have proved their usefulness for the genetic analysis of heterozygous species. This set of microsatellite markers was used to construct a *V. vinifera* map (Riaz et al. 2004) used as a reference to establish other maps (Doligez et al. 2002; Grando et al. 2003; Zyprian et al. 2003). A second set of 170 microsatellite loci, called VVI, was developed afterwards in France (Merdinoglu et al., submitted). Here we report the mapping of 222 markers from both sets on a single reference map, resulting in the most complete SSR map of *Vitis* to date. A second map is also presented, which allowed us to localise 23 additional markers.

Materials and methods

Plant material

The construction of the reference map was based on the study of 27 individuals from a cross between a Syrah N clone ENTAV73 and a Grenache N clone Mtp6 (population Mtp3298) and of 70 individuals of the reciprocal cross (population Mtp3297). All these individuals represent what we will refer to as the S × G population. The Riesling map was based on the analysis of 96 individuals derived from a selfed Riesling B clone 49 (RS_1 population). Mtp3297 and Mtp3298 populations were cultivated on their own roots in sandy soils at the INRA experimental

Table 1 SSR primer pairs developed by INRA within the VMC consortium. Amplification was tested on DNA from Syrah N, Grenache N, Cabernet Sauvignon N, Riesling B and Sultanine B

Locus	Primer name	Primer sequence
VMC8A4	VMC8A4F	TCATGAATAGCCCCTGGAAGAG
	VMC8A4R	TGAAGGATGGAGATGGGAAGAG
VMC8B5	VMC8B5F	AAAGGAGACATCTGCATCAT
	VMC8B5R	GCCTTGATCTTCCTTCTAAT
VMC8C2	VMC8C2F	AAGGAATTTGGATACTGAAGGT
	VMC8C2R	TGAAGACATCTACGTAGGTGAA
VMC8D1	VMC8D1F	AAAGCGCGTAGCTCAGACACA
	VMC8D1R	GGCGGTTGAGCTCTGCTTATC
VMC8D2	VMC8D2F	CTCAGCGGCCAAACACAC (No amplification)
	VMC8D2R	GACGGGACTGCTTTTACTCG
VMC8D3	VMC8D3F	TGGCAAGACACAATAAAACAGA
	VMC8D3R	ATAGAGTCCTGCAAATCCAAGA
VMC8D4	VMC8D4F	ACCCAAACTCCGCAAGATGT (No amplification)
	VMC8D4R	CACGACCCATTGAATTCCTG
VMC8E6	VMC8E6F	AAGGGGTTCATTTGATTGAGAG
	VMC8E6R	CTTCATCCATCCTTACAGCTTAGA
VMC8F4.2	VMC8F4.2F	GCGTAAAGCATATTCAAGCATT
	VMC8F4.2R	GAAGTTAGCGCAGATGAAAGAT
VMC8F6	VMC8F6F	AGAATTATTTTCTTCTCTCGCC (No amplification)
	VMC8F6R	TTTCGGTAAGTCATTAGAGCAA
VMC8G3.1	VMC8G3.1F	CACAAACCATGAAACATGAGGC (No amplification)
	VMC8G3.1R	AGGTATAAATGACACGCGGGAG
VMC8G3.2	VMC8G3.2F	TAGGGCGGAGATTTAACAGTCA
	VMC8G3.2R	TCAACCAAACTCATTAAAGGGG
VMC8G6	VMC8G6F	TCAGTAATCACGAGCTTCCCG
	VMC8G6R	TGGAGTGGGGATATGGAAATG
VMC8H3.1	VMC8H3.1F	TTCCCAGACACGACGTTGTAAA (No amplification)
	VMC8H3.1R	TTGCAGAGGAATGTGGAAGTTG

Table 2 Tests for heterozygosity of Syrah, Grenache and Riesling

	Syrah and Grenache (293 primer pairs tested)					Riesling (292 primer pairs tested)			
	Grenache only	Syrah only	Syrah and Grenache	Homozygous in Syrah and Grenache	Total	Heterozygous	Homozygous	Homozygous for Syrah, Grenache and Riesling	Total
Number of markers	59	58	157	36	310	207	92	20	299
Percentage (%)	19.0	18.7	50.6	11.6		69.2	30.8	6.7	

Table 3 Repartition of segregation types in the S × G population

Syrah	Grenache	Syrah and Grenache				Total
1:1	1:1	1:2:1	2:1:1	1:1:1:1	1:1:1:1	
ab × aa	aa × ab	ab × ab	ab × a0	ab × ac	ab × cd	
43	46	14	2	69	54	228

station of Vassal and the RS_1 population in greenhouses of the INRA experimental station of Colmar.

DNA extraction

DNA was extracted from 80–100 mg of young leaves using the DNeasy Plant Mini Kit (Qiagen, Courtaboeuf, France) with slight modifications of the manufacturer's protocol: addition of 1% w/v of PVP-40 to the AP1 solution and a two-step elution of the DNA with 100 and 50 µl of TE.

SSR amplification

A total of 346 primer pairs were tested on Syrah and Grenache: 140 VMC loci mainly chosen from the map published by Riaz et al. (2004) and among which only a few have been published (Sefc et al. 1999; Di Gaspero et al. 2000). Primer sequences of the VMC loci developed at INRA are given in Table 1. Laboratories interested in unpublished VMC markers should contact Agrogene SA (Moissy Cramayel, France). The other primer pairs tested targeted 170 VVI loci (available at NCBi Uni STS), 18 VVMD loci (Bowers et al. 1996, 1999b), 12 VVS and VH loci (Thomas and Scott 1993; Thomas et al. 1994).

A total of 292 primer pairs were tested on Riesling, and 111 markers were chosen for the construction of the Riesling map, based on their location on the S × G map (either well scattered on the chromosomes or unmapped), their ability to be amplified in multiplexes (Merdinoglu et al., submitted) and their heterozygosity in Riesling.

Some SSR loci were analysed using a manual sequencer according to Loureiro et al. (1998). The majority of the SSR loci were analysed on an automated sequencing apparatus. For that purpose, one of the two primers allowing the amplification of each locus was labelled with a fluorescent dye (FAM, NED, or HEX). The PCR mix used for SSR amplifications was the following: 10 ng of template DNA were added to a 12.5 µl reaction mix containing 1.5 mM $MgCl_2$, 0.4 U *Taq* polymerase (Qiagen), 200 µM dNTP, 1× *Taq* polymerase buffer (Qiagen), 0.16 pM of the labelled primer and 0.32 pM of the unlabelled primer. Amplifications were performed using a PTC100 (MJ Research) programmed as follows: 4 min at 94°C followed by 36 cycles of 1 min at 94°C, 1 min at 56 or 60°C, 1 min at 72°C followed by a final step of 6 min at 72°C. Up to four different primer pairs were mixed in the same PCR reaction (multiplex PCR), taking into account the size of the amplified fragments and/or the labelling of the primers but without modification of the PCR cycling conditions for individual loci.

Electrophoresis and data analysis

Nine to 12 amplification products were mixed according to their size and labelling. The conditions of dilution of the amplification products in water were different according to the labelling of the primers. Products amplified with a FAM-labelled primer were diluted 40 times, whereas those amplified with a NED or a HEX-labelled primer were diluted 20 times. One microlitre of each dilution was added to a mix of 10 µl of deionised formamide and 0.15 µl of an internal size standard (GENESCAN 500 ROX). The mix was then denatured for 4 min at 94°C. The samples were analysed in an ABI PRISM 3100 Genetic Analyser (Applied Biosystems) using a 36-cm capillary filled with polymer 3100 POP-4 (Applied Biosystems). The run was performed with Genescan36_Pop4 default module.

Chromatograms were analysed using the software GENESCAN 3.7 (Applied Biosystems) using the local Southern method to determine the size of peaks in basepairs. The allele calling was performed using GENOTYPER (Applied Biosystems). Alleles were then encoded according to their segregation following the method described by Doligez et al. (2002) to generate four segregation data files, one for each of Syrah, Grenache, the consensus S × G and Riesling.

Map construction

Markers were screened for their apparent heterozygosity in the three parental cultivars: they were declared apparently heterozygous when they showed a pattern with two distinct bands. The markers found to be apparently heterozygous were then scored on the appropriate population. Some primer pairs allowed the amplification of several markers that were numbered with small letters.

The segregation of each marker of the four data sets was tested for goodness-of-fit to the appropriate expected segregation using a χ^2 test. We kept the markers that showed a distorted segregation unless they were of low quality or they affected the order of their neighbours.

The CarthaGene software (Chabrier et al. 2000) was then used to construct the four maps. For this purpose, the three parental data files, Syrah, Grenache, and Riesling, were modified as follows: all loci were coded in the two possible phases. The linkage groups were then determined in these three modified data sets using the following parameters: LOD=3.0 and d_{max}=0.4 cM Kosambi. For the Riesling map, some markers were added at LOD=2.0 based on knowledge from the S × G map. For each linkage group, one of the two resulting series was chosen and used as a reference for the choice of the phase of the markers in the consensus data set for the S × G map. The maximum multipoint log likelihood was then optimised for each group of each map using the dedicated simulated annealing stochastic optimisation algorithm with the following parameters: number of assays, 50; initial temperature, 50; final temperature, 0.1; cooling, 0.9. For all maps, the Kosambi (1944) mapping function was used to convert recombination fractions into map distances. The linkage groups were drawn using the Mapchart 2.1 software (Voorrips 2002).

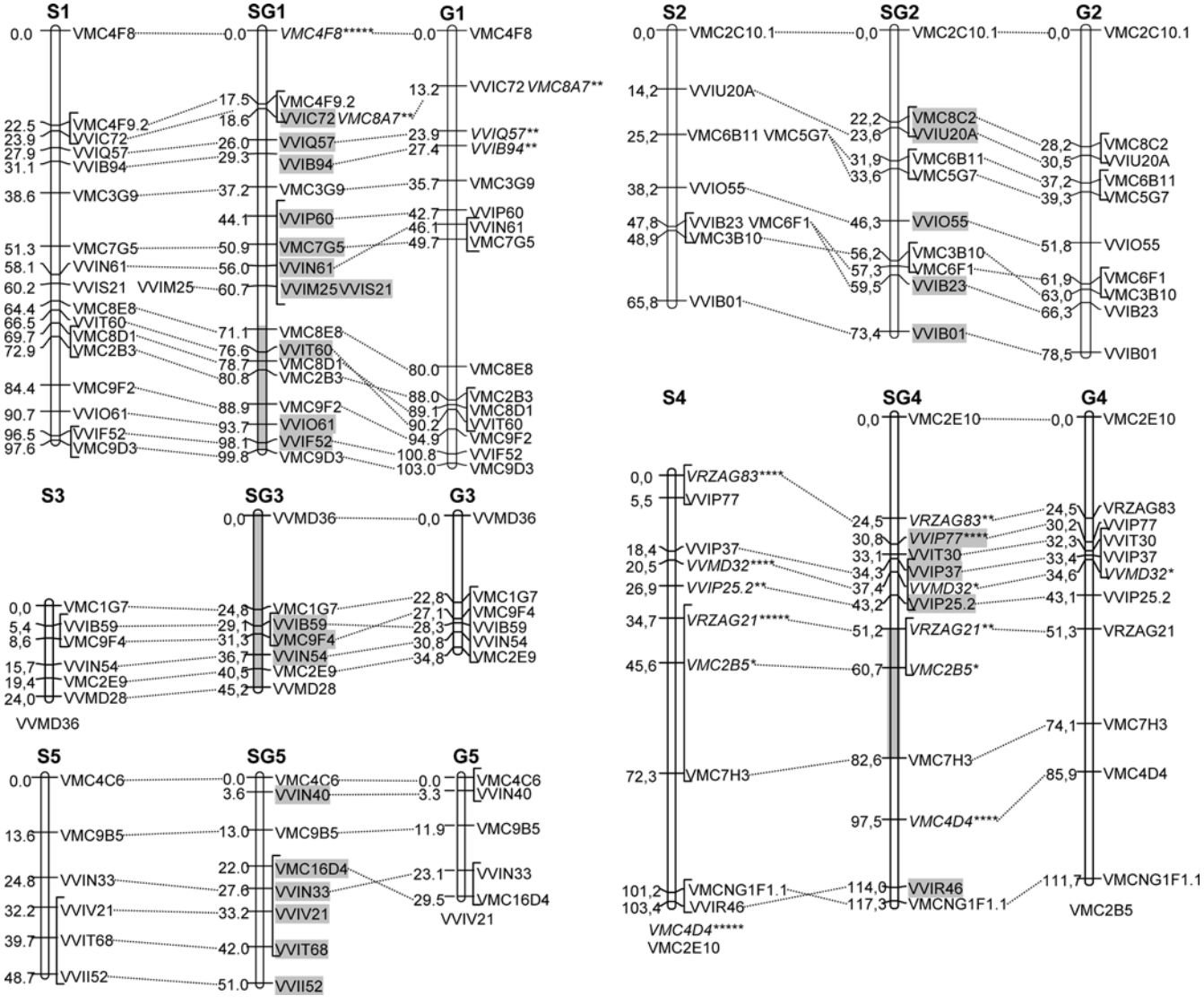

Fig. 1 Syrah, Grenache and consensus S × G maps. Linkage groups of the Syrah, Grenache and consensus maps are respectively numbered from 1 to 19 with the prefix S, G and SG. Distorted markers are in *italics* with an *asterisk* indicating the level of distortion (*P<0.05; ** P<0.02; ***P<0.01; ****P<0.001, *****P<0.0001). Markers that were ordered at LOD=2.0 are indicated with a *bracket* on the *left*. Distances of markers from the top are indicated on the *left* in cM Kosambi. Markers present only in the S × G map and not in the Riaz et al. (2004) map are indicated with *grey boxes*. *Grey zones* in the linkage group are indicating markers with a different order in the present map and the three maps of Riaz et al. (2004)

Test for a difference of recombination rates between Syrah and Grenache

A total of 89 pairs of markers were chosen along the linkage groups, as well scattered as possible. Their recombination rates were tested in Syrah and Grenache using a χ^2 test (P=0.05).

Estimation of genome size and coverage

The genome size was estimated according to the method of Hulbert et al. (1988) modified by Chakravarti et al. (1991). Confidence intervals for genome-length estimates were computed according to Gerber and Rodolphe (1994) for a bilateral type-I error rate α=5%. The expected genome coverage was estimated according to Lange and Boehnke (1982), as a function of the number of mapped markers, genome length and number of chromosomes. The observed genome length was estimated according to Nelson et al. (1994). For a comprehensive description of the method followed, see Cervera et al. (2001).

Results and discussion

Microsatellite marker heterozygosity for Syrah, Grenache and Riesling

Of the 346 primer pairs were analysed on Syrah and Grenache, 37 did not show any amplification and 16 showed low quality results and were discarded. The remaining 293 primer pairs amplified 310 markers on Syrah and Grenache. Later, 292 primer pairs were tested on Riesling, amplifying 299 markers. The results of these tests for heterozygosity are summarised in Table 2. The percentage of heterozygous markers in a particular variety is around 69% for all three varieties (Table 2). Ninety per cent of the markers can be mapped in at least one of the two parents of the S × G population (Table 2). Only 6.7% of the markers are homozygous in all three varieties (Table 2), which means that 93.3% of the markers can be mapped at least in one of the three parents.

These results, again stressing the interest of developing such markers in the grape for mapping purposes, were expected as different authors have already described the

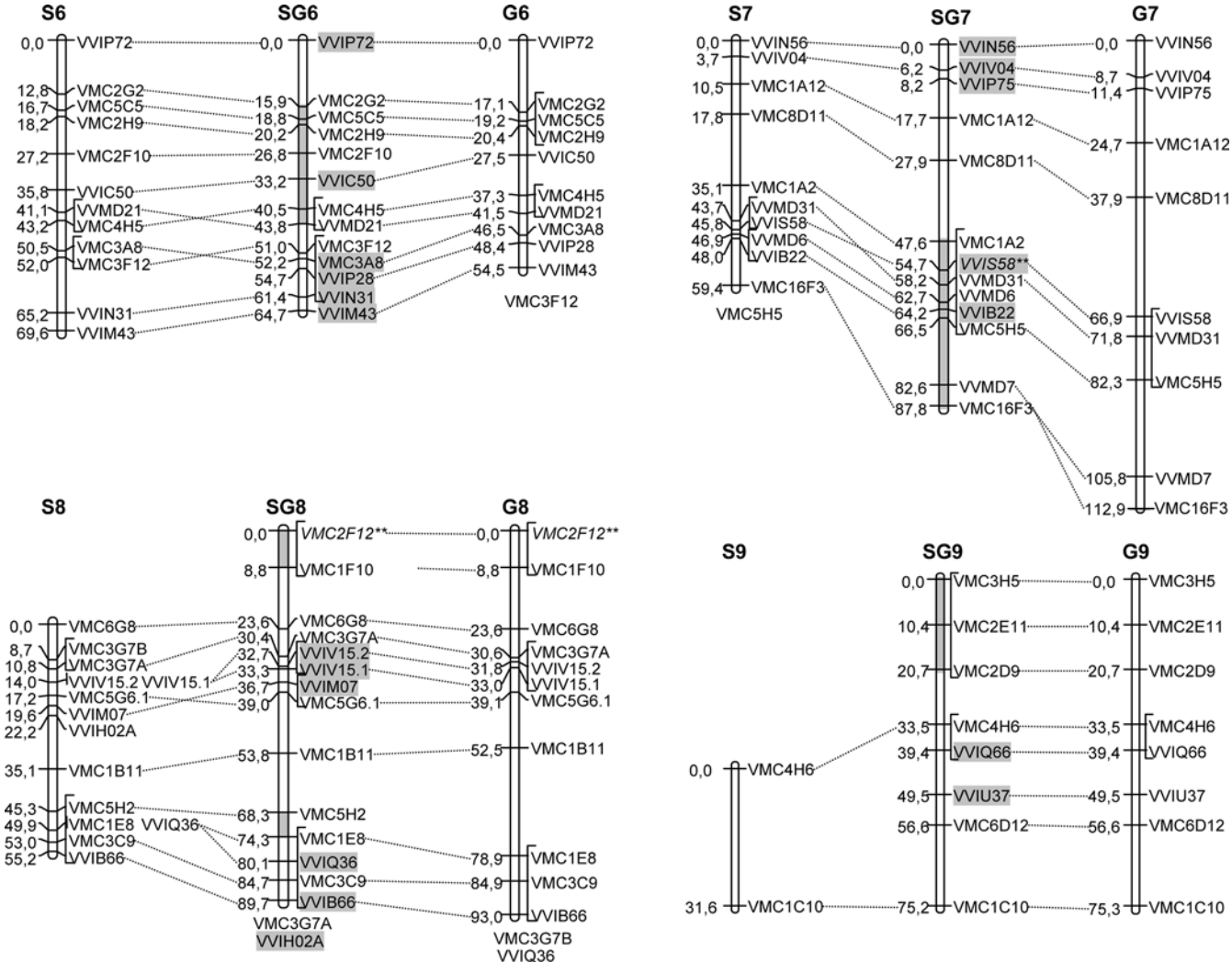

Fig. 1 (continued)

high heterozygosity level of *Vitis*-derived microsatellite loci across *V. vinifera* varieties (Thomas and Scott 1993; Bowers et al. 1996, 1999a, b; Sefc et al. 1999; Siret 2001). This high level of heterozygosity allowed a good comparison between the three parental maps that were constructed in the present work, and will also make comparisons possible with the previously published maps of *V. vinifera*. Microsatellite markers have also been shown to be valuable tools for comparative mapping of different species of the same genus, as for example the *Populus* genus (Cervera et al. 2001), or within the same families, as for the Maloideae family (Liebhardt et al. 2002). Several authors have shown a good transferability of different sets of SSR markers across the *Vitis* genus (Lin and Walker 1998; Di Gaspero et al. 2000; Scott et al. 2000; Decroocq et al. 2003) and a first comparison has been made between a *V. vinifera* map and a *V. riparia* map based on 21 SSR and 19 AFLP common loci (Grando et al. 2003). Such maps will be of special importance for the introgression of QTL for resistance to pathogens from wild *Vitis* species into *V. vinifera*.

Syrah×Grenache map construction

A total of 274 markers were genotyped on the 96 individuals of the S × G population. Two hundred and twenty-two allowed the construction of the two parental maps and the consensus map (Fig. 1). The remaining 52 markers consisted of six unlinked markers, 16 markers that were discarded because of low quality results and 30 markers not included in linkage groups because they affected the order of the neighbouring markers. Among the latter 30 markers, 12 showed distorted segregation ratios and/or the two parents carried the same alleles, and 15 corresponded to primer pairs amplifying several markers, which indicates that better PCR conditions are necessary to either eliminate non-specific amplification or to allow a better amplification of all markers. Sampling effects due to the rather small size of the population could also explain this high number of markers that we were unable to order.

The distribution of 228 markers (222 mapped and six unlinked) into different segregation types is presented in Table 3 and shows the high percentage of markers (49%) that could be mapped on both parents. This is close to the 62% of the markers mapped on both the Cabernet Sauvignon and Riesling maps generated by Riaz et al. (2004). Twenty markers showed distorted segregation ratios with a probability $P \leq 0.05$ in Syrah (10.8%), 13 in Grenache (7.2%) and 22 in the consensus segregation (9.9%, Table 4). Again, this is very close to the proportion of distorted markers observed by Doligez et al. (2002), Grando et al. (2003) and Riaz et al. (2004).

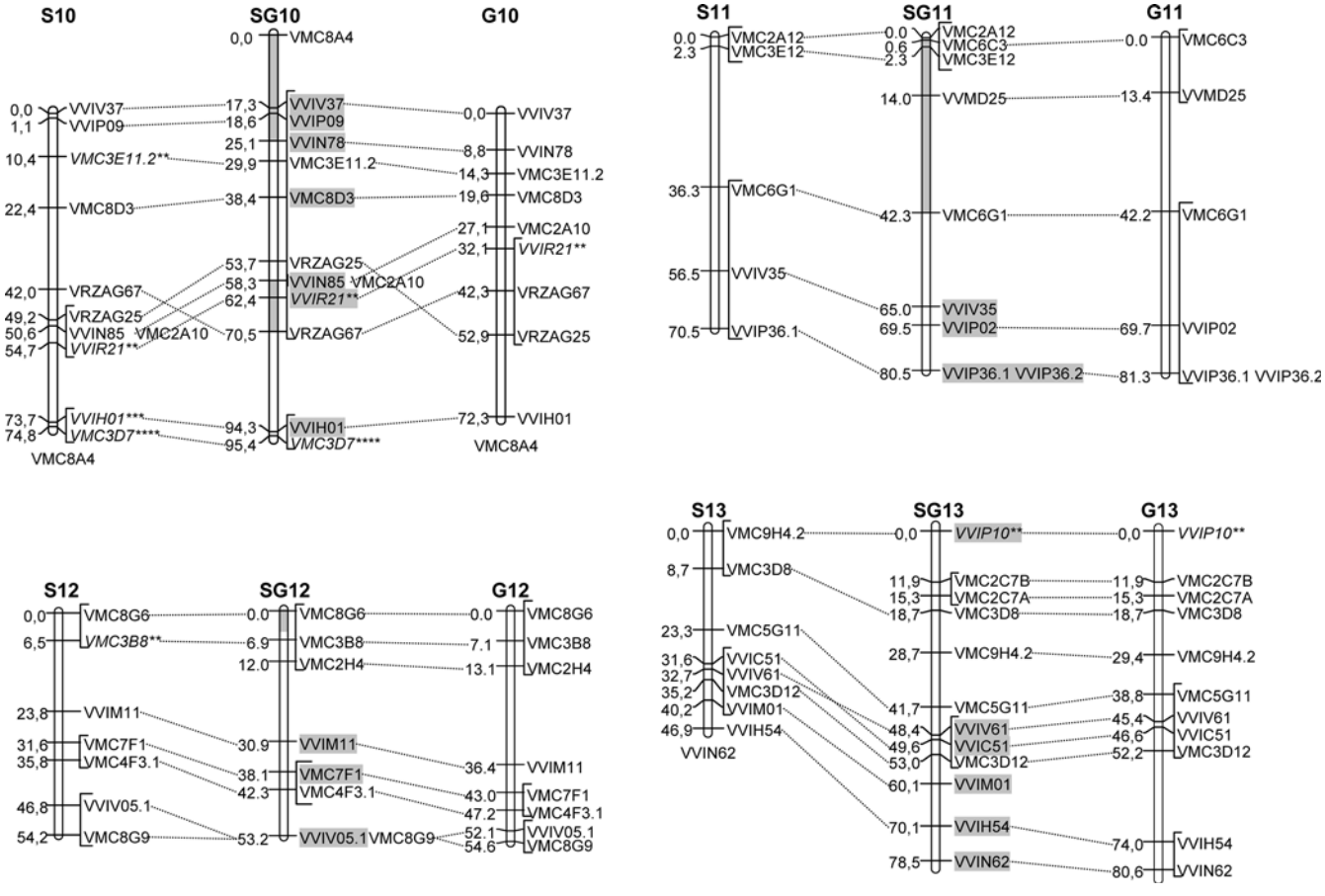

Fig. 1 (continued)

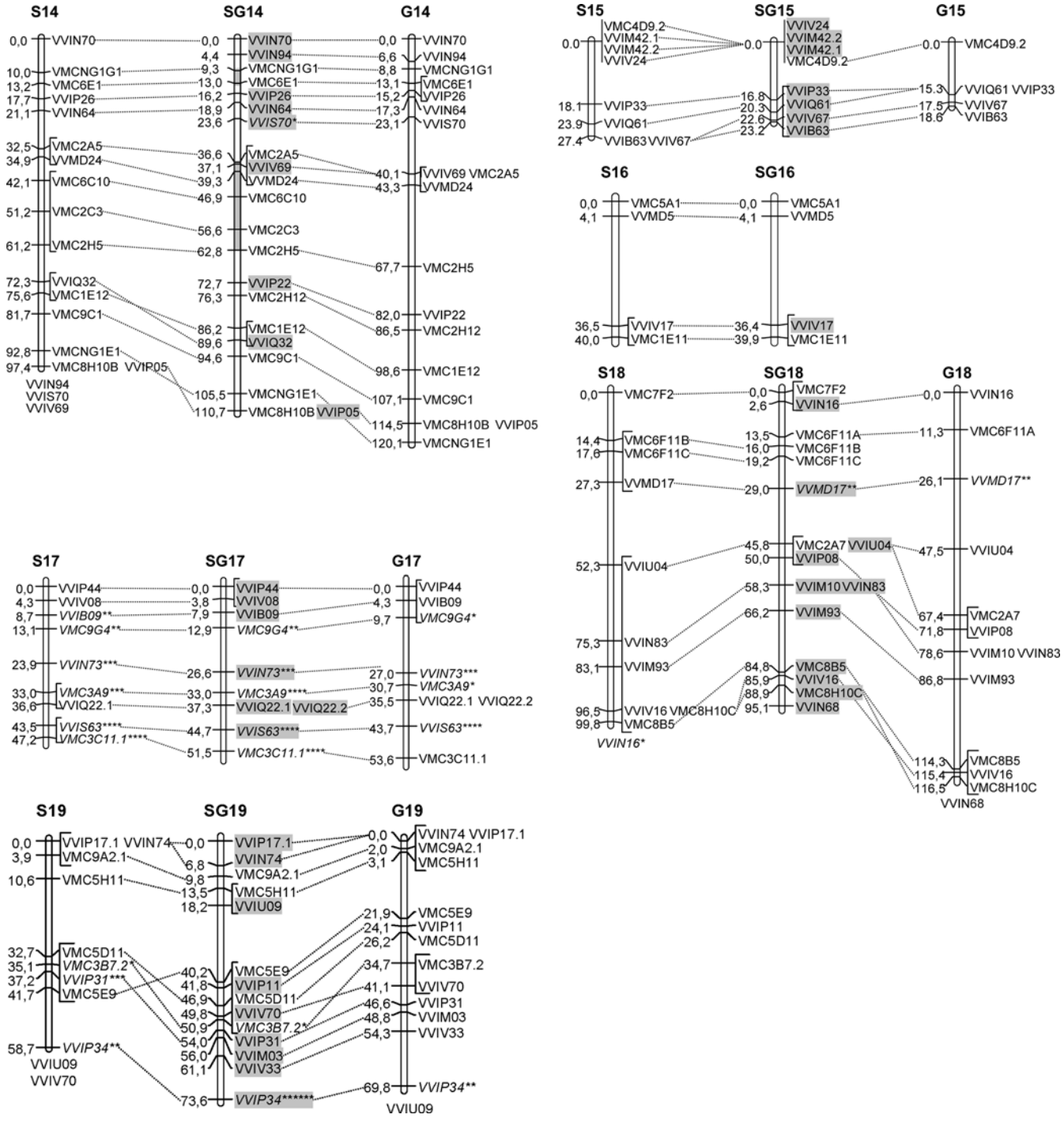

Fig. 1 (continued)

The three maps are presented in Fig. 1, the linkage groups being numbered from 1 to 19 according to the numbering of the linkage groups of the *V. vinifera* map of Riaz et al. 2004, except for groups 13 and 20. Group 13 of this previous map is lacking in our map and none of its markers were tested on the S × G population. An update of the map of Doligez et al. (2002) recently showed that groups 13 and 18 are linked (Doligez et al. 2003). We thus decided to rename group 20 group 13.

The Syrah map was constructed from the segregations of 177 markers that could be ordered into 18 linkage groups (Table 4). The total length of the Syrah map was 1,172.2 cM with an average distance between markers of 6.6 cM and ten gaps larger than 20 cM. The Grenache map was constructed with the segregations of 178 markers that could be ordered into 18 linkage groups (Table 4). The total length of Grenache map was 1,360.6 cM and the average distance between markers was 7.6 cM. Fifteen gaps larger than 20 cM were observed. Linkage group 16

Table 4 Main characteristics of the linkage groups in the four maps. Linkage groups are numbered according to the *Vitis vinifera* map of Riaz et al. (2004). Distances are in cM Kosambi

Linkage group no.	Syrah			Syrah × Grenache			Grenache			Riesling		
	Number of markers	Size	Number of distorted markers	Number of markers	Size	Number of distorted markers	Number of markers	Size	Number of distorted markers	Number of markers	Size	Number of distorted markers
1	18	97.6	0	20	99.8	2	16	103.0	3	8	63.2	1
2	9	65.8	0	10	73.3	0	10	78.5	0	6	43.6	1
3	6	24.0	0	7	45.2	0	6	34.8	0	4	47.7	0
4	10	103.4	6	13	117.7	6	11	111.7	1	5	91.6	1
5	6	48.7	0	8	51.0	0	5	29.5	0	7	52.5	0
6	12	69.6	0	13	64.7	0	10	54.5	0	10	47.1	1
7	10	59.4	0	13	87.8	1	10	112.9	0	8	84.3	2
8	14	55.2	0	14	89.7	1	11	93.0	1	8	72.7	0
9	2	31.6	0	8	75.2	0	8	75.3	0	7	82.1	2
10	11	74.8	4	13	95.4	2	9	72.3	1	5	73.2	0
11	5	70.5	0	8	80.5	0	5	81.3	0	4	64.6	1
12	7	54.2	1	8	53.2	0	8	54.6	0	6	39.2	1
13	8	46.9	0	12	78.5	1	11	80.6	1	4	66.4	2
14	19	97.4	0	21	110.7	0	18	120.1	0	8	73.7	0
15	8	27.4	0	8	23.2	0	5	18.6	0	2	22.8	0
16	4	40.0	0	4	39.9	0				4	58.9	0
17	9	47.2	6	10	51.5	5	9	53.6	4	4	74.5	0
18	10	99.8	1	16	95.1	2	13	116.5	1	4	88.3	2
19	9	58.7	2	14	73.6	2	13	69.8	1	6	45.3	0
Total	177	1,172.2	20	220	1406.1	22	178	1,360.6	13	110	1,191.7	14
Average distance between markers												
		6.5			6.4			7.6			10.8	
Average number of markers per group												
	8.9			11			9.2			5.8		
Gaps(>30 cM)												
Number			3			1			1			1
Group no.			9, 11, 16			16			1			18

Table 5 *V. vinifera* genome length estimated from four genetic maps (LOD=3.0, cM Kosambi). E(Cn) represents the expected genome coverage according to Lange and Boehnke (1982)

	Syrah	S × G	Grenache	Riesling
Genome length (cM)	1,708	2,786	1,778	2,154
Confidence interval (α=5%)	(1,585; 1,853)	(2,619; 2,975)	(1,648; 1,931)	(1,898; 2,490)
E(Cn) (%)	94.6	96.9	93.8	78.7
Observed genome coverage (%)	78.8	62.3	82.3	56.8

was missing, as all its markers were homozygous in Grenache. The consensus map was constructed with the segregations of 220 markers that were ordered into 19 linkage groups (Table 4). The total length of the consensus map was 1,406.1 cM with an average distance between markers of 6.4 cM.

Distorted markers were found in all maps for linkage groups 10, 17, 18; in the S and S × G maps for linkage groups 4, 2, 14; and in the G and S × G maps for linkage groups 1, 4, 8, 18 and 20 (Table 4, Fig. 1). The order of the markers was very consistent between the three maps except for two markers in group 10 (VrZAG25 and VrZAG67) and one marker in group 19 (VMC5E9). In both cases, this may be explained by a statistically significant difference between recombination rates in the two parents for some of the marker pairs (data not shown). The order that we propose for the whole of group 10 and the surrounding region of VMC5E9 in group 19 remains to be confirmed with the scoring of additional individuals, as most of the group was constructed with a LOD=2.0 (Fig. 1).

Significant differences were found for eight pairs in linkage groups 1 (three pairs), 8, 10, 12, 15 and 19 (data not shown). Such differences may explain some of the difficulties we had to find a consistent order for the markers between the two parental maps and the high number of markers that were discarded. However, this is certainly not the only explanation as group 1 showed differences in recombination rates for three of ten marker pairs tested, but the order of these markers was consistent in the three maps. In six of eight cases, the recombination rate was higher in Grenache than in Syrah. However, we were not able to conclude there was an effect of sex on the recombination rate as, for practical constraints, the S × G population was an unequal mixture of crosses in both orientations (around two-thirds of the population had Grenache as a maternal parent). To date, no statistically significant difference has been reported between maternal and paternal recombination rates in the grape (Doligez et al. 2002; Grando et al. 2003; Riaz et al. 2004; Fischer et al. 2004).

Riesling map construction

Of the 111 markers scored on the RS_1 population, 23 were not mapped using the S × G population (monomorphic markers, ab x ab dominant segregation type, distorted, bad quality etc.). Of these 111 markers, 110 allowed us to construct a map of a total length of 1,191.7 cM with an average distance between markers of 12.9 cM and 5.8 markers per linkage group on average (Table 4, Fig. 2). The order of the markers was consistent between the RS_1 map and the consensus S × G map except for the position of VVIB66 in group 8. This map allowed the ordering of 23 additional markers on the *V. vinifera* genome.

Estimation of genome length and coverage

The genome length of *V. vinifera* was estimated using the four maps. The results are presented in Table 5. The estimations of the genome size for *V. vinifera* made from the S × G and RS_1 were very close: 2,786 cM (S × G) and 2,154 cM (R), although their estimated coverage (both expected and observed) was very different (Table 5). Estimations of the genome size based on the S and G maps were smaller (respectively, 1,708 and 1,778 cM), which could be explained by the fact that both maps contained uncovered regions: the whole of group 16 and part of group 5 in the G map; and parts of groups 3, 4, 8, 9, and 10 in the S map (Fig. 1). The average of these four estimates of genome length (2,107 cM) was close to the average of 2,230 cM found for the map of Riaz et al. (2004) and is thus likely to be a reliable estimate of this parameter.

Additional information compared to the previous SSR map

The present work allowed us to map 123 new SSR markers on the *V. vinifera* genome (Figs. 1, 2) that were not mapped on the map of Riaz et al. 2004: 111 VVI markers and 12 others. It represents an average of 6.5 new markers per linkage group, with a minimum of two markers (groups 16) and a maximum of 13 markers (group 1) per group.

The order of the markers was consistent between our maps and that Riaz et al. (2004) within most linkage groups. The differences involved one or few markers in groups 4, 8, 9, 10, 12 and 14, and most of these regions were ordered at LOD 2.0 in our map (Fig. 1). However larger differences were observed in linkage groups 1, 3, 7 and 11, that cannot have the same origin (Fig. 1). A larger set of individuals is currently being scored to precisely define the order in some of these regions.

With the 123 newly mapped SSR markers, the present map is a valuable tool for all geneticists working on grape traits. It is already currently used as acomplement to the map of Riaz et al. (2004) for the transfer of well-scattered markers to other maps for QTL detection (Doligez et al.

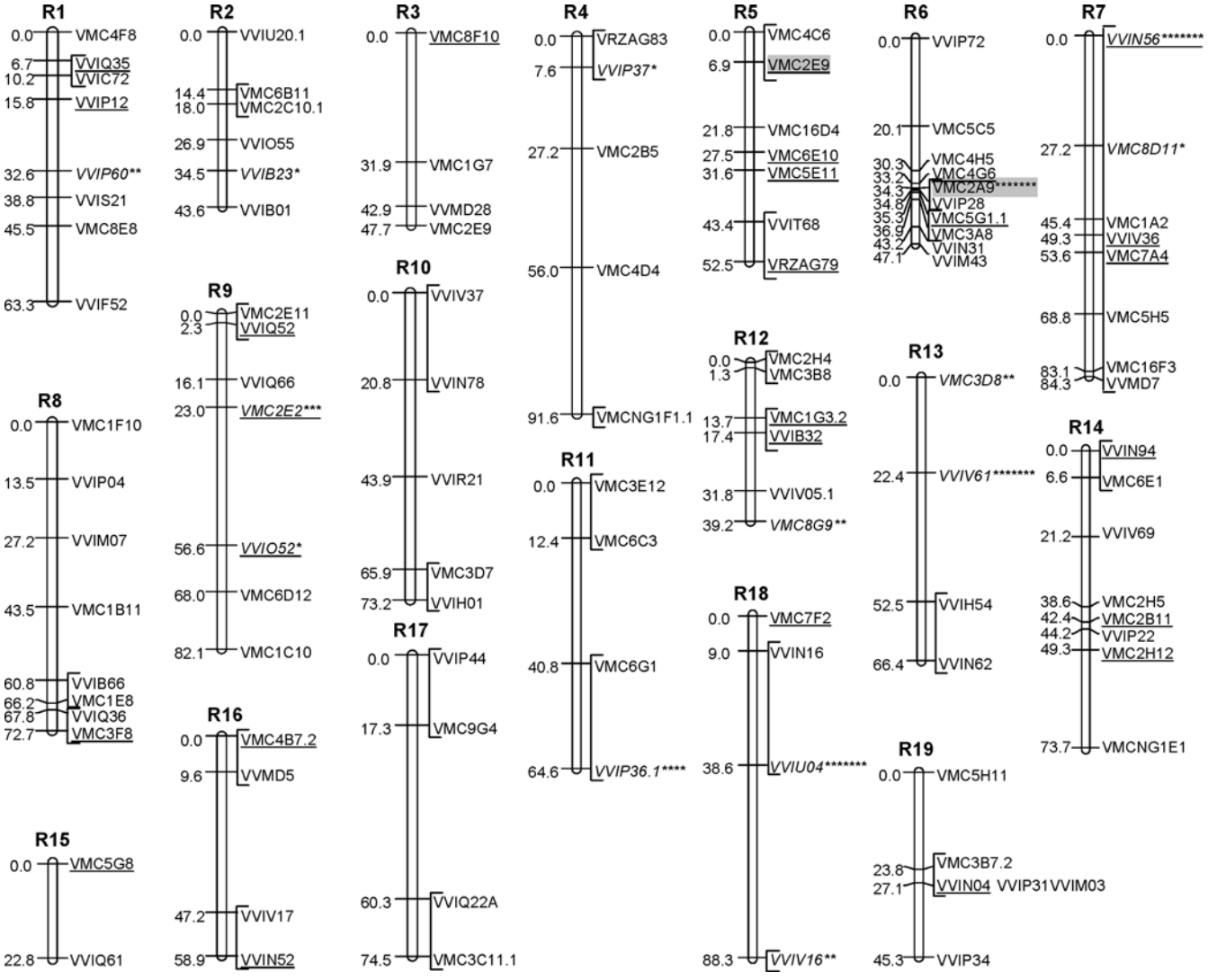

Fig. 2 The Riesling map. The linkage groups of the Riesling map are numbered from 1 to 19 respectively, with the prefix R. Distorted markers are in *italics* with an *asterisk* indicating the level of distortion (* $P<0.05$; ** $P<0.02$; *** $P<0.01$; **** $P<0.001$, ***** $P<0.0001$). Markers that were ordered at LOD=2.0 are indicated with a bracket on the *left*. Markers present only in the Riesling map and not in the S × G map are *underlined*. Markers present only in the Riesling map and not in either the S × G or the Riaz et al. (2004) maps are in *grey boxes*. The distances of the markers from the top are indicated on the *left* in cM Kosambi

2003; S. Decroocq and D. Merdinoglu, personal communication). The possibility of comparing the localisations of QTLs detected in different crosses based on common markers is of great interest, especially for the construction of genotypes accumulating different QTLs for resistance to one or several pathogens (Fischer et al. 2004). Moreover, the co-dominant status of these markers allows breeders to follow the introgression of genomic regions from both parents and to analyse their effects on the trait of interest. Finally, these mapped mono-locus markers are very useful to anchor the physical map of the grape genome on the current genetic maps (Bernole et al. 2004). The next project of the grape community will be to share the already available mapping data sets to build a consensus map.

Acknowledgements This research was funded by Génoplante grant CI1999076 and the INRA. The VMC primers were available through the VMC consortium coordinated by Agrogène. The authors are deeply indebted to Sumaira Riaz and Carole Meredith for helpful discussions, use of unpublished results (primer pairs and early versions of their map) and to Agnès Doligez for careful review of different versions of this paper. Many thanks to Mark Thomas for allowing us to test unpublished primer pairs developed at the CSIRO.

References

Bernole A, Adam-Blondon A-F, Caboche M, Chalhoub B (2004) PCR-anchoring of 700 markers and ESTs on a BAC library. Plant, animal and microbe genome conference XII, SanDiego, 10–14 January 2004 http://www.intl-pag.org/12/abstracts/P5 h_PAG12_604.htm

Bowers JE, Dangl GS, Vignani R, Meredith CP (1996) DNA fingerprint characterization of some California grape cultivars. Am J Enol Vitic 44:266–274

Bowers J, Boursiquot J-M, This P, Chu K, JohanssonH, Meredith C (1999a) Historical genetics: the parentage of Chardonnay, Gamay and other wine grapes of northeastern France. Science 285:1562–1565

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

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

Chabrier P, Gaspin C, Schiex T (2000) CarthaGene: a maximum likelihood multiple population genetic/radiated hybrid mapping software. Plant and animal genome VIII conference, San Diego, January 2000 http://www.intl-pag.org/pag/8/abstracts/pag8189.html

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

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

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

Decroocq V, Favé M-G, Hagen L, Bordenave L, Decroocq S (2003) Development and transferability of apricot and grape EST microsatellite markers across taxa. Theor Appl Genet 106:912–922

Di Gaspero G, Peterlunger E, Testolin R, Edwards KJ, Cipriani G (2000) Conservation of microsatellite loci within the genus *Vitis*. Theor Appl Genet 101:301–308

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

Doligez A, Bouquet A, Ballester J-F, Farnos M, Danglot Y, Adam-Blondon A-F, Roux C, Domergue P, This P (2003) QTLS for quality-related traits in table grapes. Plant and animal genome XI conference, San Diego, January 2003 http://www.intl-pag.org/pag/11/abstracts/P5 h_P533_XI.html

Eibach R, Diehl H, Alleweldt G (1989) Untersuchungen zur Vererbung von Resistenzeigenschaften bei Reben gegen *Oidium tuckeri*, *Plasmopara viticola* und *Botrytis cinerea*. Vitis 28:209–228

Fischer B, Salakhutdinov I, Akkurt M, Eibach R, Edwards KJ, Töpfer R, Zyprian EM (2004) Quantitative trait locus analysis of fungal disease resistance factor on a molecular map of grapevine. Theor Appl Genet 108:505–515

Gerber S, Rodolphe F (1994) An estimation of the genome length of maritime pine (*Pinus pinaster* Ait). Theor Appl Genet 88:289–292

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

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

Kosambi DD (1944) The estimation of map distances from recombination values. Ann Eugen 12:172–175

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

Levadoux L (1956) Les populations sauvages et cultivées de *Vitis vinifera* L. Ann Amelior Plant 6:59–118

Liebhardt R, Gianfranceschi L, Koller B, Ryder CD, Tarchini R, Van De Weg E, Gessler C (2002) Development and characterization of 140 new microsatellites in apple (*Malus* × *domestica* Borkh.). Mol Breed 10:217–241

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

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

Loureiro MD, Martinez MC, Boursiquot JM, This P (1998) Molecular marker analysis of Vitis vinifera 'Albariño' and some similar grapevine cultivars. J Am Soc Hortic Sci 123:842–848

Nelson CD, Kubisiak M, Stine M, Nance WL (1994) A genetic linkage map of the longleaf pine (*Pinus palustris* Mill.) based on random amplified polymorphic DNAs. Theor Appl Genet 87:145–151

Olmo HP (1976) Grapes. In: Simmonds NW (ed) Evolution of crop plants. Longman, London, pp 294–298

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

Scott KD, Eggler P, Seaton G, Rossetto M, Ablett EM, Lee LS, Henry RJ (2000) Analysis of SSR derived from grape ESTs. Theor Appl Genet 100:723–726

Sefc KM, Regner F, Tureschek E, Glossl J, Steinkellner H (1999) Identification of microsatellite sequences in *Vitis riparia* and their application for genotyping of different *Vitis* species. Genome 42:367–373

Siret R (2001) Etude du polymorphisme génétique de la vigne cultivée (*Vitis vinifera* L.) à l'aide de marqueurs microsatellites: application à la caractérisation des cépages dans les vins. Dissertation, Université de Montpellier

Thomas MR, Scott NS (1993) Microsatellite repeats in grapevine reveal DNA polymorphisms when analysed as sequence-tagged sites (STSs). Theor Appl Genet 86:985–990

Thomas MR, Cain P, Scott NS (1994) DNA typing for grapevine: a universal methodology and database for cultivar identification. Theor Appl Genet 86:173–180

Voorrips RE (2002) MapChart: software for the graphical presentation of linkage maps and QTLs. J Hered 93:77–78

Zyprian E, Eibach R, Töpfer R (2003) Comparartive molecular mapping in segregating populations of grapevine. Acta Hortic 603:71–77