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Molecular Linkage Maps: Strategies, Resources and Achievements

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ABSTRACT

The development of genetic maps in grapevine started in the late 90s. It greatly benefited from the development of SSR markers by an international consortium, leading to the construction of integrated genetic maps. Software was improved for facilitating map construction in full sib families. A review of the different strategies that were developed for mapping in grapevine, including mapping populations, software and markers is provided here together with a discussion on their interest and limitations. Thanks to all these resources and experience, genetic mapping is now easy to handle in grapevine and is now widely used for QTL detection, map-based cloning, comparative mapping across species and genome sequence anchoring.

Keywords: Genetic map, SSR, SNP, integrated map, full-sib family, heterozygosity

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5.1 Introduction

Genetic maps based on molecular markers emerged on the scene of plant genetics in the 1980s (Beckmann and Soller 1983; Tanksley et al. 1989) and in the course of time became indispensable tools for grapevine geneticists.

In perennials, the development of the first genetic maps occurred later than in annuals due to the higher difficulty of generating large progeny from controlled crosses (e.g., Tulsieram et al. 1992) and to the difficulty of extracting large amounts of high quality DNA, which was required for the production of the first-generation molecular markers, such as restriction fragment length polymorphism (RFLP) markers (e.g., Lodhi et al. 1994). The first grapevine linkage map was constructed in 1995 (Lodhi et al. 1995), while the first genetic maps in tomato and maize were published in 1986 and 1988, respectively (Bernatzky and Tanksley 1986; Helentjaris et al. 1988).

Genetic mapping provided significant benefit to the grapevine community only after the development of molecular markers based on polymerase chain reaction (PCR). In parallel, improvement of statistical methods and software required for handling segregation data in various genetic contexts (full-sib families, back-crosses, F_2) were regularly achieved.

In the following paragraphs we will review how and when molecular markers were introduced in grapevine genetics, the evolution of grapevine linkage maps, as well as the algorithms and mapping populations used.

5.2 Evolution of Molecular Markers for Genetic Mapping: from RFLPs to SNPs

The development and use of molecular markers in grape followed the development of molecular biology that began in the 1970s. Initially, markers were used for the analysis of genetic diversity in the grapevine germplasm (see Chapter 2).

5.2.1 Isozymes

Isoenzymes became very popular in the 1980s as markers capable of discriminating grape accessions and to confirm parentage (Loukas et al. 1983; Arulsekar and Parfitt 1986). Segregation data provided by this kind of biochemical marker can be found in the first grapevine linkage maps (Lodhi et al. 1995; Dalbò et al. 2000; Doligez et al. 2002; Grando et al. 2003).

5.2.2 RFLP

The first kind of DNA-based molecular markers that came into play for genetic mapping was RFLP marker (Beckmann and Soller 1983). RFLP

markers have never received the same attention from grape breeders as they got for instance in humans and in other crop species. This was mainly due to the high cost of using this technique, the large amount of DNA required, and the necessity to access and store large collections of probes. Most of these probes were heterologous, that is developed from the DNA of other plant species. In spite of these technical constraints, RFLP contributed to the construction of the earliest grape maps (Lodhi et al. 1995; Dalbò et al. 2000; Doligez et al. 2002; Grando et al. 2003).

5.2.3 RAPD, AFLP, SAMPL, and S-SAP

The first revolution in grape molecular genetics took place with the advent of the PCR technique and the use of arbitrarily designed primers that do not require *a priori* knowledge of the DNA sequence of the species of interest (Williams et al. 1990). Random amplified polymorphic DNA (RAPD; Williams et al. 1990) markers were easy to produce, and their cost was affordable in terms of labor and investment, even in small laboratories.

In grapevine, RAPD markers were used in several maps (Lodhi et al. 1995; Lahogue et al. 1998; Dalbò et al. 2000), but they were very quickly replaced by another type of marker produced with a combination of endonucleases and arbitrarily designed primers: the amplified fragment length polymorphism (AFLP) marker (Vos et al. 1995). AFLPs were more reproducible and yielded a greater number of polymorphic loci per experiment, with a single gel displaying as many as one hundred different bands per lane, a dozen of which could segregate in a mapping population. AFLP-based linkage maps were produced between 2002 and 2007 (Doligez et al. 2002; Grando et al. 2003; Doucleff et al. 2004; Fischer et al. 2004; Riaz et al. 2004; Cabezas et al. 2006; Troggo et al. 2007).

Both RAPD and AFLP markers have several drawbacks, such as dominance, reproducibility issues, and low transportability across distantly related genotypes. The latter is a severe limitation, as geneticists and especially breeders frequently need to share knowledge acquired in a particular mapping population with others.

Two marker classes, based on the AFLP technology and thus showing the same major drawbacks, deserve mentioning, although their use was mostly aimed at distinguishing grape accessions rather than at producing linkage maps: selective amplification of polymorphic loci (SAMPL), which combines the AFLP technology with the abundance and polymorphism of simple sequence repeat (SSR) markers, and sequence-specific amplified polymorphism (S-SAP), in which the AFLP technology is combined with the retrotransposon long terminal repeat (LTR) (Pelsy et al. 2003; Labra et al. 2004; Pelsy 2007). A single paper reports the use of SAMPL and S-SAP markers in grapevine mapping (Cabezas et al. 2006).

5.2.4 SSR

RAPD, AFLP, and their derived classes of markers were never adopted in humans, where genetic maps were initially produced using RFLP and then microsatellite or SSR markers. A highly saturated linkage map based on 5,264 SSRs was published in 1996 to anchor the human physical map in the frame of the Human Genome Sequencing project (Dib et al. 1996).

Microsatellite repeats are also abundant in plant genomes, and show a preferential distribution to non-repetitive regions (Morgante et al. 2002). They are amplified as single loci in diploid genomes, they are codominant, and are highly polymorphic in grapevine (see for, e.g., Aradhya et al. 2003). In addition, they have conserved flanking regions that make them transferable to related species (Moore et al. 1991), which is particularly true within the Vitaceae family (Sefc et al. 1999; Di Gaspero et al. 2000; Decroocq et al. 2003). All of these positive features made them the ideal markers for the construction of linkage maps that are transferable from one cross to another.

The SSR markers were first isolated in grapevine in the early 1990s (Thomas and Scott 1993; Bowers et al. 1996; Bowers et al. 1999). During the International Conference on Grape Genetics and Breeding in 1998 held at Montpellier (France), Mark Thomas, Carol Meredith, and Patrice This launched the *Vitis* Microsatellite Consortium (VMC), a project aiming to develop a large number of microsatellite markers under the coordination of Agrogene, SA. The proposal stimulated the cooperative effort of numerous institutions called to sequence groups of 48 cloned fragments produced by Agrogene from grape genomic libraries, enriched with 10 different types of di- and tri-nucleotide repeats. This work resulted in the production of nearly 1,000 SSR markers. Several hundreds of these markers were polymorphic and segregated in mapping populations (Doligez et al. 2002; Grando et al. 2003; Adam-Blondon et al. 2004; Fisher et al. 2004; Mandl et al. 2006; Riaz et al. 2006; Di Gaspero et al. 2007). Since 90% of the VMC SSRs were AG repeats, several institutions produced new genomic libraries enriched for different repeats, which were used to isolate a substantial number of new SSRs (Sefc et al. 2000; Lefort et al. 2002; Di Gaspero et al. 2005; Merdinoglu et al. 2005), while other groups isolated new SSRs from expressed sequence tag (EST) collections (Scott et al. 2000; Decroocq et al. 2003; Riaz et al. 2006). An added advantage of SSR markers is that several of them can be multiplexed by different dyes and by adjacent allele range sizes, and analyzed in a single run with an automatic sequencer, increasing the throughput of the analysis (Merdinoglu et al. 2005). After the completion of the grapevine genome sequencing projects, several thousand SSR markers became available and retrievable from the NCBI database with simple programs like Sputnik (<http://cbi.labri.fr/outils/Pise/sputnik.html>). The most abundant class of SSRs

in the grapevine genome are di-nucleotide repeats, representing 46% of the total 75,185 SSRs identified so far. Tri-, tetra-, and penta-nucleotide repeats are also cumulatively well represented in the genome, but the relative frequency of each individual type of repeat is low, except for AAT (19%), AAAT (10%), and AAAT (0.07%; Table 5-1).

A set of SSR markers with 3-nt to 5-nt core repeat, the type of choice in human forensics, were selected for covering all grapevine chromosomes and validated for their power of discrimination among individuals (Cipriani et al. 2008), in view of their potential use in grapevine fingerprinting (Cipriani et al. 2010).

Table 5-1 SSR markers identified in the 12X assembly of the PN40024 grape genome sequence (Jaillon et al. 2007) using a modified version of the Sputnik software (<http://cbi.labri.fr/outils/Pise/sputnik.html>).

Type of repeat	Average repeat length (bp) ^a	No. of occurrences	Fraction of the total
<i>di-nucleotide</i>	12.02	34,449	0.46
-AT	14.09	23,674	0.31
-AG/CT	13.00	8,349	0.11
-AC/GT	12.01	2,423	0.03
-CG	9.00	3	0.00
<i>tri-nucleotide</i>	7.47	18,747	0.25
-AAT	9.08	14,522	0.19
-AAG	8.01	1,982	0.03
-ATC	7.09	710	0.01
-ACC	7.08	486	0.01
-AAC	7.07	363	0.00
-others	7.27	684	0.01
<i>tetra-nucleotide</i>	5.68	11,279	0.15
-AAAT	5.07	7,715	0.10
-AAAG	5.08	862	0.01
-AATT	5.04	721	0.01
-ACAT	7.03	470	0.01
-AATC	5.06	446	0.01
-others	5.73	1,065	0.01
<i>penta-nucleotide</i>	4.08	10,710	0.14
-AAAAT	4.05	5,623	0.07
-AAAAG	4.06	1,809	0.02
-AAATT	4.04	651	0.01
-AAAAC	4.06	525	0.01
-AATAT	4.05	348	0.00
-others	4.09	1,754	0.02
TOTAL	6.94	75,185	1.00

^aMinimum no. of repeat fixed for each search: 8 for di-nucleotide repeats, 6 for tri-nucleotide repeats, 5 for tetra-nucleotide repeats, and 4 for penta-nucleotide repeats.

All these sets of SSR markers are listed in Table 5-2, and most of them are available in public databases (see Chapter 13).

Table 5-2 Grapevine SSR markers listed according to their date of publication.

Code	No. of sequences	Type of repeat	Source (a)	Reference
VVS	5	GA and GT	EGL	Thomas and Scott 1993
VVMD	4	mainly CT	EGL	Bowers et al. 1996
VVMD	22	CT	EGL	Bowers et al. 1999
ssrVrZAG	18	GA	EGL	Sefc et al. 1999
scu[xx]vv	10	2- and 3-nt	EST	Scott et al. 2000
ssrVvUCH	7	mainly AG	EGL	Lefort et al. 2002
VVC	8	CA and CA	EST	Decroocq et al. 2003
VMC, VMCN _g	357	mainly AG	EGL	Agrogene, unpublished (b)
VVI	169	various	EGL	Merdinoglu et al. 2005
UDV	108	AC	EGL	Di Gaspero et al. 2005
ctg, CF, AF, BM, VEST	unknown	unknown	EST	Riaz et al. 2006
uncoded	239,634	all	SG	Jaillon et al. 2007
VChr	38	3- to 5-nt	SG	Cipriani et al. 2008

(a) EGL, Enriched genomic libraries; EST, Expressed sequence tag libraries; SG, Shot-gun sequencing.

(b) Partially published in Di Gaspero et al. 2000; Pellerone et al. 2001; Adam-Blondon et al. 2004; Arroyo-Garcia and Martinez-Zapater 2004; Riaz et al. 2004; Goto-Yamamoto et al. 2006; deposited in NCBI STS database.

5.2.5 SNP

Single nucleotide polymorphism (SNP) markers are the most recently developed class of markers. They target a single base mutation in the DNA sequence. Small indels (insertion or deletion events) are also assimilated as SNP markers since they can be handled with many of the technologies designed to identify SNPs. Their discovery relies on resequencing the same DNA region from different haplotypes. In grapevine, two research groups sequenced 25 and 230 gene fragments in 9 and 11 *Vitis vinifera* genotypes, respectively and observed a frequency of one SNP every 47 to 64 bp (Salmaso et al. 2004; Lijavetzky et al. 2007). Sequencing the heterozygous Pinot Noir led to the discovery of one SNP every 250 bp, by comparing the two haplotypes (Velasco et al. 2007), however, a significant amount of variation in this frequency exists along the genome: from one SNP/60 bp to one SNP/250 bp or less. Authors estimated that the number of potential SNP markers in the Pinot Noir genome could reach 2 million, with many present in coding regions, covering approximately 87% of annotated genes (Velasco et al. 2007).

SNPs are easily amenable to massive parallel automatic detection (Rapley and Harbron 2004; Steemers et al. 2006), making them particularly useful for saturating maps, especially in coding regions (Pindo et al. 2008),

or as an approach to gene discovery through linkage disequilibrium studies. Lijavetzky et al. (2007) estimated an 83% success rate for the conversion of a candidate variable nucleotide position into a technically scorable SNP marker. This conversion rate fell to 61% when SNPs were initially identified in the Pinot Noir sequence without resequencing any additional genotype (Pindo et al. 2008; Vezzulli et al. 2008a). Some 30% of SNP markers developed from sequencing *V. vinifera* cultivars are estimated to be heterozygous in any other *V. vinifera* cultivar (Lijavetzky et al. 2007; Vezzulli et al. 2008a). This rate could fall to 19% in feral accessions of *V. vinifera* and to 2% in wild species (Vezzulli et al. 2008b).

The transferability of SNPs across species is, therefore, much less likely than the transferability of SSRs and this is a major issue in mapping many traits, such as those for resistance to biotic and abiotic stress which usually entail genes present in wild species. However, due to their high density in the grapevine genome and the flexibility of multiplexing systems, it is possible to envisage working with complementary sets of SNP markers, some of which could be informative in *V. vinifera* and others in wild relatives, as it has been proposed for rice (http://mlorieux.free.fr/Rice_Genomics/Research/RiceDiversity/CoreMap/index.html).

Next-generation sequencing technologies now allow to rapidly identify customized sets of SNPs, for maximizing the informative content of the DNA chip in the gene pool of interest or in the parents of mapping populations. Accurate filtering of true SNPs from sequencing and alignment errors is still a challenging step, before embarking on a SNP chip experiment for linkage mapping. More confidence on this issue was generated by a case study of Myles et al. (2010), in which a 9K SNP array design from a set of 71K high-quality SNPs provided 97.7% concordance between genotype calls from the sequencing data and from the DNA chip.

5.3 Mapping Populations and Mapping Strategies

As mentioned above, perennial species pose specific biological problems in the development of mapping populations. The generation time is long (3–5 years from seed to seed), growing many individuals is cost and labor demanding, species are heterozygous, and several of them, grapevine included, are sensitive to inbreeding depression (Einset and Pratt 1975). Yet, large cross populations can be prepared without the limits of animal brood sizes.

The genetic structure of the mapping population and the dominant/co-dominant nature of the markers are the two factors that determine the observed genotypic classes and the information provided by each individual of the family (Allard 1956).

Traditionally, linkage studies in plants are carried out using the classical test cross, where two phenotypically or genotypically contrasting inbred lines are crossed to produce an F_1 , which in turn is either backcrossed to one of the parents resulting in a 1:1 segregation of markers, or selfed to produce an F_2 , resulting in a 1:2:1 segregation of co-dominant markers and a 3:1 segregation of dominant markers. Ritter et al. (1990) proposed the formulas for calculating the linkage between different classes of markers (dominant, co-dominant, with or without null allele) segregating in F_1 families obtained from heterozygous parents. In the case of dominant markers, the segregation of markers heterozygous in either parent is the same as in a classical backcross or testcross. This approach was adopted in 1994 by Grattapaglia and Sederoff to produce a linkage map of *Eucalyptus* species based on dominant RAPD markers (Grattapaglia and Sederoff 1994). They named this type of mapping approach the “pseudo-testcross”. Lodhi et al. (1995) introduced such a “pseudo-testcross” in grapevine, which was widely used for the construction of RAPD- and AFLP-based maps (Table 5-3). However, the drawback of this strategy is that the correspondence between the homologous linkage groups in the two parents can not be established. It can only be established through markers that are heterozygous in both parents and segregate in a ratio of 3:1 (Ritter et al. 1990; Lodhi et al. 1995). Co-dominant markers, such as SSR markers, that often have segregating alleles from both parents, provide a better solution to bridging homologous linkage groups, and have been widely used for that reason since 2003 (Table 5-3). From this point of view, SNP markers are in most cases bi-allelic (Troggio et al. 2007; Vezzulli et al. 2008a), segregate from a single parent like in the pseudo-testcross, and do not contribute to the identification of homologous linkage groups.

In grapevine, mapping populations usually consist of full-sib families (F_1) derived from a cross between two highly heterozygous parents, but some have also been produced by selfing a single genotype (Table 5-3). This unexpectedly reduced segregation biases (Adam-Blondon et al. 2004; Duchêne et al. 2009), with the percentage of segregating markers falling to around 50% against 75% of markers segregating in a typical full-sib family. Parents of biparental populations were chosen for their high heterozygosity and loose genetic relatedness with the objective of minimizing the occurrence of common alleles, which hampers the identification of which parent transmits them to the progeny and in turn makes them less informative. Contrasting phenotypes for the trait(s) to be mapped are also necessary factors in the choice of parents, in order to follow the inheritance of the trait in the progeny.

The number of meioses, and thus the size of the progeny, necessary to reach an acceptable level of precision in the estimation of the genetic distance between two markers depends on the informative content of the markers,

Table 5-3 Grapevine (*Vitis* spp.) linkage maps listed according to their publication date. All but two mapping populations were F₁ from heterozygous parents and the others were obtained by selfing one heterozygous individual.

Mapping population (a)	No. of progeny	No. of markers (b)	Types of markers	No. of linkage groups (b)	Map lengths cM (b)	Software used	Reference
Cayuga White (<i>It</i>) x Aurore (<i>It</i>)	60	438	most RAPD	20-22	1,196-1,477	MapMaker	Lodhi et al. 1995
Horizon (<i>It</i>) x Illinois 547-1(<i>It</i>)	58	438	most RAPD	20	1,199-1,470	MapMaker	Dalbò et al. 2000
MTP2223-27 (<i>Vv</i>) x MTP2121-30 (<i>Vv</i>)	139	301	AFLP/SSR & other	22-23 (20)	767-816 (1,002)	MapMaker, JoinMap	Doligez et al. 2002
Moscato bianco (<i>Vv</i>) x <i>V. riparia</i> (<i>Vri</i>)	81	338-429	AFLP/SSR & other	20-19	1,639-1,518	MapMaker, JoinMap	Grando et al. 2003
Syrah (<i>Vv</i>) x Grenache (<i>Vv</i>)	96	177-178 (220)	SSR	19-18 (19)	1,172-1,361 (1,406)	CartTaGene	Adam-Blondon et al. 2004
Riesling (<i>Vv</i>) selfed	96	110	SSR	19	1,192	CartTaGene	Adam-Blondon et al. 2004
<i>V. rupestris</i> (<i>Vru</i>) x <i>V. arizonica</i> (<i>Va</i>)	116	475	most AFLP	17-19	756-1,082	JoinMap, PGRI	Doucleff et al. 2004
Regent (<i>It</i>) x Lemberger (<i>Vv</i>)	153	429	AFLP/ RAPD/SSR/ SCAR	20-26	1,277-1,158	JoinMap	Fischer et al. 2004
Riesling (<i>Vv</i>) x Cabernet Sauvignon (<i>Vv</i>)	153	152	SSR	20-20 (20)	1,431-1,450 (1,728)	MapMaker, JoinMap	Riaz et al. 2004
Dominga (<i>Vv</i>) x Autumn Seedless (<i>Vv</i>)	118	595	AFLP/ SAMPL/S-SAP, SSR	19	1,173-1,131	JoinMap	Cabezas et al. 2006
Five mapping populations (c)	46 to 153	502	most SSR	19	1,154-1,570 (1,647)	CartTaGene	Doligez et al. 2006

Table 5-3 contid....

Table 5-3 cont'd....

Mapping population (a)	No. of progeny	No. of markers (b)	Types of markers	No. of linkage groups (b)	Map lengths cM (b)	Software used	Reference
MTP2687-85 (<i>Vv</i>) x Muscat de Hambourg (<i>Vv</i>)	174	139	SSR	>19	935-1,365 (1,265)	CarHTaGene	Doligez et al. 2006b
<i>V. Champinii</i> x <i>V. riparia</i>	188	205	SSR	19	1,245-1,095 (1,305)	JoinMap	Lowe et al. 2006
Welschriesling (<i>Vv</i>) x Sirius (<i>It</i>)	92	251	most SSR	20-20	not reported	MapMaker	Mandl et al. 2006
D8909-15 (<i>Vru</i> x <i>Va</i>) x F8909-17 (<i>It</i>)	188	159-158 (210)	most SSR	18-19 (19)	865-1,055 (1,154)	JoinMap	Riaz et al. 2006, then implemented by Xu et al. 2008
Chardonnay (<i>Vv</i>) x Bianca (<i>It</i>)	46 (116)*	332-353 (709)	SSR/RGA	19-19	1,210-1,425 (1,676)	CarHTaGene/JoinMap	Di Gaspero et al. 2007, *then implemented by Bellin et al. 2009
Cabernet Sauvignon (<i>Vv</i>) x 20/3 (<i>It</i>)	46	379-349 (709)	SSR/RGA	19-19	1,254-1,418 (1,676)	CarHTaGene	Di Gaspero et al. 2007
Syrah (<i>Vv</i>) x Pinot Noir (<i>Vv</i>)	94	994	SNP/AFLP/SSR	19	1,245	TMAP	Troggio et al. 2007
Regent (<i>It</i>) x Lemberger (<i>Vv</i>)	153	398	SSR/RGA/SCAR	19	1,631	JoinMap	Welter et al. 2007
Italia (<i>Vv</i>) x Big Perlon (<i>Vv</i>)	163	276-210 (341)	AFLP/SSR & other	19-19 (18)	1,353-1,130 (1,426)	JoinMap	Costantini et al. 2008
Moscato bianco (<i>Vv</i>) x <i>V. riparia</i> (<i>Vri</i>)	171						Battilana et al. 2009
Merzling (<i>It</i>) x Teroldego (<i>Vv</i>)	89	166-168 (247)	SNP/SSR	20-21 (19)	914-1,174 (1,309)	MapMaker/JoinMap	Salmaso et al. 2008
Three mapping populations (d)	87 to 94	1,134	AFLP/SSR/SNP	19	1,443	TMAP	Vezzulli et al. 2008

Muscat Ottonel (<i>Vv</i>) selfed	121	84	most SSR	19	569 (e)	CarHTaGen/ JoinMap	Duchêne et al. 2009
Cabernet S x <i>Vitis riparia</i> Gloire de Montpellier (<i>Vri</i>)	138	212	SSR/SSCP	19	1,270–1,410 (1,249)	CarHTaGene/JoinMap	Marguerit et al. 2009
V3125 (<i>Vv</i> 'Schiava grossa' x 'Riesling') x 'Börner' (<i>Vri</i> x <i>Vci</i>)	188	174–190 (235)	Most SSR	19–21 (19)	1,116–1,070 (1,155)	JoinMap	Zhang et al. 2009

(a) species: *Va* = *Vitis arizonica*, *Vc* = *Vitis champiniii*, *Vci* = *Vitis cinerea*, *Vri* = *Vitis riparia*, *Vru* = *Vitis rupestris*, *Vv* = *Vitis vinifera*, *Ith* = *Interspecific hybrids*.

(b) Individual maps, and in parentheses the consensus/integrated map.

(c) Syrah (*Vv*) x Grenache (*Vv*), Riesling selfed (*Vv*), Chardonnay (*Vv*) x Bianca (*Ith*), MTP2223-27 (*Vv*) x MTP2121-30 (*Vv*), Riesling (*Vv*) x Cabernet Sauvignon (*Vv*).

(d) Syrah (*Vv*) x Pinot Noir (*Vv*), Syrah (*Vv*) x Grenache (*Vv*), Cabernet Sauvignon (*Vv*) x Riesling (*Vv*).

(e) The short length of map is due to the reduced number of markers used to produce a framework map.

(f) D8909-15 (*Vru* x *Va*) x F8909-17 (*Ith*), D8909-15 (*Vru* x *Va*) x B90-116 (*Vv*), D8909-15 (*Vru* x *Va*) x Airen (*Vv*).

which can be highly variable in an F_1 family depending on the segregation type (Ritter et al. 1990). The size of mapping populations in grape has ranged from 46 to 181 individuals, with most maps based on a number of individuals close to 100. Such a progeny size is a good compromise, in order to avoid inflation of the cost of map production while still providing enough statistical strength for the estimation of the correct marker order and distances. It also depends on the objective of the mapping project. For instance, to quickly get a genome-wide picture of marker order for as many markers as possible, it is possible to combine mapping data from two or more populations (Di Gaspero et al. 2007; Doligez et al. 2006a), sometimes very small (46 individuals in Di Gaspero et al. 2007). This trick allowed the mapping of an extraordinarily high proportion of markers in at least one parent (91%), while only 75 and 80% of total markers would have segregated in either population separately (Di Gaspero et al. 2007). Hence, although the two mapping populations were of limited size, the number of informative meioses per marker was 46 to 184, and 130 on average, depending on the heterozygosity of the parents for each marker (Di Gaspero et al. 2007). This high number of informative meioses per marker mitigated the conflicts in finding the most likely linear order of markers (Doligez et al. 2006a; Di Gaspero et al. 2007; Vezzulli et al. 2008a). The drawbacks of this strategy will be discussed later. When the purpose of mapping is to identify a trait locus, marker orders and estimation of genetic distances can be refined around the locus, once the chromosomal region controlling a trait of interest has been identified, by adding markers designed on the homologous region in the reference genome sequence and by using additional individuals of the same or related mapping populations (Riaz et al. 2008; Bellin et al. 2009; Coleman et al. 2009; Hwang et al. 2010; Di Gaspero and Cattonaro 2010).

5.4 Mapping Algorithms and Software Used

The process of map construction can be separated into three phases: identification of groups of linked markers, ordering these markers, and estimation of the genetic distance between two adjacent markers. Of these tasks, the second is the most challenging. The ways in which these issues have been addressed in the construction of genetic maps in grapevine is discussed in the following paragraphs.

5.4.1 Individual Parental Maps

Whatever the type of marker used (dominant or co-dominant), it is possible to follow the segregation of heterozygous markers in either parent of an F_1 population like in a backcross population, and to construct two independent maps (Lodhi et al. 1995; Dalbo et al. 2000). If the population is derived from

a selfed individual, then the segregation behaves like a F_2 (Adam-Blondon et al. 2004; Duchêne et al. 2009).

The first very popular software adopted by plant geneticists and then by grape geneticists, was MapMaker (Lander et al. 1987). MapMaker calculates the probabilities of association between markers and the probabilities of marker order by the maximum likelihood approximation (Allard 1956; Lander et al. 1987). To handle the marker phase, some authors have suggested a strategy which consists of duplicating the whole set of data, inverting the allele code in the duplicated matrix, and merging the two mirror datasets. The program then constructs groups of linked markers and each group has a mirror group that is removed from the subsequent analyses. This strategy was first used in woody plants by Grattapaglia and Sederoff (1994) and in grape by Lodhi et al. (1995). Marker distances are finally calculated using functions that take into account the non-additive nature of mapping distances when they are large (Haldane 1919; Kosambi 1944).

5.4.2 Consensus Map from the Two Parental Segregations of a Cross

When a high number of co-dominant markers segregating in both parents are available, it is possible to construct a single consensus map from the genotypic dataset of the F_1 family, and the software must process heterogeneous types of segregation as described in Ritter et al. (1990). This kind of dataset cannot be handled by MapMaker. JoinMap is the first popular software that was developed for this purpose (Stam and Van Ooijen 1995; Van Ooijen and Voorrips 2001).

JoinMap automatically calculates the phase of the markers before conducting linkage analysis for full-sib families. Version 4.0 of the program includes either the classical maximum likelihood or a new Monte Carlo maximum likelihood algorithm (ML). The latter is very fast in computation and can easily make calculations from large sets of markers (100 or more) per linkage group (Van Ooijen 2006). Unfortunately it cannot be used for out-breeding full-sib families, which are the most commonly used populations in grapevine (Table 5-3). An original algorithm was developed for the calculation of distances between markers (Stam 1993). Fifteen papers report the use of different versions of JoinMap to produce linkage maps in grapevine (Table 5-3). Such popularity is likely due to the flexibility in handling different marker segregations and the user-friendly interface.

CarHTaGene is mentioned in six papers (Table 5-3) and was the second most popular software used in grapevine. TMAP was the last software to be adopted (documented in two papers listed in Table 5-3). TMAP but not CarHTaGene calculates the phase of the alleles, but both of them

can build consensus maps with or without the assumption of conserved recombination ratios across different individuals (de Givry et al. 2005; Cartwright et al. 2007). In both, marker ordering is based on a Monte Carlo algorithm to find the maximum multipoint-likelihood order with automated heuristic methods for testing the orders and managing missing data (several in CarHTaGene and one in TMAP; Scheix and Gaspin 1997; de Givry et al. 2005; Carthwright et al. 2007). CarHTaGene is the only one among the three packages managing outbred populations that gives scientists the option of choosing between different final orders. However, this package is computationally more power-demanding. TMAP is the only package that calculates a probability of error for each marker, which is accounted for in the calculation of the maximum likelihood of the map order (Cartwright et al. 2007). The efficient removal of false double recombination has been shown to reduce map inflation. Finally, from a more practical point of view, TMAP is able to read data files compatible with MapMaker, JoinMap, and CarHTaGene, and has very useful procedures for automatic transformation of the data matrix (for parental maps, consensus maps, etc.) that does not exist in the other packages.

The simultaneous use of segregation from both parents is advantageous when one wants to compile a unique map, which consists of markers alternatively heterozygous in either parent, and also wants to increase the number of informative meioses for the estimate of marker distance. In the process of integrating maps, the way in which an analyst copes with heterogeneous recombination rates and with duplicated marker loci is crucial for the correct reconstruction of marker order. Low resolution due to genotype-specific suppression of recombination in one parent is compensated by the crossovers that could have occurred in the other parent. Recombination rates are averaged over the two individuals, thus providing a more reliable expectation for any other genotype of the species. If this is desirable at a genome wide level, it may be misleading for predicting the efficiency for marker-assisted selection of alleles linked to a trait in a certain parent. Integrated maps tend to wobble in regions with a low density of common markers, in stretches of linked markers with distorted segregation in one parent, and across segmental duplications in which duplicated markers are heterozygous in the two parents at either locus.

5.4.3 Integration of Maps developed from Different Populations

As the first SSR-based genetic maps became available, they were soon merged into single consensus maps (Doligez et al. 2006a; Vezzulli et al. 2008a). In this case, the problem is handling data sets where the segregation type can be heterogeneous not only between markers, but also within markers. JoinMap, CarHTaGene, and TMAP can all handle such data. The

integrated map is constructed with different algorithms depending on the software used, which may lead to different outputs. Several papers describe the difference between the outputs of JoinMap and CarHTaGene (Scheix and Gaspin 1997; Doligez et al. 2006a).

Merging maps is very attractive because it provides a snapshot of the markers available at a given chromosomal region, but such consensus maps have some inherent weaknesses. For instance, map distances are less accurate if only a few markers are shared among individual maps, and map length is inflated by genotyping errors that add up. Also, the location of “private” markers, which are markers segregating in only one or a few parents tends to wobble, and their distance from common markers is not defined (Doligez et al. 2006a). These drawbacks are mainly due to the unbalanced number of meioses used to calculate the recombination among pairs of markers, which creates friction in the estimate of map distances. In some instances, instead of merging datasets, it would be preferable to build individual maps based on specific recombination rates, and then to align them using common markers, thus bringing markers mapped in different individuals into a unique map by a projection of distances (Arcade et al. 2004).

5.5 Different Generation of Maps in the *Vitaceae*

To date, genetic maps have been developed only in the *Vitis* genus, with several aims: QTL detection, physical map/chromosome anchoring, and map based cloning.

5.5.1 Maps Based on Anonymous Markers

The first grapevine linkage map was produced by the research group based at the Geneva Experimental Station in the United States (Lodhi et al. 1995). The mapping population was an F_1 obtained by crossing two highly heterozygous interspecific hybrids produced by the same research group, Cayuga White and Aurore, and consisted of 60 progeny. The authors were able to place 428 markers in total, mostly RAPD, and the maps resulted in 20 and 22 linkage groups, with total lengths of 1,196 and 1,477 cM, respectively (Table 5-3). The markers, being dominant, segregated from either parent and were recorded as presence/absence of amplified bands. MapMaker was adopted for the association analysis and construction of the linear series of markers along each linkage group. Lodhi’s map was developed as an exercise for showing the potential of molecular markers in rapidly saturating genetic maps, without the need for hundreds of phenotypic characters. It is mentioned here for its historical value, being the first map ever produced in grape and the first application of the pseudo-testcross method in this species.

Several other maps were developed mainly from RAPD or AFLP markers, with a progressive introduction of SSR markers as they became available from the *Vitis* Microsatellite Consortium (e.g., Doligez et al. 2002; Grando et al. 2003). The main objective of these maps was to quickly cover all of the chromosomes of both parents in order to detect QTLs for traits of interest (e.g., Doligez et al. 2002; Fischer et al. 2004; Cabezas et al. 2006; Welter et al. 2007; Costantini et al. 2008). Some single locus traits were also mapped, such as the main locus for berry color and the locus controlling flower sex (Dalbò et al. 2000; Doligez et al. 2002).

5.5.2 Maps Based on SSR and SNP Markers

The publication of the first map completely developed from SSR markers was in 2004 (Riaz et al. 2004). Many genetic maps were published later, based mainly, if not exclusively, on SSR markers (Adam-Blondon et al. 2004; Fisher et al. 2004; Mandl et al. 2006; Riaz et al. 2006; Di Gaspero et al. 2007; Welter et al. 2007). SSR markers allowed the unification of linkage group numbering (Riaz et al. 2004; Adam-Blondon et al. 2004) and the definition of sets of markers located at regular intervals, suited for comparative QTL detection, and highly heterozygous across the existing maps. Some improvement is expected in the near future by defining an additional group of markers to better cover the chromosomal extremities and a few remaining gaps (Doligez et al. 2006a). This may require new marker development, as the main problem in these areas has been the homozygosity of the parents for the markers available (Doligez et al. 2006a). The genome sequence will help to provide many targeted candidates. The grape genome is very heterozygous on average, but some regions contain homozygous blocks that are not covered by informative markers (Velasco et al. 2007). It is still unclear if localized low heterozygosity occurred specifically and by chance in the few genotypes in which it was noticed, or if it is a common feature with a biological significance in certain regions across the genotypes.

The first map predominantly based on SNP markers appeared in 2007 (Troggio et al. 2007). The authors observed a non-random distribution of the SNP markers derived from non-coding regions (BAC-end sequences), whereas SNPs derived from coding regions were randomly distributed, like SSR markers. This map is also the most dense genetic map based on a single population (average distance between two markers of 1.3 cM), with 615 transferable markers (483 SNP and 132 SSR) and 379 AFLP markers (Troggio et al. 2007). It spans a 1,245-cM genome length, which means that 1 cM would correspond to 390 kb on average, although the correlation between genetic and physical distances is variable across the genome

(Lamoureux et al. 2006; Troggio et al. 2007). A comparison between the SSR-based linkage map of linkage group 2 from Doligez (Doligez et al. 2006a) and the same linkage group based on SSR and SNP markers published by Vezzulli (Vezzulli et al. 2008a) is shown in Fig. 5-1.

Finally, SSR markers and SNPs have also allowed gene mapping in grapevine, with a strong emphasis on candidate genes for disease resistance (Di Gaspero et al. 2007; Welter et al. 2007), as well as other traits (Salmaso et al. 2008), and targeting genes has allowed the development of markers that are more transferable across species (Lowe and Walker 2006).

5.5.3 Use of Linkage Maps in Genome Sequencing Projects

Genetic maps with dense saturation and high resolution are fundamental tools in genome sequencing projects to assist the anchorage of the genome sequence to the chromosomes, and as such, the Doligez et al. (2006a) and Vezzulli et al. (2008a) integrated maps were used to anchor respectively the 8.4X assembly of the PN40024 (Jaillon et al. 2007) and the Pinot Noir (Velasco et al. 2007) genome sequences, respectively.

Recently, an improved version of the Doligez et al. (2006a) map has been produced in order to anchor and order the sequence scaffolds of the final PN40024 12X assembly, by increasing the size of two mapping populations, and by genotyping a well chosen set of markers in each of them: 358 individuals of the Chardonnay x Bianca population were scored with 379 SSR markers, and 179 individuals of the Syrah x Grenache population were scored with 325 SSR markers (unpublished data). The two consensus maps were constructed using Tmap, and the Syrah x Grenache map was projected onto the Chardonnay x Bianca population using Biomecator (Arcade et al. 2004). The resulting integrated map has 514 SSR markers, 174 of them newly developed from BAC-end sequencing and scaffold sequences, and is presented in Fig. 5-2.

While the sequence assembly of the nearly homozygous genotype PN40024 made use of genetic map information mainly for anchoring ready-to-map sequence scaffolds (Jaillon et al. 2007), the assembly of the heterozygous Pinot Noir sequence required marker information to assist the sequence assembler in the construction of metacontigs, sorting out chimeric metacontigs that merged contigs from different linkage groups (Velasco et al. 2007). The linkage map was also used to find the metacontigs position and orientation. SNP markers were then developed on sequence contigs with unassigned map position and placed back onto the genetic map (Troggio et al. 2007; Velasco et al. 2007; Vezzulli et al. 2008a).

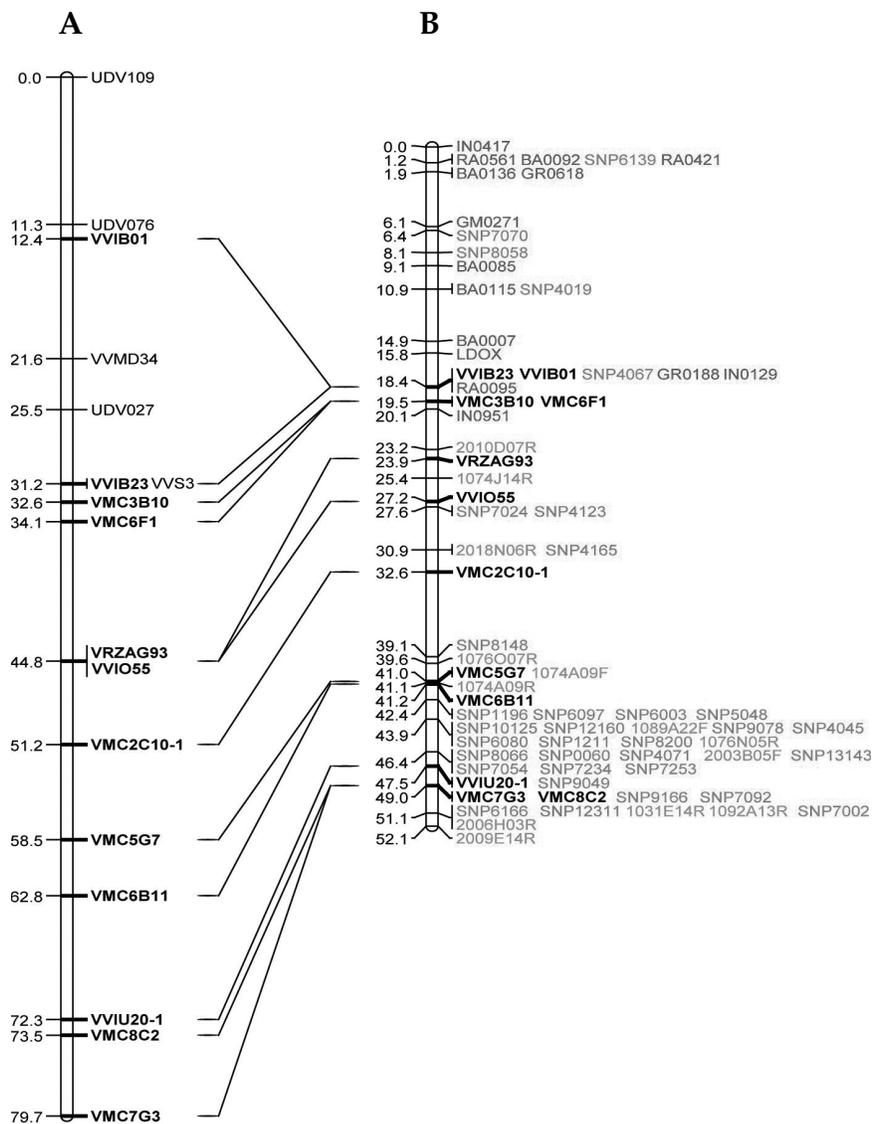


Figure 5-1 Grapevine linkage map of chromosome 2. (A) Reference map based exclusively on SSR markers (Doligez et al. 2006a); (B) the same linkage group saturated with SNP markers (Vezzulli et al. 2008a). SSR markers common to both maps are connected by lines. EST-derived SNPs are in red; BES-derived SNPs are in green (from Troglio et al. 2007); SNPs based on the heterozygous sites in the Pinot Noir sequence are in cyan (Velasco et al. 2007).

Color image of this figure appears in the color plate section at the end of the book.

5.5.4 Comparative Genetic Mapping across the Vitaceae

Local marker order and estimation of their relative distances are occasionally inconsistent across different maps, but most of the time these discrepancies involve closely linked markers or markers for which the most probable order is not clear (see Doligez et al. 2006a; Di Gaspero et al. 2007; and Vezzulli et al. 2008a for discussion). The possibility of mis-ordering due to local heterogeneity in recombination rates between individuals, to genotyping errors, to intra-chromosomal segmental duplications, or to regions with skewed segregation ratio is discussed in Doligez et al. 2006a; Lowe and Walker 2006; Di Gaspero et al. 2007; and Riaz et al. 2008. Frequently, these points of attrition are associated with markers embedded into intrachromosomal segmental duplications or regions with tandemly arrayed genes (Doligez et al. 2006a; Di Gaspero et al. 2007).

Despite small local deviations from conservation of marker order, grape genetic maps are easily compared and there is so far no evidence of major disruption of synteny either between species of the genus *Vitis* (Grando et al. 2003; Doucleff et al. 2004, Lowe et al. 2006; Welter et al. 2007; Riaz et al. 2008) or within *Vitis vinifera* (Doligez et al. 2006a; Vezzulli et al. 2008a). One region with a distorted segregation on chromosome 14 was consistently found across several populations. Riaz et al. (2008) proposed that this region might contain gametophytic factors and called it *V-SDR1* for *Vitis-Segregation Disorder Region 1*.

The main use of genetic maps is to find the chromosomal region where the genetic determinants of any qualitative or quantitative trait reside. This goal may be initially achieved with a minimal set of evenly spaced markers. Some 150–200 markers covering the entire grape genome are enough for detecting the large linkage blocks, transmitted by the parents to a biparental mapping population. Once the region of interest is identified one can select sequence scaffolds anchored by the markers of the region of interest, and develop new markers in the sequence that will be used in turn to saturate the map. The methods and issues associated to map-based cloning are discussed in Chapter 8. The annotated grapevine genome can aid in the search for genetic determinants of phenotypic traits. The two-pronged process of jumping from the genetic map to the sequence scaffolds (and back) helps to integrate the positional cloning strategy with the candidate gene approach as exemplified by the linkage maps used to find the candidate gene for terpenol biosynthesis (Battilana et al. 2008; Duchêne et al. 2009) or for the powdery mildew resistance gene at the *REN1* locus (Coleman et al. 2009).

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