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ORIGINAL ARTICLE

Introgression reshapes recombination distribution in grapevine interspecific hybrids

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Abstract

Key message **In grapevine interspecific hybrids, meiotic recombination is suppressed in homeologous regions and enhanced in homologous regions of recombined chromosomes, whereas crossover rate remains unchanged when chromosome pairs are entirely homeologous.**

Abstract *Vitis rotundifolia*, an American species related to the cultivated European grapevine *Vitis vinifera*, has a high level of resistance to several grapevine major diseases and is consequently a valuable resource for grape breeding. However, crosses between both species most often lead to very few poorly fertile hybrids. In this context, identifying genetic and genomic features that make cross-breeding between both species difficult is essential. To this end, three mapping populations were generated by pseudo-backcrosses using *V. rotundifolia* as the donor parent and several *V. vinifera* cultivars as the recurrent parents. Genotyping-by-sequencing was used to establish high-density genetic linkage maps and to determine the genetic composition of the chromosomes of each individual. A good collinearity of the SNP positions was observed between parental maps, confirming the synteny between both species, except on lower arm of chromosome 7. Interestingly, recombination rate in *V. rotundifolia* × *V. vinifera* interspecific hybrids depends on the length of the introgressed region. It is similar to grapevine for chromosome pairs entirely homeologous. Conversely, for chromosome pairs partly homeologous, recombination is suppressed in the homeologous regions, whereas it is enhanced in the homologous ones. This balance leads to the conservation of the total genetic length of each chromosome between *V. vinifera* and hybrid maps, whatever the backcross level and the proportion of homeologous region. Altogether, these results provide new insight to optimize the use of *V. rotundifolia* in grape breeding and, more generally, to improve the introgression of gene of interest from wild species related to crops.

Introduction

Wild species related to crops have provided plant breeders with a wide range of genetic resources which have contributed to the emergence of modern agriculture and to the agricultural economy worldwide. Wild relatives have proved useful in breeding for different traits, including abiotic stress tolerance, yield, quality, male sterility. Among these traits, pest and disease resistance account for 80% of the cases where genes derived from wild relatives have been transferred to cultivated species over the last century (Hajjar and Hodgkin 2007). Despite the undeniable importance of wild species in crop improvement for disease resistance, breeders have often faced the challenge of obtaining viable and fertile interspecific hybrids, which has consequently limited the optimal use of wild genetic resources (Brar and Khush 1997; Atlagić 2004; Bradshaw and Ramsay 2005; Garg et al. 2007; Hajjar and Hodgkin 2007; Wulff and Moscou 2014; Singh and Nelson 2015).

Grapevine is a crop particularly threatened by many severe pests and diseases. Indeed, from the nineteenth century, European viticulture has undergone radical changes as a result of the introduction of major pests and diseases from North America: powdery mildew, downy mildew and

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phylloxera, respectively, caused by the fungus *Erysiphe necator*, the oomycete *Plasmopara viticola* and the insect *Daktulosphaira vitifoliae*. Almost all European grapevine cultivars (*Vitis vinifera* ssp. *sativa*) are susceptible to these pathogens. Many sources of resistance were found in Asian and American wild *Vitis* species (Boubals 1966; Cadle-Davidson 2008; Cadle-Davidson et al. 2011), and several genetic factors conferring protection against downy mildew, powdery mildew and phylloxera have been recently identified (Marguerit et al. 2009; Zhang et al. 2009; Blasi et al. 2011; Riaz et al. 2011; Schwander et al. 2012; Pap et al. 2016). Aside from the *Vitis* species that belong to the *Vitis* subgenus (also called true *Vitis*), *Vitis rotundifolia* is a North American grapevine which belongs to the *Muscadinia* subgenus. *V. rotundifolia* has the unique feature of displaying a high resistance level to many aggressive bio-agents affecting grapevine: *E. necator*, *P. viticola* and *D. vitifoliae* but also *Guignardia bidwellii*, the fungus responsible for black-rot disease, *Xiphinema index*, the nematode vector of grapevine fanleaf virus and *Xylella fastidiosa*, the bacteria responsible for Pierce's disease (Bouquet 1981; Olmo 1986; Goldy and Onokpise 2001; Ruel and Walker 2006).

Vitis rotundifolia being particularly interesting for grape breeding, crosses with *V. vinifera* were attempted as soon as the end of the nineteenth century and a first success was obtained by Detjen (1919). In the 1980s, successful crosses produced fertile hybrids and allowed the use of *V. rotundifolia* in French breeding programmes (Bouquet 1980). Backcross-based introgressions were attempted to transfer resistance factors from *V. rotundifolia* into cultivated backgrounds while eliminating the unwanted cultural traits and off-flavours. Meanwhile, genetic factors conferring resistance to powdery and downy mildew were identified and molecular makers allowing marker-assisted selection were developed (Pauquet et al. 2001; Merdinoglu et al. 2003; Barker et al. 2005; Wiedemann-Merdinoglu et al. 2006; Riaz et al. 2011; Blanc et al. 2012; Feechan et al. 2013). However, the poor vigour and low fertility of the *V. vinifera* × *V. rotundifolia* hybrids and backcrosses hampered a broader use of *V. rotundifolia* in breeding programmes. The low rate of successful crosses and the sterility of interspecific hybrids were first attributed to the difference in chromosome numbers between both species, $2n = 38$ chromosomes for *V. vinifera* versus $2n = 40$ for *V. rotundifolia*. Cytogenetic studies showed that some mismatches between chromosomes of the same pair occur during meiosis of F1 hybrids and of individuals derived from the first pseudo-backcross (Patel and Olmo 1955; Jelenkovic and Olmo 1968). But the small size of the chromosomes did not allow to determine which ones were really involved in these mismatches. Blanc et al. (2012) established a genetic linkage map of *V. rotundifolia* using SSR markers that showed a high level of macrosynteny with *V. vinifera*. The same work also revealed that chromosome 7 of *V. vinifera* matches with chromosomes 7 and 20 of *V. rotundifolia*, thus explaining the origin of the difference of chromosome number between both species. Although the higher genetic distance existing between *Vitis* and *Muscadinia* compared to the genetic distance between *Vitis* subgenus species (Wan et al. 2013) could explain the low prolificity and fertility of interspecific crosses, the underlying mechanisms are still unknown.

In order to understand the compatibility between the genomes of *V. rotundifolia* and *V. vinifera,* we decided to study the recombination rate between homeologous chromosome pairs (i.e. composed of one chromosome from *V. vinifera* and one from *V. rotundifolia*) in interspecific hybrids. Our study is based upon pseudo-F1 hybrids derived from crosses between *V. rotundifolia* and *V. vinifera,* and pseudo-backcross populations derived from successive crosses between pseudo-F1 hybrids and various *V. vinifera* cultivars. The genetic maps previously developed with these populations were mainly built thanks to microsatellite markers and did not offer an adequate marker density for a fine analysis of recombination rate along the chromosomes (Merdinoglu et al. 2003; Wiedemann-Merdinoglu et al. 2006). Genotyping-by-sequencing (GBS) appears to be a simple and robust method allowing the discovery of thousands of SNPs for hundreds of individuals rapidly and at low cost (Elshire et al. 2011). Here, we use GBS to perform a fine-scale analysis of the recombination rate along the genome in various interspecific contexts represented by three pseudo-backcross populations derived from *V. rotundifolia* and *V. vinifera* at different level of introgression.

Materials and methods

Plant material

Three pseudo-backcross (BC) populations derived from two sources of *V. rotundifolia* were used in this study (Fig. 1). A BC1 mapping population of 177 individuals was obtained from the cross between 8624 ((*V. vinifera* cv. Carignan × *V. vinifera* cv. Cabernet Sauvignon) × *V. rotundifolia* cv. Trayshed) as female parent and *V. vinifera* cv. Cabernet Sauvignon as pollen donor. A BC4 mapping population of 79 individuals, derived from the BC1 by three consecutive pseudo-backcrosses, was then obtained from the cross between 1771P ((BC1 × *V. vinifera* cv. Barbera) × *V. vinifera* cv. Tempranillo) and *V. vinifera* cv. Nebbiolo. A BC2 mapping population of 248 individuals was obtained from a cross between 22-8-78 ([(*V. vinifera* cv. Cabernet Sauvignon × *V. vinifera* cv. Alicante Bouschet) × *V. rotundifolia* cv. NC184-4] × [(*V. vinifera* cv. Mourvèdre × *V. vinifera* cv. Cabernet Sauvignon) × *V. vinifera* cv. Pinot meunier]) as female parent and *V. vinifera* cv. Syrah as pollen donor.

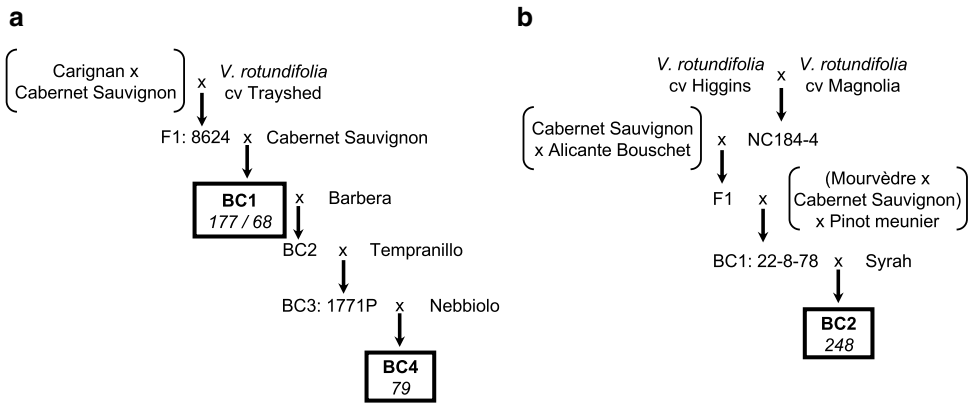

Fig. 1 Pedigrees of the **a** BC1, BC4 and **b** BC2 populations. Cultivar and accession names are in normal font, population names in bold and population sizes in italics. For BC1 population, number 177 corresponds to the initial number of individuals available for SSR genotyping, whereas 68 is the number of individuals available for GBS analysis

SSR marker and GBS analyses

Genomic DNA was extracted from 80 mg of young expanding leaves using the Qiagen DNeasy® 96 Plant Kit (Qiagen S.A., Courtaboeuf, France) as described by the supplier. Microsatellite (SSR) analysis was performed as described in Blasi et al. (2011). DNA samples following quality prerequisites were sent to the Genomic Diversity Facility at the Institute of Biotechnology of Cornell University (Ithaca, NY, USA) where GBS analysis, sequence alignment to *V. vinifera*'s reference genome (PN40024 12Xv2; https://urgi.versailles.inra.fr/jbrowse/gmod_jbrowse/?data=myData%2FVitis%2Fdata_gff&loc=chr1%3A9694432.14541139&tracks=Vitis%20vinifera%20cv.PN40024%20assembly%2012XV2%2CCRIBI_V1%2CREPET_TE%2Cscaffolds%2CSNP_Discovery_Vitis_vinifera&highlight=) and SNP calling were achieved. The reference-based pipeline used for bioinformatics analysis was the "discovery" pipeline described in TASSEL 3.0 documentation and in Glaubitz et al. (2014). The number of individuals analysed in each BC population by SSR and GBS is given in Table S1.

Chromosome painting

The aim of chromosome painting is to identify introgressed regions remaining in each BC individual thanks to SNPs detected in *V. rotundifolia*. Illumina resequencing data were available for seven *V. rotundifolia* accessions: Trayshed, Fry, Carlos, Dulcet, Regale, Yuga × Carlos and 8085Mtp1 (https://bioweb.supagro.inra.fr/collections_vigne/Home.php). Resequencing datasets were deposited under https://doi.org/10.15454/1.5009976984187844e12 and are available through the following web page: https://urgi.versailles.inra.fr/Projects/Achieved-projects/Muscares. They will be made available through the EMBL database for final publication. For BC1 and BC4 populations, both derived from Trayshed, chromosome painting was performed with the Trayshed data. *V. rotundifolia* parent of the BC2 population being not available, resequencing data from all the seven *V. rotundifolia* accessions were used for the analysis.

First, SNPs from *V. rotundifolia* that could be found in the offspring were determined. Illumina reads from the seven accessions of *V. rotundifolia* were aligned against the *V. vinifera* reference genome (PN40024 12Xv2), using the alignment GSNAP software (Wu and Nacu 2010). We performed the analysis using the following parameters: –batch = 4, –npaths = 3, –max-mismatches = 12. Reads were then parsed with an edit distance of 15, and we retained only the alignments that were paired and that matched with a unique location on the reference genome. SNPs from these reads were called, using the default parameters for SAMtools mpileup (Li et al. 2009), followed by bcftools, as described on the SAMtools webpage (http://samtools.sourceforge.net/mpileup.shtml) and with the following settings: –multiallelic-caller, –skip-variants = Indel and –pval-threshold = 1. SNP calling for *V. rotundifolia* accessions was performed at the same positions than those provided in the GBS vcf file, and a vcf file was generated for each accession. Data were then cleaned for their quality: QUAL score lower than 30, DP score lower than 4 or higher than 25 were considered as missing data. A pool of *V. rotundifolia* SNPs was established using each position with at least one alternative allele in Trayshed, for BC1 and BC4, or in one of the 7 cultivars, for the BC2.

Second, SNPs from *V. rotundifolia* identified in the previous step were searched in the offspring. After eliminating the positions with missing data, SNPs that were actually present were determined for each individual of each population. These SNPs were heterozygous and had one allele from *V. rotundifolia*.

Finally, a rate of the SNPs coming from *V. rotundifolia* was calculated on sliding windows of 20 consecutive SNPs as:

$$\tau_i = \frac{Nr_i}{Np_i}$$

where τ_i was the rate of SNPs coming from *V. rotundifolia* for the window i, Np_i the potential number of SNPs from *V.*

rotundifolia that could be found in the window i, Nr_i the real number of SNPs from *V. rotundifolia* detected in the window i. The sliding window was moved one SNP at a time. A threshold of τ_i beyond which the region was assigned to *V. rotundifolia*, was fitted for each population according to the BC level and the quality of the data to balance the chance to detect a false homeologous region and the chance not to detect a true homeologous region.

Genetic mapping

SNP markers obtained by GBS were filtered to only keep the most informative and reliable markers. The first step consisted in cleaning the data. For each genotype, SNPs with read depth (DP) lower than 4 or allelic depths (AD) for the reference and alternate alleles lower than 2 were set as missing data.

Then, for each population of backcross, SNP markers were eliminated if: (1) they had more than 5% of missing data in the population, (2) their segregation in the population was strictly identical to the segregation of another SNP, (3) the origin of their segregation was unknown because genotypes of both parents were missing, (4) they did not segregate in the population because both parents were homozygous. The goodness-of-fit between observed and expected Mendelian ratios was analysed for each marker locus using a χ^2 test. Markers showing segregation distortion (p value < 0.05) were not included in the final maps. The same filters were applied to SSR markers.

Parental genetic linkage maps were established for each population. We distinguish the maps of the F1, BC1 and BC3 parents, called hybrid maps from the maps of the *V. vinifera* parents, called *V. vinifera* maps. Distribution of markers in linkage groups and phase analysis was performed with JoinMap 3.0 (Van Ooijen and Voorrips 2001). The threshold value of logarithm of odd (LOD) score was set at 3.0 to claim linkage between markers. Linkage analysis was then performed with the R/qtl package of the R software (version 3.3.2, © 2016 The R Foundation for Statistical Computing) (Broman et al. 2003). Linkage groups were numbered according to the reference genome (Jaillon et al. 2007).

A coverage rate was calculated for each chromosome as the ratio of physical distance between distal mapped markers on the total physical chromosome length. A contraction rate, allowing to link genetic distance and physical size, was also calculated per chromosome as:

$$C = \frac{D_g}{D_p} \times 300{,}000$$

where D_g is the genetic distance in cM between the two distal markers, D_p the physical distance in bp between the same two markers and 300,000 an arbitrary conversion factor between centimorgans and base pairs. Calculated in this way, the contraction rate of the framework reference linkage map published by Doligez et al. (2006) is 0.9.

Results

Chromosome painting

Resequencing and GBS data were used to locate introgressed regions remaining in each BC individual. GBS was performed on 357 individuals belonging to three mapping populations derived from backcrosses between *V. rotundifolia* and *V. vinifera* (BC1, BC2 and BC4, Table S1), leading to the detection of 263,034 SNPs. In parallel, in order to identify *V. rotundifolia*-specific regions, we searched for *V. rotundifolia*-specific SNPs using sequencing data available for seven accessions of *V. rotundifolia.* Finally, we selected for *V. rotundifolia*-specific SNPs that were identified as polymorphic in the GBS data. Out of the 263,034 SNPs detected by GBS, 91,865 SNPs were found in *V. rotundifolia* cv. Trayshed, which is the accession at the origin of BC1 and BC4 populations, and 110,815 SNPs in the seven accessions of *V. rotundifolia* altogether.

To perform chromosome painting, a rate of SNPs coming from *V. rotundifolia*, τ_i, was calculated as the ratio of the number of detected SNPs from *V. rotundifolia* on those potentially found, using sliding windows of 20 SNPs. A threshold was set to balance the chance to detect a false homeologous region and the chance not to detect a true homeologous region. When the rate was above the threshold, the region was identified as homeologous (*V. vinifera*–*V. rotundifolia*). In the F1 hybrid, the threshold was set to 0.6, the highest value which allowed to detect the entire genome as homeologous, as expected. For the BC1 and BC4 populations, analysis was performed using the 91,865 SNPs from Trayshed and a τ_i threshold of 0.6. Because of a lack of information on the *V. rotundifolia* parent of the BC2 population, analysis was performed using the 110,815 SNPs set and the τ_i threshold was set to 0.5.

In individual 1771P, parent of BC4 population, five regions derived from *V. rotundifolia* were identified, on chromosomes 8, 13, 14, 18 and 19 (Fig. 2a). Analysis in the BC4 population revealed the recombination events taking place in homeologous regions. (Table 1). In chromosomes 8, 18 and 19, which bore a large *V. rotundifolia* region covering over 80% of the chromosome length, respectively, 15%, 32% and 13% of the individuals showed a recombination event in these homeologous regions. Conversely, in chromosomes 13 and 14, carrying a smaller *V. rotundifolia* region covering, respectively, 24.7% and 30.3% of the chromosome length,

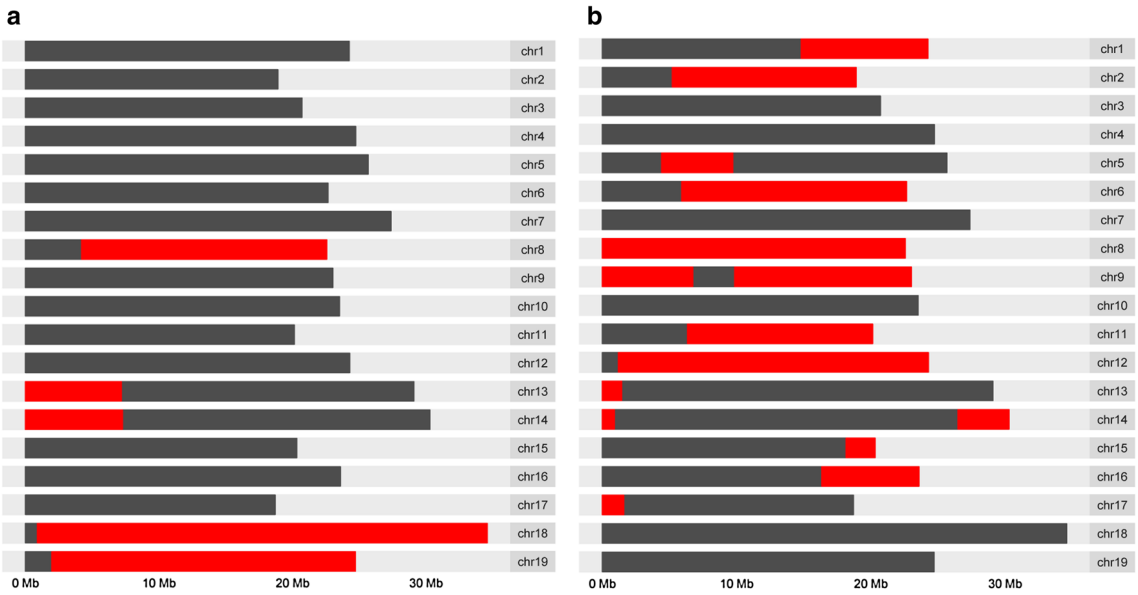

Fig. 2 Chromosome painting of parental hybrids 1771P and 22-8-78. Chromosomes are horizontally represented with their physical length on the *x*-axis. Homeologous regions (*V. vinifera*–*V. rotundifolia*) are in red; homologous regions (*V. vinifera*–*V. vinifera*) are in dark grey. **a** Individual 1771P, hybrid parent of the BC4 population. **b** Individual 22-8-78, hybrid parent of BC2 population (colour figure online)

Table 1 Relative length of homeologous regions of 1771P parent and recombination in BC4 population

Chromosome	CL (Mb)	LHr (Mb)	PHr (%)	IR1
8	22.55	18.34	81.3	12
13	29.08	7.17	24.7	1
14	30.27	7.25	30.3	1
18	34.57	33.66	97.4	25
19	24.70	22.71	92.0	10

For each chromosome, the proportion of homeologous regions (PHr) is calculated as the ratio between the length of the homeologous region (LHr) and the chromosome length (CL). IR1: number of individuals having one recombination event in the homeologous region. Population size: 79 individuals

Table 2 Relative length of homeologous regions of 22-8-78 parent and recombination in BC2 population

Chromosome	CL (Mb)	LHr (Mb)	PHr (%)	IR1	IR2
1	24.23	9.42	30.4	10	0
2	18.89	13.67	54.6	0	0
5	25.65	5.29	24.4	2	0
6	22.65	16.73	66.9	10	0
8	22.55	22.55	100.0	98	3
9	23.01	19.87	86.4	62	0
11	20.12	13.77	68.4	3	0
12	24.27	23.07	95.1	40	1
13	29.08	14.30	4.9	0	0
14	30.27	4.66	15.4	1	0
15	20.30	2.14	10.6	0	0
16	23.57	7.22	30.6	1	0
17	18.69	1.58	8.5	0	0

For each chromosome, the proportion of homeologous regions (PHr) is calculated as the ratio between the length of the homeologous regions (LHr) and the chromosome length (CL). IR1–IR2: number of individuals having one recombination event (IR1) or more (IR2) in the homeologous region. Population size: 210 individuals

only one individual out of 79 displayed a recombination event in these homeologous regions.

In individual 22-8-78, parent of BC2 population, regions from *V. rotundifolia* were identified on 13 out of 19 chromosomes (Fig. 2b). Chromosomes 3, 4, 7, 10, 18 and 19 derived entirely from *V. vinifera*, whereas chromosomes 8, 9 and 12 were almost totally from *V. rotundifolia*. Interestingly, chromosome 14 carried two distal telomeric regions derived from *V. rotundifolia*, revealing two likely crossovers for the meiosis of the corresponding pseudo-F1 parent. Analysis in the BC2 population revealed the recombination patterns in the homeologous regions (Table 2). For chromosomes 8, 9 and 12, characterized by a homeologous region covering over 85% of the chromosome, a substantial number of recombination events were observed, with, respectively, 47%, 29% and 19% of the individuals displaying at least one or more crossovers. Conversely, for chromosomes carrying small homeologous regions, i.e. covering less than 25% of the chromosome length, less than 1% of the individuals displayed a recombination event. Finally, in cases where the homeologous region covered between 25 and 85% of the chromosome, the number of recombination events

observed was variable (0–5% of the individuals depending on the chromosome) but never high.

Genetic mapping

The 263,034 SNPs detected by GBS were filtered as follows. Only SNPs having less than 5% of missing data were kept. Next, SNPs showing redundancy (providing the same information for genetic mapping) were discarded. Non-redundant SNPs whose parental origin could not be clearly determined and those that were homozygous in both parents were also removed. Finally, SNPs showing a distorted segregation were discarded. After performing all the filtering steps, 3415, 2210 and 2222 SNPs were available for genetic mapping for BC1, BC2 and BC4 populations, respectively.

Table 3 SNP filtering for genetic mapping

Filtering steps	Mapping populations		
	BC1	BC2	BC4
Total SNPs detected	263,034		
SNPs ≤ 5% missing data	9006	9132	8489
Non-redundant SNPs	5396	6677	4068
SNPs with known parental genotype	5266	6460	3868
SNPs with at least one heterozygous parent	4270	2943	2427
SNPs with segregation distortion	855	733	205
SNPs available for genetic mapping	3415	2210	2222

Number of SNP markers after each filtering step for each population. SNPs with segregation distortion were removed for genetic mapping but not for calculation of segregation distortion represented in Fig. S2

Results of the different filtering steps are presented in Table 3.

Parental genetic linkage maps were established for the BC1, BC2 and BC4 populations using the above-described SNPs and, respectively, 87, 31 and 106 SSR markers. The number of mapped markers depended on the parent types and the backcross levels (Table 4). Overall, hybrid maps showed a higher number of mapped markers than *V. vinifera* maps. The genetic distance covered was quite similar between maps, from 1153.7 cM (1771P) to 1455.8 cM (Cabernet Sauvignon), regardless of the parent and the population and was not correlated to the number of mapped markers. The average physical spacing between two successive markers was rather low, especially for hybrid maps which displayed a density of one marker per 0.17–0.32 Mbp. Despite the overall high marker density, some local gaps devoid of markers were observed (Fig. S1).

A coverage rate was calculated for each chromosome as the ratio of physical distance between distal mapped markers on the total physical chromosome length. The coverage rates were high, varying from 88 to 96% (Table 4), but singularities were identified. Chromosomes 7, 8 and 12 of BC1 hybrid map and chromosome 8 of BC2 hybrid map were entirely covered (Table S2). Conversely, chromosome 8 of BC2 *V. vinifera* map and chromosome 11 of BC2 hybrid map were poorly covered with, respectively, 22% and 11% coverage. Despite quite high covering rates, some chromosomes show badly covered regions: chromosomes 1, 11, 13 and 15 of the BC1 hybrid map, chromosomes 6 and 16 of the BC2 *V. vinifera* map and chromosomes 1 and 6 of the BC2 hybrid map (Fig. S1). Low coverage rates have two main origins: (i) a low density of SNPs detected in the corresponding

Table 4 Statistics for parental maps of BC1, BC2 and BC4 populations

Mapping population	BC1		BC2		BC4	
Population size	68		248		79	
Parent	♂ Cabernet Sauvignon	♀ 8624	♂ Syrah	♀ 22-8-78	♂ Nebbiolo	♀ 1771P
Number of mapped markers	927	2860	996	1516	1177	1521
Total length of the map (cM)	1455.8	1325.3	1242.8	1272.1	1368.5	1153.7
Average spacing between single genetic positions (cM)	2.0 (0.9–4.9)	1.6 (0.3–21.2)	1.8 (0.9–7.5)	1.1 (0.3–2.8)	1.2 (0.8–1.8)	1.0 (0.3–1.7)
Average spacing between markers (Mbp)	0.46 (0.25–1.32)	0.15 (0.09–5.25)	0.41 (0.28–2.66)	0.27 (0.13–0.54)	0.37 (0.28–0.59)	0.28 (0.13–0.52)
Extrema of the maximum spacing between markers per chromosome (cM)	6.2–32	3.0–43.9	5.4–18.6	3.5–57.5	5.5–41.9	5.4–47.4
Coverage rate	0.93 (0.79–0.98)	0.96 (0.75–1.00)	0.88 (0.22–0.99)	0.89 (0.11–1.00)	0.95 (0.78–0.99)	0.94 (0.79–0.99)
Contraction rate	1.02 (0.69–1.45)	0.90 (0.62–1.18)	0.85 (0.43–1.19)	0.93 (0.63–1.95)	0.94 (0.76–1.41)	0.80 (0.52–1.39)

Minimum and maximum values observed in individual chromosomes are shown in brackets. Grey background indicates *V. vinifera* maps and white background hybrid maps. A single genetic position is defined as a genetic position at which several markers co-localize

regions, e.g. chromosome 6 of both parental maps of the BC2 population, (ii) a segregation distortion which led us to eliminate markers from mapping, especially chromosome 13 in the BC1 population and chromosomes 8 and 11 in the BC2 population (Fig. S2).

A contraction rate was calculated for each chromosome as the ratio of genetic distance and physical distance between the two distal markers. The *V. vinifera* reference map has a calculated contraction rate of 0.9 (Doligez et al. 2006). Accordingly, a contraction rate above 0.9 means that recombination rate is higher than observed in the grapevine reference map. No difference was observed in average contraction rate between *V. vinifera* and hybrid maps, both reaching a mean value (0.93) very close to the reference linkage map (Table 4). Most chromosomes displayed a contraction rate between 0.8 and 1.0 in most populations, close to the reference, but some particular cases were observed. For instance, chromosome 14 was contracted at different levels in the majority of parental maps, either hybrid or *V. vinifera*. Chromosome 9 was contracted in the hybrid maps, especially in the BC1 and BC2 populations with contraction rates of 0.69 and 0.63, respectively. On the other side, chromosome 7 was dilated in all the *V. vinifera* maps, with contraction rates of 1.45, 1.14 and 1.41 for BC1, BC2 and BC4 populations, respectively (Table S3).

Transmission rate of *V. rotundifolia* alleles

To analyse the bias of transmission of *V. rotundifolia* genome in homeologous regions, a transmission rate was calculated for each SNP as the percentage of individuals carrying the *V. rotundifolia* allele of its hybrid parent, for each population. As expected, transmission rates were around 0.5 in the majority of regions (Fig. 3). However, some regions showed an unbalanced transmission. For instance, in BC1 population, alleles from *V. rotundifolia* were transmitted in 62% of the offspring on chromosome 1, whereas, on chromosome 11, they were transmitted in only 39% of the offspring. The same pattern of segregation distortion was observed in the BC2 population, with alleles from *V. rotundifolia* being conserved in 68% of the individuals on chromosome 1 and in 17% of the individuals on chromosome 11. A second example is the upper part of the chromosome 13, where alleles from *V. rotundifolia* were present in near than 80% of the BC1 individuals. It is worth noting that the segregation distortion was not always in favour of the *V. vinifera* allele.

Relationship between recombination distribution and level of introgression

The study of recombination showed different behaviours depending on the population.

Both maps of BC1 population showed a high level of collinearity between genetic and physical maps for all chromosomes (Fig. S1). In the BC1 population, recombination rate was overall similar between both parents, with some exceptions like chromosome 7, which displayed a fall in recombination rate in its lower half (Fig. 4a).

In the BC2 population, changes in terms of recombination rates were observed depending on chromosomes. Consequently, marker order was not conserved between BC2 maps in the region where the recombination rate was abnormally low in the hybrid map. In chromosomes completely homologous or homeologous, recombination rates were similar in both parents, like for instance chromosome 4, which is entirely homologous (Fig. 4b), and chromosome 8, which is entirely homeologous. Chromosomes that are partially homeologous showed a particular pattern: the hybrid map revealed a higher recombination rate than the *V. vinifera* map in homologous regions, whereas homeologous regions displayed a low recombination rate or, even, did not recombine at all (see chromosomes 2, 12 and 14, Fig. 4b). It is worth noting that both parent maps have a similar total genetic length.

The BC4 population showed the same pattern of recombination as observed in the BC2 population not necessarily in the same chromosomes (Fig. 4c). For instance, variations in recombination rate along chromosome 5 were similar between both parents. On the contrary, chromosome 14 showed a lower recombination rate on hybrid map than on *V. vinifera* map in the homeologous region of the chromosome and a reversal of trend in the homologous part of the chromosome.

To confirm this observation, the relationship between the recombination fractions and the nature of regions, homologous or homeologous, was studied at the genome scale: as a general rule, low recombination regions matched homeologous regions on the BC2 and BC4 hybrid maps on chromosomes partially homeologous (Fig. 5).

Discussion

Although *V. rotundifolia* is highly resistant to numerous grapevine diseases, its use in breeding programmes has been limited due to the difficulty to obtain fertile progeny in interspecific crosses. In our study, we used three populations of different level of pseudo-backcross to establish high-density genetic linkage maps of the parents of the populations, allowing studying marker order, segregation distortion and recombination rates across the genome and in introgressed regions. The heterogeneity of population sizes between the three examined generations (Fig. 1; Table S1) and, especially, the lower number of recombination events sampled in BC1 and BC4 could have affected the genetic

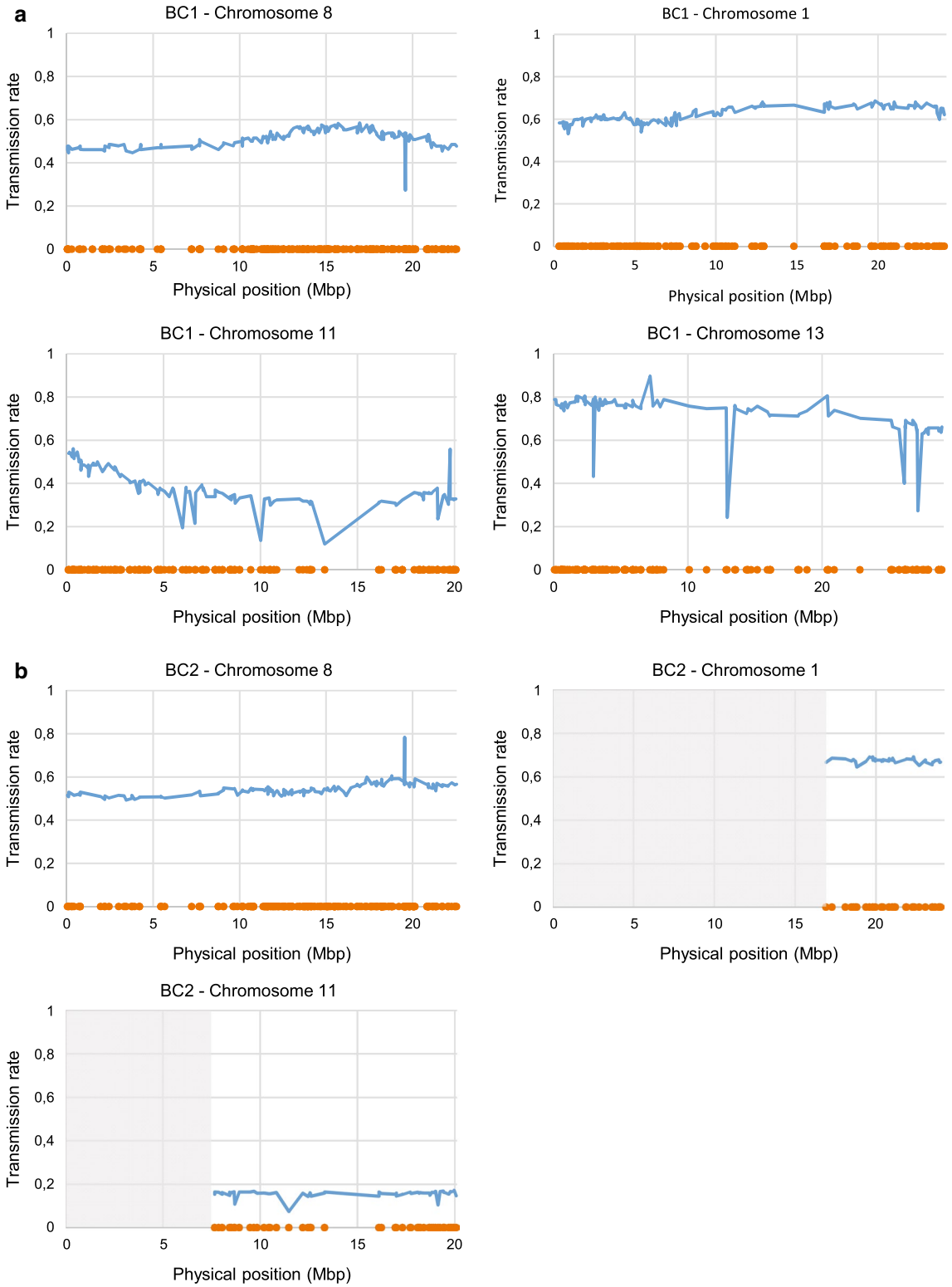

Fig. 3 Transmission rate of *V. rotundifolia* SNPs in the BC populations. The transmission rate is calculated for each SNP as the percentage of individuals in a population harbouring the allele from *V. rotundifolia*. **a** Chromosomes 8, 1, 11 and 13 of BC1 population. **b** Chromosomes 8, 1 and 11 of BC2 population. Dots on the *x*-axis show the positions of SNPs used to calculate transmission rate. Homologous regions, lacking *V. rotundifolia* SNPs, are represented with a grey background

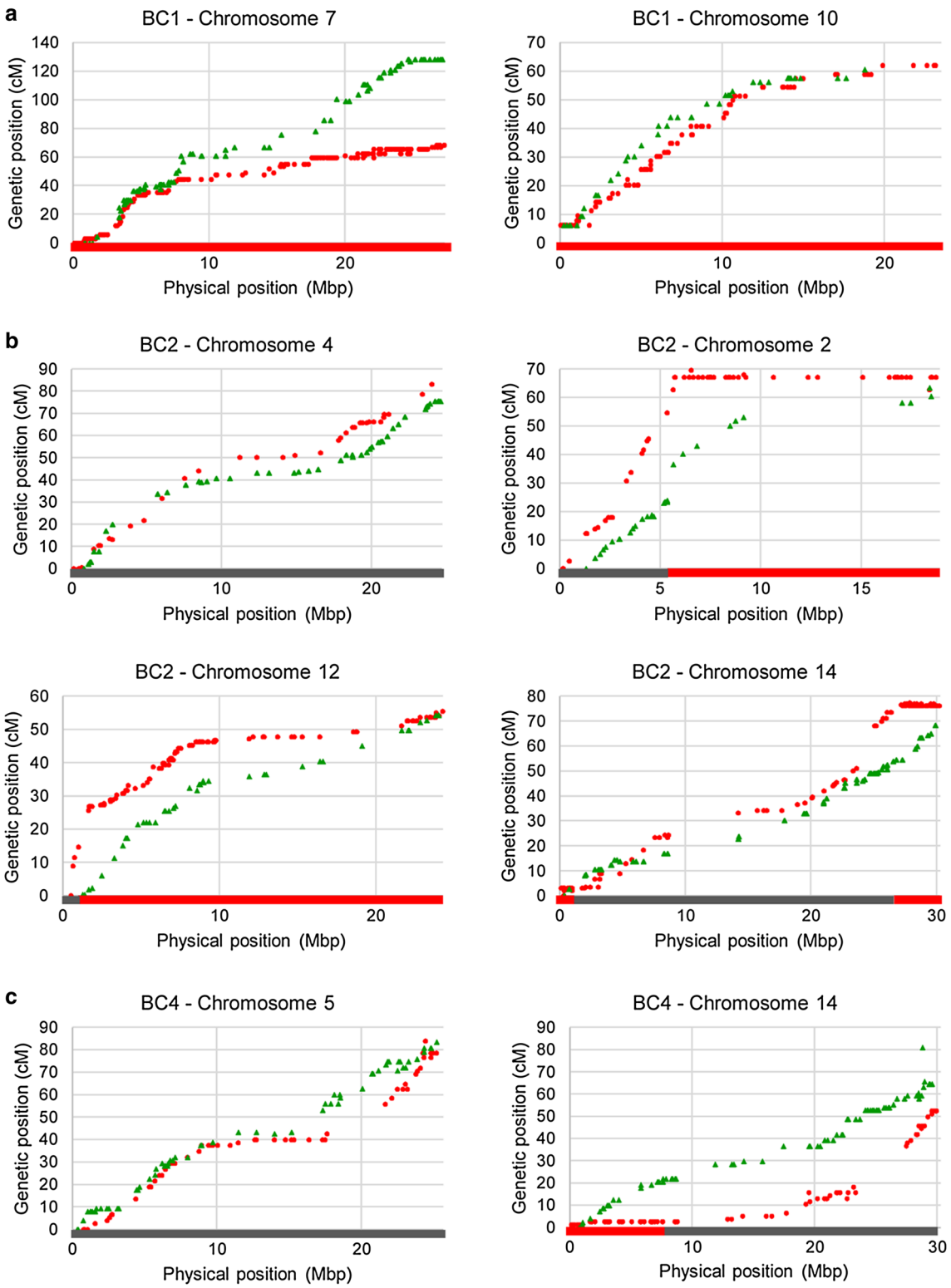

Fig. 4 Analysis of recombination rate in homologous versus homeologous regions. Variation of genetic distance depends on physical distance on the hybrid and *V. vinifera* maps. Recombination rate is represented by the slope of the plots. For each cross, *V. vinifera* maps are represented with green triangles, and hybrid maps with red dots. Bars below the graphs show the regions covered by homeologous pairs of chromosomes (in red) and homologous regions (in dark grey) in the hybrid parent of each population. Gaps in the graphs correspond to regions lacking SNPs. **a** Chromosomes 7 and 10 of the BC1 population. **b** Chromosomes 4, 8, 2, 12 and 14 of the BC2 population. **c** Chromosomes 5 and 14 of the BC4 population (colour figure online)

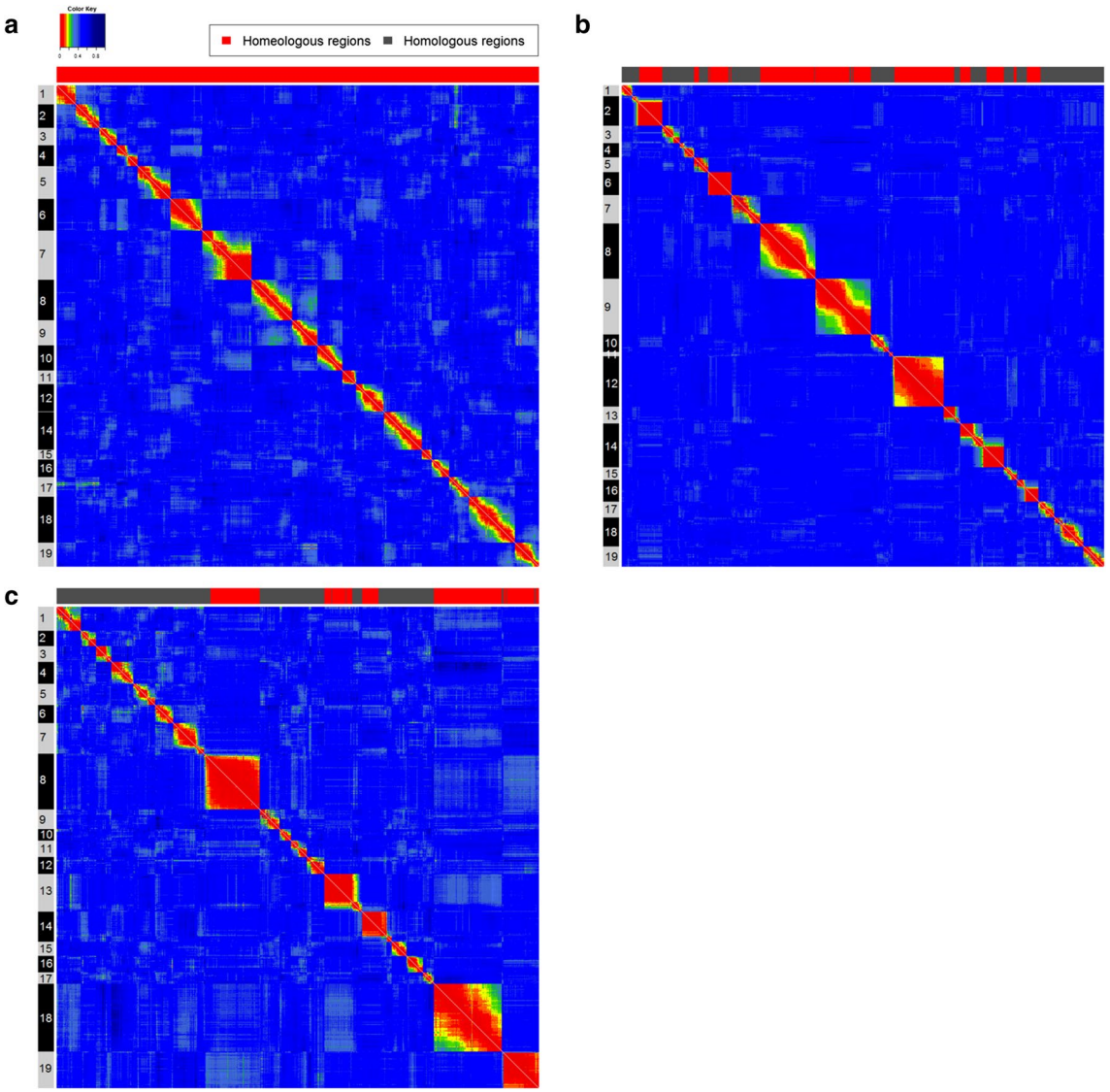

Fig. 5 Genome-wide analysis of recombination rates. Plot of estimated recombination fractions for all pairs of markers of the BC1 (**a**), BC2 (**b**) and BC4 (**c**) hybrid genetic maps. Markers are plotted ordered on the *x*-axis and on the *y*-axis. Recombination fraction is plotted as a colour scale. Red indicates that the two markers, respectively, on *x*-and *y*-axis, are highly linked (low recombination fraction), and blue indicates that the markers are poorly or not linked (high recombination fraction). Chromosome limits are identified at the left of the heatmap. Homeologous and homologous regions are identified at the top (colour figure online)

mapping precision and, more generally, the conclusions of this study. Despite this potential limit, we observed a high level of collinearity, at the same time, between genetic and physical maps, but also, between the different parental maps, which makes the information acquired throughout this study consistent and conclusive.

Synteny and divergence between *V. vinifera* and *V. rotundifolia* genomes

GBS allowed detecting SNPs all over the genome in the three crosses analysed. SNPs were identified aligning sequences against the *V. vinifera* reference genome (PN40024 12Xv2). More SNPs were mapped in the hybrid maps than in the *V. vinifera* ones (Table 4), and homeologous regions were better covered than homologous ones (Fig. S1). As expected, the more advanced is the backcross, the lower is the number of detected SNPs, and the higher is the homozygosity. All these observations confirm that sequences are close enough to allow alignment of *V. rotundifolia* reads against the *V. vinifera* genome and show that the divergence between *V. rotundifolia* and *V. vinifera* genomes leads to the identification of higher number of SNPs in homeologous regions.

The comparison of marker order with their physical position in the hybrid map and in the *V. vinifera* linkage map of the BC1 population confirms the high level of macrosynteny between *V. vinifera* and *V. rotundifolia* genomes already observed by Blanc et al. (2012). Moreover, no translocation or chromosomal inversion was identified.

Our genetic linkage maps allowed establishing 19 linkage groups. This number is consistent with the 19 pairs of chromosomes of *V. vinifera* and not with the 20 pairs of chromosomes of *V. rotundifolia*. Cytogenetic studies showed that the F1 hybrid carries 39 chromosomes, 19 inherited from *V. vinifera* and 20 inherited from *V. rotundifolia* (Patel and Olmo 1955). Blanc et al. (2012) showed that linkage groups 7 and 20 of *V. rotundifolia* match, respectively, the upper and lower arms of chromosome 7 of *V. vinifera*. We showed that allele segregation of chromosome 7 fitted the expected Mendelian ratios. This signifies that, during F1 meiosis, chromosomes 7 and 20 from *V. rotundifolia* and chromosome 7 from *V. vinifera* are equally distributed in the gametes. In addition, recombination rate on the upper part of the chromosome 7 of the BC1 hybrid map was similar to the BC1 *V. vinifera* map, while in the lower part of the same chromosome, the recombination rate in the hybrid map was extremely low compared to the *V. vinifera* map (Fig. 4a). Consequently, we hypothesize that, during meiosis of fertile F1 hybrid, the number of crossovers between *V. rotundifolia* chromosome 7 and the upper part of *V. vinifera* chromosome 7 is normal, while it is extremely low between *V. rotundifolia* chromosome 20 and the lower part of *V. vinifera* chromosome 7. This observation reveals a high divergence between both genomes on the lower arm of the chromosome 7 which could partly explain the low level of fertility of pseudo-F1 hybrids (Patel and Olmo 1955; Jelenkovic and Olmo 1968). In addition, this mismatch allows us to predict the difficulty to introgress in *V. vinifera* a genetic factor carried by *V. rotundifolia* chromosome 20.

Distortion of segregation

Many segregation distortions were observed in the hybrid maps of BC1 and BC2 populations but also in the BC2 *V. vinifera* map (Fig. S2). This phenomenon is common in interspecific crosses (Myburg et al. 2004; Kullan et al. 2012; Brennan et al. 2014; Liu et al. 2016), but it has also been observed in crosses between *V. vinifera* cultivars (Adam-Blondon et al. 2004; Troggio et al. 2007). However, some particular patterns arise from our results. In fact, the majority of distorted markers that we observed were clustered in large region covering several Mbp, allowing us to discard technical causes, such as multilocus SNPs or scoring errors, which generally generate isolated distorted markers.

Interestingly, the same clusters of distorted markers are shared between BC mapping populations although they derived from two accessions of *V. rotundifolia* and several accessions of *V. vinifera*. Markers on chromosomes 1 and 11 were distorted exactly in the same way in BC1 and BC2 hybrid maps (Fig. 3). These distorted clusters are probably caused by genomic specificities of each species rather than to particular accession features. Thus, the risk to observe these distortions in a major part of the crosses between *V. rotundifolia* and *V. vinifera* whatever the accessions used seems high. This kind of bias could have an impact on the efficiency of the introgression by potentially reducing the number of individuals with interesting traits, when the favourable allele determining a trait is in coupling with the weakly transmitted region. In addition to species-related distortions, our results also show that specific interactions between accessions may unpredictably arise in a particular cross and create a strong distortion, as observed on chromosome 13 of the BC1 hybrid map.

In interspecific backcrosses, alleles from the recurrent parent are expected to be favoured over alleles from the donor parent (Myburg et al. 2004). Surprisingly, we observed that in homeologous distorted regions, *V. rotundifolia* alleles were not systematically eliminated in favour of *V. vinifera* alleles (Fig. 3). For instance, *V. rotundifolia* alleles were preferentially conserved on chromosomes 1 and 13, whereas they were preferentially eliminated on chromosome 11. Conservation of donor alleles in a recurrent background was already observed in other interspecific backcrosses and pseudo-backcrosses (Jiang et al. 2000; Lacape et al. 2003; Myburg et al. 2004). Myburg et al. (2004) explained this phenomenon by "the alleviation of genetic load by donor alleles in the recurrent genetic backgrounds", i.e. the increase of fitness in hybrids. Rieseberg et al. (2000) gave an alternative explanation involving the existence of "selfish" genes "that enhance the success of gametes they inhabit even if they pose a significant fitness cost during the diploid phase of the life cycle".

BC1 population provided an interesting case to study segregation distortion. Initially composed of 177 individuals, its size was dramatically reduced through the years due to developmental abnormalities which increased after establishment in the vineyard. SSR data acquired on seedlings initially growth in the greenhouse allowed us to compare segregation distortions before and after size reduction (Table S4) and to distinguish three different cases: (i) segregation distortion was already present in SSR data acquired on seedlings and stayed stable after population size reduction, as observed on chromosome 15; (ii) segregation distortion was not present in data acquired on seedlings but appeared after population size reduction, as observed on chromosome 13; (iii) segregation distortion was present in data acquired on seedlings but evolved by increasing (chromosomes 4 and 11) or decreasing (chromosome 1) after population size reduction. Several causes can be associated with the first

case of distortion: issues during gametogenesis, fertilization or seed germination. In the second case, the distortion is caused by developmental and abiotic stress leading to the death of plants after establishment in the vineyard, and most likely the distorted regions are involved in sustaining those stresses. The third case combines both causes: alleles or genotypes favoured during gametogenesis, fertilization or germination were either favoured again after establishment in the vineyard or eliminated.

In BC2 population, both maps, *V. vinifera* and hybrid, displayed distortion of segregation on the same chromosomes (chromosomes 4, 6, 8 and 18). These clusters were located either in a homologous region (chromosomes 4 and 18) or in a homeologous one, where *V. rotundifolia* alleles were either eliminated (chromosome 6), or favoured (chromosome 8). We assume that post-zygotic lethal interactions occurred between alleles of the two parents, influencing the viability of zygotes, the germination rate of seeds or the seedling survival. This phenomenon, which has already been reported in other plant species (Myburg et al. 2004), is thus not specific to grapevine.

Our results show that a special attention has to be given to regions harbouring unbalanced segregations, particularly to species-related distortions. Indeed, by favouring some alleles or genotypes at the expense of others, segregation distortion impacts qualitatively and quantitatively the introgression of interesting or unfavourable traits. Some interesting alleles may be lost quickly if they are in a region preferentially eliminated. Moreover, if these alleles are present in few individuals, the probability to have interesting genotypes with a desired combination of traits is low.

Recombination suppression in homeologous regions

The difference in recombination rate observed between populations is the most surprising result of our study (Fig. 5). In the BC1 hybrid map, recombination pattern was similar to that of the BC1 *V. vinifera* map, except for chromosome 7. Genetic distances were conserved between both parental maps, even if variations of the ratio between genetic distance and physical distance were observed all along the chromosomes. Conversely, in the BC2 and BC4 hybrid maps, recombination patterns were different from those of the *V. vinifera* maps: recombination is suppressed in homeologous regions and enhanced in homologous regions of recombined chromosomes.

In an F1 parent, all chromosome pairs are homeologous, i.e. associate one chromosome from *V. vinifera* to one chromosome from *V. rotundifolia*. The similar genetic distance between BC1 hybrid and *V. vinifera* maps can be explained by two phenomena. First, a failure to form at least one crossing-over between chromosomes of the same pair during meiosis may lead to sterility by creating unbalanced gametes (Li et al. 2011; Mercier et al. 2015; Dumont 2017). During F1 meiosis, only cells with at least one crossing-over per chromosome are able to produce viable gametes and then generate a progeny. Second, *V. vinifera* and *V. rotundifolia* have small chromosomes and, according to the mean distance of genetic linkage maps, only 1–2 recombinations occur per chromosome during meiosis. The similar size of the F1 and *V. vinifera* maps obtained in this study is probably the consequence of these two limitations: a minimum of one recombination per chromosome and a maximum of two recombinations per chromosome. Moreover, the distances between markers are conserved in both BC1 parental maps, which tend to indicate that the divergence between both species is stable along the genome, except the lower arm of chromosome 7, as discussed above.

In the 22-8-78 BC1 and 1771P BC3 hybrid parents, each pair of chromosome is composed of one chromosome from *V. vinifera* and one chromosome from their hybrid parent (F1 and BC2) introgressed with a variable-length segment from *V. rotundifolia*, according to the individuals, i.e. some parts of their genome are homologous, while some others remain homeologous (Fig. 2). We observed that recombination rate in 22-8-78 BC1 and 1771P BC3 meiosis depended on the level of introgression. When the chromosome was entirely inherited from *V. rotundifolia*, it behaved as in the 8624 F1 meiosis: the recombination events occurred along the chromosome as expected in intraspecific crosses (Fig. 5), leading to a linkage map with genetic distances similar to *V. vinifera*. When the pairs of chromosomes are composed of both homologous and homeologous regions, recombination occurs preferentially in homologous regions rather than in homeologous regions. This recombination suppression in homeologous regions in interspecific hybrids has already been observed in tomato (Canady et al. 2006), lettuce (den Boer et al. 2013), barley (Johnston et al. 2013) and cotton (Zheng et al. 2016). Plant materials used in these studies were introgression lines or substitution lines selected for their unique introgressed region. Canady et al. (2006) established that recombination suppression observed in tomato is not due to chromosomal rearrangements and proposed as a cause the lack of sequence identity. As discussed above, macrosynteny between *V. rotundifolia* and *V. vinifera* is conserved, but sequence divergence between the two genomes is substantial (Wan et al. 2013). Thus, sequence divergence could explain the recombination suppression observed in our crosses. Regarding the distribution of recombination, according to Kauppi et al. (2004), it can be explained by three reasons: 1) "the balance between the need for recombination and the need to minimize the breakdown of favourable haplotypes", 2) "the facilitation of the optimal mechanical/biochemical function of chiasmata in chromosome segregation", 3) "the efficiency of the region

to repair". Moreover, several studies have shown that DNA mismatch repair system restricted homeologous recombination in model systems, such as yeast (Hunter et al. 1996) and Arabidopsis (Li et al. 2006).

In addition, we observed that the recombination suppression was more important when the proportion of homeologous region on a given chromosome was lower than a threshold estimated to 80–85% and seemed total under 10%. Here, we first report about the effect on recombination of variable-length introgressed regions. Our results highlight that the behaviour at meiosis in terms of crossovers of each chromosome pair depends on its proportion of homeologous region and is unrelated to the behaviour of the other chromosome pairs.

Interestingly, in our study, even if recombination is suppressed in homeologous regions, total genetic length of each chromosome is conserved between *V. vinifera* and hybrid maps whatever the backcross level and the proportion of homeologous region. The higher recombination rate in homologous regions balances the lack of recombination in homeologous regions. Consequently, at a chromosome scale, the presence of a homeologous segment amplifies recombination in the remaining homologous region. Analysis of allotriploids in *Brassicas* led Pelé et al. (2017) to similar conclusions. As observed in the F1 meiosis, the need for at least one recombination on the chromosome to produce viable gamete is probably responsible for this offsetting mechanism.

Our results bring new insight to address and overcome the difficulties met in the exploitation of wild species through interspecific crosses in grape breeding. In particular, phenomena uncovered about the behaviour of homeologous regions in various genetic contexts open concrete and practical perspectives to make the introgression process more effective and select individuals which display the most favourable recombination pattern, i.e. carrying the targeted genetic factor of interest surrounded by an as small as possible homeologous region. Indeed, long introgressed regions being more favourable to recombination than shorter ones, the strategy that we can recommend to introgress a gene of interest from a wild relative is, first, to select in the BC1 progeny individuals with a large segment including the target locus at one end. Then, the selection for recombinants on the other side of the target locus is performed, in a second step, in BC2 progeny. The significant advantage of this two-step strategy is to be particularly adapted to the elimination of wild residual genetic material in species producing low amount of seeds, for which a one-step strategy based on double recombinant selection would be most probably inefficient.

Author contribution statement DM contributed to conceptualization and to funding acquisition, and administered the project. MD and SB were involved in formal analysis. MD, EP and PM were involved in investigation. DM, MD, CR and GR contributed to methodology. CS contributed to resources. DM, CR and MD were involved in supervision. MD contributed to visualization. MD and DM wrote the original draft. MD, DM, CR, EP and PM were involved in writing reviewing and editing.

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Compliance with ethical standards

Conflict of interest On behalf of all authors, the corresponding author states that there is no conflict of interest.

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