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ORIGINAL PAPER

Selective sweep at the *Rpv3* locus during grapevine breeding for downy mildew resistance

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Abstract The *Rpv3* locus is a major determinant of downy mildew resistance in grapevine (*Vitis* spp.). A selective sweep at this locus was revealed by the DNA genotyping of 580 grapevines, which include a highly diverse set of 265 European varieties that predated the spread of North American mildews, 82 accessions of wild species, and 233 registered breeding lines with North American ancestry produced in the past 150 years. Artificial hybridisation and subsequent phenotypic selection favoured a few *Rpv3* haplotypes that were introgressed from wild vines and retained in released varieties. Seven conserved haplotypes in five descent groups of resistant varieties were traced back to their founders: (1) 'Munson', a cross between two of Hermann Jaeger's selections of *V. rupestris* and *V. lincecumii* made in the early 1880s in Missouri, (2) *V. rupestris* 'Ganzin', first utilised for breeding in 1879 by Victor Ganzin in France, (3) 'Noah', selected in 1869 from intermingled accessions of *V. riparia* and *V. labrusca* by Otto Wasserzieher in Illinois, (4) 'Bayard', a *V. rupestris* × *V. labrusca* offspring generated in 1882 by George Couderc in France, and (5) a wild form closely related to *V. rupestris* accessions in the Midwestern United States and introgressed into 'Seibel 4614' in the 1880s by Albert Seibel in France. Persistence of these *Rpv3* haplotypes across many of the varieties generated by human intervention indicates that a handful of vines with prominent resistance have laid the foundation for modern grape breeding. A rampant hot spot of NB-LRR genes at the *Rpv3* locus has provided a distinctive

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advantage for the adaptation of native North American grapevines to withstand downy mildew. The coexistence of multiple resistance alleles or paralogues in the same chromosomal region but in different haplotypes counteracts efforts to pyramidise them in a diploid individual via conventional breeding.

Introduction

The cultivation of *Vitis vinifera* originated in Eurasia before the introduction of New World diseases. Ancient varieties have been immortalised for centuries through vegetative propagation, still persisting today because of their distinguished wines. Grapevine improvement has scarcely advanced even since the foundation of plant genetics (Owens 2008). Intentional breeding has been documented since the early 1800s, when amateur breeders in Southern France crossed *V. vinifera* varieties and selected red flesh grapes (Viala 1886), and when European settlers in North America struggled against the odds in the attempt to establish colonial viticulture (reviewed in Pinney 1989). Before that, the introduced *V. vinifera* was susceptible to local diseases, and indigenous grapes of the Atlantic Coast were unsuitable for wine making. Early colonists first encountered the fox grape (*V. labrusca*), which had an unpleasant flavour dominated by methyl anthranilate, and the river bank grape (*V. riparia*) which yielded small berries. Both species thrived in humid climates, climbed over trees, and grew vigorously when seedlings were brought into cultivation (Galet 1988). They hybridised naturally, and several purely indigenous hybrids were selected by colonists (e.g. 'Noah' and 'Clinton'). The summer grape *V. aestivalis* was also widespread throughout the East Coast of North America.

The creation of hybridised forms of *V. labrusca* or *V. aestivalis* with Old World varieties imported into Virginia and New England marked the dawn of grapevine breeding for disease resistance (Hedrick 1908; Pinney 1989). 'Isabella' and 'Catawba' resulted from the earliest crosses between *V. labrusca* and *V. vinifera*, generated in 1816 and 1819, respectively. 'Norton' is an alleged cross of *V. aestivalis* × *V. vinifera* that has been commercially available in Virginia since 1830. 'Concord' originated in 1849 in Massachusetts most likely from the hybridisation of *V. labrusca* and *V. vinifera*. Crude characteristics possessed by the parents were refined in these seedlings, providing proof of concept that improvement was achievable through hybridisation.

Colonists became aware of a new range of species when they ventured westward (reviewed in Pinney 1989). The flow of German immigrants to the central Mississippi basin established viticulture in the Midwestern US in the 1850s. In 1860, seedlings with sufficient frost hardiness to survive cold winters (e.g. 'Elvira') were selected from local *V. riparia* and *V. labrusca* (Pinney 1989). It was not before Hermann Jaeger, a Swiss winemaker who settled in the Ozarks region of Missouri in 1865, and Thomas Volney Munson, a horticulturist who became familiar with grapes in Kentucky before moving to Northwestern Texas in 1876, that systematic exploration of wild vines was undertaken in Missouri, Oklahoma, Arkansas, and Texas (Munson 1909; Tarara and Hellman 1990; McLeRoy and Renfro 2004). A few accessions of *V. rupestris* and *V. lincecumii*, which withstood infestations of the pathogens that flourished in their native habitat, also produced acceptable wine in the absence of *V. vinifera* genes. The rock grape *V. rupestris* was once a common groundcover in the harsh environment of gravely creek banks, and is now threatened by genetic erosion (Pavek et al. 2003). The post-oak grape *V. lincecumii* climbed over *Quercus stellata* trees, which remained preferentially associated with poor soils of dry woodlands (Munson 1909).

By the time the North American phylloxera had invaded French vineyards in 1867 and began its devastation of *V. vinifera* roots across Europe, the Midwestern US viticultural industry became vitally important to winegrowing in the Old World by furnishing millions upon millions of American cuttings (Husmann 1880). George Husmann and Hermann Jaeger led trials in Missouri for the selection of resistant rootstocks upon which to graft *V. vinifera*. Dormant wood was dispatched to France, where the incoming accessions were frequently misnamed in the rush to propagate cuttings and replant vineyards. Threatened by new diseases, which were introduced by the carryover of the pathogens *Plasmopara viticola* and *Erisyphe necator* on imported cuttings (Demaree et al. 1937), French viticulturists did not limit their use of North American vines to rootstocks. Some phylloxera resistant vines were also tolerant to mildews and were grown as direct producers, in particular those selected for wine making in the North American Atlantic Coast, such as 'Noah' and 'Clinton'. In the 1880s, experts in the Midwestern US were again called on to supply downy mildew (DM) resistant native grapevines that could provide better fruit quality than hybrids of *V. labrusca* and *V. riparia*. In parallel, hybridisation efforts intensified in Southern France. Victor Ganzin had been generating hybrids between a vine of *V. rupestris* called 'Ganzin' and *V. vinifera* 'Aramon' since 1879. In 1882, the amateur viticulturist Eugène Contassot received cuttings from Hermann Jaeger, including the selection 'Jaeger 70' which impressed him with the size and health of the fruit (Galet 1988; Volle et al. 2007). Contassot saved the seeds from the female 'Jaeger 70', which had been pollinated by

neighbouring *V. vinifera* vines, and donated them to his neighbours, Georges Couderc and Albert Seibel, who selected their first hybrids out of those seedlings and later became the most prolific French breeders (Paul 1996). Couderc extensively used accessions of *V. rupestris* with the primary goal of improving rootstocks. Seibel focused more on mildew resistance, by selecting and selling more than a thousand new varieties in his commercial nursery. 'Ganzin 1', 'Jaeger 70', and 'Noah' were Seibel's favourite parents for donating DM resistance, from which he obtained 359, 234, and 79 varieties, respectively (http://www.vivc.de/index.php). Early French crosses were made with *V. vinifera* varieties once valued in Southern France for their high yield (e.g. 'Aramon', 'Clairette', 'Alicante Bouschet', 'Folle blanche', 'Piquepoul', 'Bourrisquou', and others). At the dawn of the twentieth century and during the period between the World Wars, other French hybridisers (e.g. Bertille Seyve, Bertille Seyve Jr. also known as Seyve-Villard, Joannès Seyve, Joanny Burdin, Pierre Landot, and Jean-Louis Vidal) used the most promising Ganzin, Couderc, and Seibel selections for producing crossbreeds or for backcrossing them with noble cultivars of *V. vinifera* possessing distinctive varietal aromas (e.g. 'Traminer', 'Riesling', the 'Muscat' family). This work was continued after World War II in many European national breeding programs, in France, Germany, Hungary, and former Czechoslovakia, Yugoslavia, and Soviet Union, with the goal of further improving wine properties and retaining resistance.

What grape breeders did not know until the advent of molecular genetics was that they were selecting for particular alleles of distinctive disease resistance genes. In the case of DM resistance, the major locus is today designated *Rpv3* (Bellin et al. 2009). Downy mildew resistance is a quantitative trait in North American grapevines. The *Rpv3* locus is associated with the major component of defence, and it controls the ability to trigger a race-specific hypersensitive response (HR) to *Plasmopara viticola* (Casagrande et al. 2011). A resistant *Rpv3* haplotype has been introgressed from North American wild ancestors to *V. vinifera* through the 'Villard Blanc' lineage (Bellin et al. 2009). The *Rpv3*-mediated HR response correlates well with the number of leaves affected by sporulation, the density of sporangiophores on the abaxial surface of the leaf, and the severity of symptoms on the whole plant, which are all parameters used by breeders for phenotypic selection in the field. Positive directional selection primarily acts on genes with a strong influence on the major component of the trait, leading to reduced variability and increased linkage disequilibrium in the respective region. Thus, the extent of the selective sweep at the *Rpv3* locus may disclose the relevance of *Rpv3* haplotypes for DM resistance in modern grapevine breeding. The *Rpv3* locus resides on the lower arm of chromosome 18 within a genomic region rich in NB-LRR genes, and it is traceable by its association with rare alleles at two microsatellite markers (UDV305 and UDV737) that flank both sides of *Rpv3* within an interval of 1.4 cM (Moroldo et al. 2008, D. Copetti, unpublished data).

In this paper, we present the extent to which different haplotypes at the *Rpv3* locus have been selected during grapevine breeding for DM resistance in the past 150 years, and we trace the most conserved alleles further back to their North American ancestry.

Materials and methods

Plant material

Leaf samples were collected from the germplasm repositories of the Experiment Stations of the United States Department of Agriculture Plant Genetic Resources Unit (USDA-PGRU) in Geneva, NY, and in Davis, CA; the Institut National de la Recherche Agronomique (INRA), Centre of Grapevine Genetic Resources, Département de Génétique et Amélioration des Plantes, Unité Expérimentale du Domaine de Vassal, Marseillan-plage, France; the Julius Kühn-Institut (JKI), Institute for Grapevine Breeding Geilweilerhof, Siebeldingen, Germany; the Institute of Viticulture and Enology, University of Pécs, Hungary; the Experiment Station of the University of Udine, Italy, the Experiment Station of 'Centro Pilota per la Vitivinicoltura', Gorizia, Italy; and the Kuban State Agrarian University, Krasnodar, Russia. A few samples were also collected at the vineyard 'Museum of viticulture' held at the winery of Lieselehof, Kaltern, Italy. Breeding lines of the MM series came from the Centre of Grape Breeding, Kishinev, Moldova, and were selected by the breeders N.I. Guzun, M.V. Cüpko, F.A. Olar, and P.N. Nedov. The genotypes at the *Rpv3* locus reported are based on the plants associated with the respective accession names in those collections. Cases of mismatch between names and their reported genetic relatedness were resolved by comparing multiple samples from different collections. However, occasional curation errors and naming inaccuracies in each repository cannot be completely excluded. The reader should take this cautionary note into consideration when associating these genotypic data with accessions maintained in other collections. Wild accessions of *V. rupestris* were collected from USDA-PGRU-designated in situ preservation sites in Southern Missouri (Pavek et al. 2003). GPS coordinates of the collection sites are given in Supplementary Material S1. Other accessions of *V. rupestris* were collected from the John

Grinstead Collection at the Fruit Experiment Station of Missouri State University in Mountain Grove, Missouri.

Genealogy and pedigree reconstruction

Pedigrees of breeding lines and descent groups were reconstructed according to the passport data reported in the Vitis International Variety Catalogue (VIVC, http://www.vivc.de/index.php). Statistics on grapevine breeding activity and likelihood of a given seedling to generate progeny that passed all steps of selection for resistance and wine quality (registered varieties) are based on VIVC records as of May 2010 (Supplementary Material S2).

Genotyping

Genomic DNA was extracted using a CTAB-based method. PCR reactions were carried out in a 10-µL volume containing 200 µM of each dNTP, 0.2 µM of each primer, 10 ng of genomic DNA, and 0.5 U of HotMaster Taq polymerase (Eppendorf). The forward primers were labelled with a 6-FAM fluorescent dye. The PCR reactions were carried out in a GeneAMP 9700 thermal cycler (Applied Biosystems), with the following thermal profile: 95°C for 2 min, followed by 10 touchdown cycles at 94°C for 20 s, 55°C (–0.5°C/cycle) for 20 s, 65°C for 40 s, followed by 25 cycles at 94°C for 20 s, 50°C for 20 s, 65°C for 40 s, and a final elongation of 30 min at 65°C. PCR products were diluted in 30–60 µl dH_2O, then 2 µl (diluted PCR products) were added to 0.2 µL LIZ 500 size standard and 7.98 Hi-Di Formamide (Applied Biosystems) and separated by capillary electrophoresis using an ABIPrism 3730xl DNA analyzer (Applied Biosystems). Alleles were called and sized using GeneMapper Software (Applied Biosystems), with supervised user annotation of the identified peaks. The presence of null alleles was determined based on the lack of Mendelian transmission of seemingly homozygous alleles in documented pedigrees. The primer pairs of the markers used for genotyping are reported in Supplementary Material S3.

Haplotype analysis

Haplotypes were inferred from alleles at the *Rpv3*-flanking loci UDV305 and UDV737 using genotypes of 580 diploid accessions without any a priori assumption on their kinship. Conserved *Rpv3* haplotypes were named using superscript numbers reporting the corresponding UDV305-UDV737 allele sizes. Conserved *Rpv3* haplotypes in the founders of the major descent groups were confirmed using the internal markers UDV730, UDV732, UDV734, and UDV736.

Results

Rpv3 haplotypes in downy mildew resistant varieties

A total of 233 breeding lines selected by different breeders to withstand grape diseases, and in particular downy mildew, were genotyped at the *Rpv3* locus (Supplementary Material S4). The genetic diversity is significantly reduced in this set, as compared to wild vines and *V. vinifera*, because of a few overrepresented haplotypes, summarised in Table 1. $Rpv3^{299-279}$ is the most frequent haplotype. It is present in 106 accessions, in 92 of them in combination with a *V. vinifera* haplotype, and in 14 of them in combination with another North American haplotype. The second most conserved haplotype is $Rpv3^{null-297}$, which is present in 50 accessions, and in 20 of these, it is combined with another North American haplotype. Less frequent haplotypes are $Rpv3^{321-312}$, $Rpv3^{null-271}$, $Rpv3^{361-299}$, $Rpv3^{299-314}$, and $Rpv3^{null-287}$. All of these haplotypes are absent from the entire set of 265 varieties of *V. vinifera*, indicating that multiple *Rpv3* haplotypes were introgressed from wild species (Supplementary Material S4). Identity by descent of each *Rpv3* haplotype within kinship groups of DM resistant lines was confirmed using additional microsatellite markers (Supplementary Material S5). A graphical overview of the conservation of major *Rpv3* haplotypes within groups of DM resistant lineages related by their pedigree is given in Supplementary Material S6.

The 'Seibel 4614' lineage of downy mildew resistant grapevines and the $Rpv3^{299-279}$ haplotype

The most common haplotype $Rpv3^{299-279}$, which corresponds to the one previously mapped in 'Bianca' (Bellin et al. 2009), a backcross of the variety 'SV12-375' ('Villard blanc'), is present in all 48 breeding lines analysed in this study that were obtained from the backcross of 'Villard blanc' to different varieties of *V. vinifera* (Supplementary Material S6). These lines were phenotypically selected for DM resistance in France, Germany, Italy, Eastern Europe, and Russia.

The $Rpv3^{299-279}$ haplotype was passed to 'Villard blanc' by its female parent 'Seibel 6468' and, further back, by its grandparent 'Seibel 4614' (Supplementary Material S6). 'Seibel 4614' is the earliest documented ancestor of $Rpv3^{299-279}$ that has persisted in germplasm repositories until the present. 'Seibel 4614' gave rise to only five recorded offspring (Supplementary Material S6). Along the lineages of four of them, $Rpv3^{299-279}$ became extinct. 'Seibel 6468' is the only offspring of 'Seibel 4614' that disseminated $Rpv3^{299-279}$. 'Seibel 6468' was rarely used by breeders in crosses with varieties of *V. vinifera*, but it was widely utilised in combination with other resistant lineages, resulting in 72 selected progeny.

Table 1 Conserved *Rpv3* haplotypes of North American origin in mildew resistant grape varieties

Rpv3 haplotype[a]	Founder of descent group	Wild ancestor	Haplotype frequency[b]
$Rpv3^{299-279}$	'Seibel 4614'	*V. rupestris* [c]	106
$Rpv3^{null-297}$	'Munson' ('Jaeger 70')	*V. rupestris* or *V. lincecumii*	50
$Rpv3^{321-312}$	'Noah'	*V. labrusca* or *V. riparia*	17
$Rpv3^{null-271}$	'Noah'	*V. labrusca* or *V. riparia*	17
$Rpv3^{361-299}$	*V. rupestris* 'Ganzin'	*V. rupestris*	16
$Rpv3^{299-314}$	*V. rupestris* 'Ganzin'	*V. rupestris*	10
$Rpv3^{null-287}$	'Bayard' ('Couderc 28-112')	*V. rupestris* or *V. labrusca*	10

[a] Superscript numbers indicate the allele size at UDV305 and UDV737 microsatellites, respectively

[b] Number of accessions that possess the corresponding haplotype out of 233 mildew resistant lines

[c] Based not on recorded ancestry, but inferred from occurrence of this haplotype exclusively in wild *V. rupestris* accessions

The cross 'Seibel 6468' × 'Seibel 6905' was the most prolific, yielding 18 registered seedlings that are full-sibs of 'Villard blanc'. We genotyped 'SV12-286', 'SV12-303', 'SV12-358', 'SV12-364', 'SV12-390', and 'SV23-657' ('Varousset') and they all retained $Rpv3^{299-279}$ (Supplementary Material S6), as did all resistant lines obtained from subsequent generations of backcrossing (e.g. 'SV20-347' also known as 'Perle noire', 'Zala Gyöngye', 'Medina', and 'Rösler'). $Rpv3^{299-279}$ is also present in 'JS26-205' ('Chambourcin'), a popular resistant variety with an uncertain parentage. 'Chambourcin' has long been suspected to be an offspring of 'SV12-417' and 'Seibel 7053' ('Chancellor'): the presence of $Rpv3^{299-279}$ is compatible with the female gamete and the resistance phenotype being donated by 'SV12-417', a full-sib of 'Villard blanc', while the allelic haplotype is incompatible with the hypothesis that 'Chancellor' is the other parent. 'Regent' is another important DM resistant variety which had inherited $Rpv3^{299-279}$ from 'Chambourcin'.

'Seibel 6468' was also used in parental combinations with other resistant lineages (e.g. 'Plantet', 'Seibel 6746', 'Seibel 5408', 'Seibel 10096', and 'Seibel 5813'). All DM resistant varieties selected from their progeny have retained $Rpv3^{299-279}$, as well as those obtained from the subsequent generation of backcrossing (Supplementary Material S6). This occurred for instance in well-known varieties such as 'Seibel 13663', 'Seibel 14514' ('Dattier précoce de Seibel'), 'SV20-473' ('Muscat de Saint-Vallier), 'Seibel 14514' ('Johanniter'), and 'Helios'.

$Rpv3^{299-279}$ was also found in eleven varieties with unknown pedigree and four lines in which the presence of $Rpv3^{299-279}$ was unexpected based on their reported genealogy (Supplementary Material S6).

Ancestry of the $Rpv3^{299-279}$ haplotype

'Seibel 4614' is the earliest ancestor to which we could trace back $Rpv3^{299-279}$. The parentage of 'Seibel 4614' is questionable based on historical records. 'Seibel 752' is one of the reported parents, but the 'Seibel 752' accession analysed in this study has *Rpv3* haplotypes incompatible with it being the genotype that gave rise to 'Seibel 4614'. 'Seibel 752' has a non-*vinifera* haplotype $Rpv3^{361-299}$, which is shared with its wild ancestor *V. rupestris* 'Ganzin', not casting doubt on the trueness-to-type of 'Seibel 752'. The second alleged parent of 'Seibel 4614' is extinct: it was reported to be a seedling of 'Seibel 2003' ('Vivarais'), which itself does not have $Rpv3^{299-279}$, and an unknown accession of *V. berlandieri*. Since the genealogy of 'Seibel 4614' is fragmentary, deep ancestry of $Rpv3^{299-279}$ was investigated by an allelic survey using the extremely polymorphic microsatellite UDV305.

We have genotyped a set of 265 varieties of *V. vinifera* representative of the current geographical distribution and covering 35 countries from the European shores of the Atlantic to the oases of Central Asia. There are 52 alleles of UDV305 in the *V. vinifera* germplasm (Fig. 1), but the $UDV305^{299}$ allele associated with $Rpv3^{299-279}$ is absent from this sample. There are 39 alleles of UDV305 in a set of 82 accessions of wild grapevines representative of 33 non-*V. vinifera* species. The $UDV305^{299}$ allele associated with $Rpv3^{299-279}$ is present exclusively in accessions of *V. rupestris*, in particular, in several *V. rupestris* from Southern Missouri. Assuming a stepwise model of evolution for the $(AT)_n$ microsatellite UDV305, the closest allelic sizes to $UDV305^{299}$ are expected to be present within the same ecological niche rather than in unrelated populations. The alleles $UDV305^{297}$ and $UDV305^{301}$ in fact are only present in local accessions of *V. rupestris* sampled from the same geographical area of the Midwestern US (Fig. 1, Supplementary Materials S1 and S4).

The 'Munson' lineage of downy mildew resistant lines and the $Rpv3^{null-297}$ haplotype

$Rpv3^{null-297}$ is the second most conserved haplotype in the set of DM resistant varieties analysed in this study.

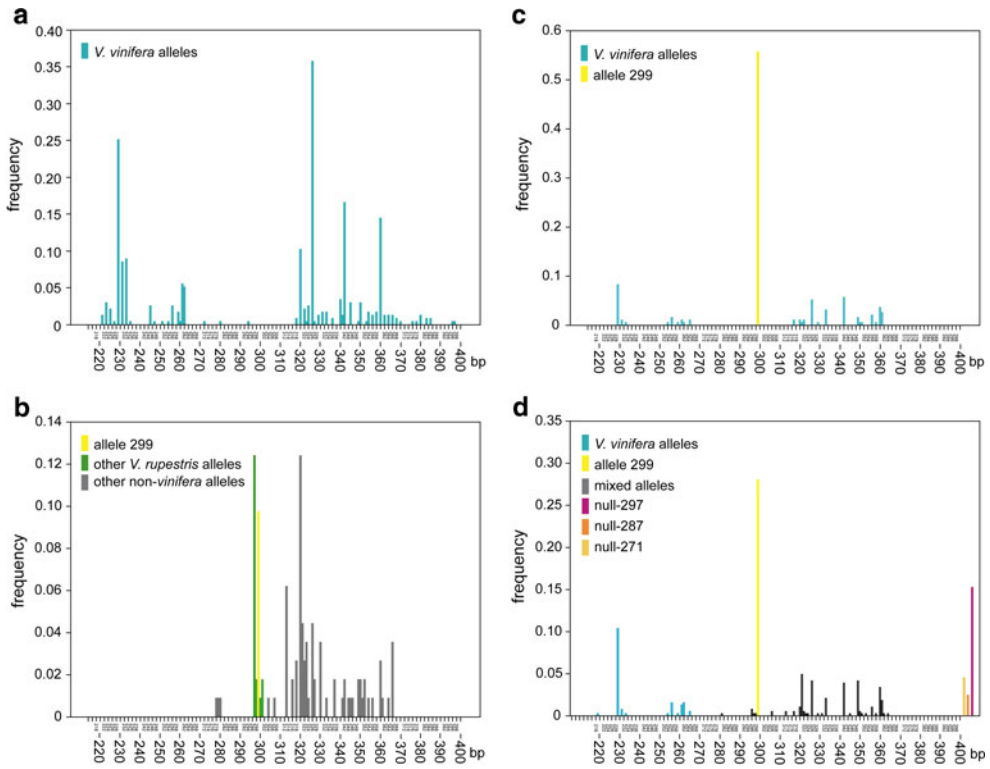

Fig. 1 Allele sizes and their frequency at the microsatellite locus UDV305 in a set of 265 varieties of *V. vinifera* (**a**); in a set of 82 accessions of 33 wild species enriched in 27 samples of *V. rupestris* (**b**); in descendants of 'Seibel 4614' selected for mildew resistance over a variable number of generations of backcrossing to *V. vinifera* (**c**); and in the whole set of 233 breeding lines (**d**). In **d**, null alleles at the UDV305 locus are differentiated from each other based on the allele at the adjacent locus UDV737

Rpv3$^{null-297}$ descended from 'Munson', also known as 'Jaeger 70', which was selected by Hermann Jaeger from a cross between '*V. lincecumii* No. 43', a Post Oak grape found in Southwestern Missouri, and a male seedling of his '*V. rupestris* No. 60' (Munson 1909). *Rpv3*$^{null-297}$ has been invariably retained in DM resistant descendants of 'Munson' at the expense of the allelic haplotype *Rpv3*$^{306-290}$ (Supplementary Material S5 and S6).

'Munson' became one of the favourite parental lines for several French breeders, who selected many resistant seedlings from its crosses with varieties of *V. vinifera* and other resistant lineages, 247 of which have been introduced into cultivation or further used for crossing (Supplementary Material S2). Among those reported in Supplementary Material S6, *Rpv3*$^{null-297}$ is present for instance in 'America', 'Seibel 2', 'Seibel 29', and 'Vivarais' which is itself the parent of another 208 resistant varieties. Through 'Seibel 29' and 'Vivarais', *Rpv3*$^{null-297}$ was transmitted to many lines from which popular DM resistant varieties were selected, such as 'Seibel 5279' ('Aurore') and Seibel 5455' ('Plantet'). 'Plantet' then generated 136 registered offspring (Supplementary Material S2) and those analysed in this study indicate that phenotypic selection was associated with the preferential retention of *Rpv3*$^{null-297}$ (Supplementary Material S6).

Rpv3$^{null-297}$ has also been retained by offspring originating from the crosses between varieties of the 'Munson' lineage and other resistant lineages (Supplementary Material S6). Among those seedlings is 'Seibel 6905' ('Subéreux'), which gave rise to 53 offspring that were retained for cultivation or further utilised in breeding. The parentage of 'Subéreux' is uncertain, since neither parent has survived until the present, and its presumed grandparents have incompatible *Rpv3* haplotypes with the genotype of 'Subéreux'. Far back in its genealogy, the *Rpv3*$^{null-297}$ haplotype present in 'Subéreux' is compatible with descent from 'Munson' along the maternal lineage and the allelic *Rpv3*$^{361-299}$ haplotype traces back to 'Ganzin 1' along the paternal lineage.

As to which wild species donated *Rpv3*$^{null-297}$ to 'Munson', neither parent ('*V. lincecumii* No. 43' or '*V. rupestris* No. 60') is still in existence for providing DNA. However, two F1 hybrid seedlings registered as offspring of the cross *V. rupestris* × *V. vinifera* ('Couderc 241-123' and 'Couderc 199-88' also known as 'Panache blanc'), without any details as to which accession of *V. rupestris* was used, do have the same *Rpv3*$^{null-297}$ haplotype present in 'Munson' (Supplementary Material S5). This suggests that *Rpv3*$^{null-297}$ might have originated from Jaeger's *V. rupestris* No. 60, and this valuable accession also became available

to George Couderc. Additional evidence is provided by the observation that the UDV737^{297} allele of *Rpv3*$^{null-297}$ is absent from the set of *V. vinifera* varieties tested in this study and, among wild species, it is most frequently found in *V. rupestris*.

V. rupestris 'Ganzin' and the haplotypes *Rpv3*$^{361-299}$ and *Rpv3*$^{299-314}$

The haplotypes *Rpv3*$^{361-299}$ and *Rpv3*$^{299-314}$ trace back to the wild accession *V. rupestris* 'Ganzin' (Supplementary Materials S4, S5, and S6). DNA of this accession could not be tested in the present study, but indirect evidence from its descendents indicate this male stock of *V. rupestris* crossed by Victor Ganzin with *V. vinifera* 'Aramon' as the founder of two distinct lineages.

Rpv3$^{361-299}$ is present in the offspring 'Ganzin 1' (*V. rupestris* 'Ganzin' × 'Aramon') in combination with a distinctive *V. vinifera* haplotype inherited from 'Aramon'. 'Ganzin 1' gave rise to 442 offspring selected by several breeders, and in particular it was extensively used by Albert Seibel. The *Rpv3*$^{361-299}$ haplotype is present also in some of Couderc's hybrids, indistinctively registered as *V. rupestris* × *V. vinifera* hybrids, as well as in Seibel's hybrids 'Seibel 752' and 'Subéreux', which were themselves used to breed many DM resistant varieties (Supplementary Material S2).

Rpv3$^{299-314}$ is present in 'Ganzin 2' (*V. rupestris* 'Ganzin' × 'Aramon'), a full-sib of 'Ganzin 1', and it is absent from the entire set of *V. vinifera* analysed in this study. *Rpv3*$^{299-314}$ is fairly common in Couderc's hybrids of *V. rupestris* × *V. vinifera* origin (e.g. 'Couderc 10', 'Couderc 901', 'Couderc 93-5', 'Couderc 16', 'Couderc 162-5', and 'Couderc 503' also known as 'Oiseau bleu'), which were mainly used for breeding rootstocks and were not specifically selected for DM resistance.

'Bayard' and the *Rpv3*$^{null-287}$ haplotype

The *Rpv3*$^{null-287}$ haplotype is commonly found in DM resistant varieties that descended from 'Bayard' (Supplementary Material S6). 'Bayard', also known as 'Couderc 28-112', was obtained by George Couderc in 1882 by crossing an unrecorded accession of *V. rupestris* with 'Emily', a reportedly *V. labrusca* × *V. vinifera* hybrid selected by Peter Raabe in the US. A few plants of 'Bayard' have survived worldwide. The accession of 'Bayard' available for this study conflicts with the *Rpv3* genotype expected from the DNA typing of its progeny, in the cases for which the offspring and the second parent are unequivocally identified, such as 'Flot d'or' ('Bayard' × 'Afus ali') and 'Seibel 880' ('Bayard' × 'Vivarais'). The 'Bayard'-derived *Rpv3*$^{null-287}$ haplotype was thus inferred from the preferential retention of this haplotype by its DM resistant descendants. Albert Seibel bred many varieties from 'Bayard' most frequently in combination with 'Vivarais' resulting in 123 registered selections. One of the most important is 'Seibel 880', which combines the *Rpv3*$^{null-287}$ inherited from 'Bayard' with the *Rpv3*$^{null-297}$ donated by 'Munson' through 'Vivarais' (Supplementary Material S6). 'Seibel 880' has originated 71 offspring disseminating the *Rpv3*$^{null-287}$ haplotype, for instance to the varieties 'Seibel 8745' ('Seinoir') and 'Seibel 7053' ('Chancellor').

The 'Noah' lineages of downy mildew resistant lines and the haplotypes *Rpv3*$^{321-312}$ and *Rpv3*$^{null-271}$

The haplotypes *Rpv3*$^{321-312}$ and *Rpv3*$^{null-271}$, which characterise two distinct lineages of DM resistance varieties, trace back to 'Noah' (Supplementary Material S6). 'Noah' was selected by Otto Wasserzieher from Illinois in 1869. Its parents are 'Taylor', a hybrid of *V. labrusca* with *V. riparia* found in Kentucky in the mid-1800s, crossed with another *V. labrusca* suspected to be 'Hartford'. 'Noah' was one of the earliest North American selections introduced in France, and was intensively used for breeding, giving rise to 152 varieties.

Rpv3$^{321-312}$ has been retained along the lineage of 'Gaillard 2' and 'Seibel 5163'. 'Seibel 5163' gave rise to 106 breeding lines, and it was frequently used in crosses with other resistant lineages. Among its descendents, *Rpv3*$^{321-312}$ was retained by 'Accent' and 'Hibernal', by 'Chancellor' in combination with the 'Bayard'-derived *Rpv3*$^{null-287}$, by 'Villard noir' in combination with the 'Munson'-derived *Rpv3*$^{null-297}$ (Supplementary Material S6) and by several selections for which the pedigree is unknown or questionable. The SSR alleles characterising *Rpv3*$^{321-312}$ are absent from the *V. vinifera* germplasm, confirming the introgression of this DNA region from a wild species.

The other haplotype present in 'Noah', *Rpv3*$^{null-271}$, was inherited by 'Seibel 867', which carries it in combination with the 'Munson'-derived *Rpv3*$^{null-297}$ (Supplementary Material S6). 'Seibel 867' generated 64 registered seedlings, in some cases the 'Munson'-derived *Rpv3*$^{null-297}$ haplotype was retained (e.g. in 'Gloire de Seibel'), while in other cases the 'Noah'-derived *Rpv3*$^{null-271}$ was passed on (e.g. 'Seibel 5450'). More interestingly, *Rpv3*$^{null-271}$ is present in 'Seibel 4986' ('Rayon d'or') and 'Seibel 4643' ('Roi des noirs'), though this is discordant with their reported pedigrees. The evidence that 'Rayon d'or', 'Roi des noirs', and 'Noah' share *Rpv3*$^{null-271}$ by descent is confirmed by additional SSR markers in the *Rpv3* locus (Supplementary Material S5). 'Rayon d'or' also generated a significant descent group of DM resistant varieties, among which *Rpv3*$^{null-271}$ is maintained e.g. in 'Seyve-Villard

1-72', 'Saphira', 'Duna Gyöngye', and notably in 'Vidal blanc', which became popular for the production of Cognac in France and then in Ontario for the production of ice wine (Supplementary Material S6). 'Rayon d'or' also transmitted $Rpv3^{null-271}$ to 'Seyve-Villard 5276' ('Seyval'), which carries it in combination with the 'Munson'-derived $Rpv3^{null-297}$ haplotype. In DM resistant backcrosses of 'Seyval', such as 'Merzling', 'Solaris', 'Bronner', 'Monarch', and 'Baron', $Rpv3^{null-271}$ was selected at the expense of $Rpv3^{null-297}$.

Discussion

Allele mining: a proof of concept for disease resistance genes in grapevine

Crop improvement involves the deliberate selection of beneficial alleles by breeders. This decreases diversity at the selected loci compared to more ancient cultivars or wild relatives from which the favourable alleles have been introgressed. Thus far, the genetic diversity in breeding material obtained from North American *Vitis* spp. and *V. vinifera* gene pools has been fragmentarily investigated (Pollefeys and Bousquet 2003). In this study, we compared a highly diverse set of 265 *V. vinifera* varieties whose origin predated the spread of DM from North America, 82 accessions of wild species, and 233 registered breeding lines with North American ancestry selected by human intervention in the past 150 years with the purpose of developing DM resistant varieties. An allele mining strategy was adopted in order to identify conserved haplotypes at a known locus associated with the desired phenotype. Allele mining is particularly suitable for disease resistance genes because they tend to colocalise in the same genomic region across unrelated individuals. Thus, when applied to disease resistance, allele mining often leads to the discovery of linked paralogous genes. We identified seven conserved haplotypes at the *Rpv3* locus, which are overrepresented in grapevine breeding lines that were historically selected for DM resistance relative to their wild grape relatives, and are absent from the susceptible varieties of *V. vinifera*. These grapevine haplotypes may carry *Rpv3* alleles or adjacent *Rpv3* paralogues that consistently remained associated with the diagnostic markers.

Novel gene products with conserved function may arise through allelic variation or evolution of initially identical duplicate genes. With respect to NB-LRR genes, local duplication is common, resulting in large series of disease resistance alleles or paralogues originating from a functional member within that locus. Proliferation of functional genes may continue in divergent lineages, preserving the original gene function in new alleles/paralogues while undergoing some specialisation, most frequently the recognition of specific strains of the pathogen. Consequently, an allele mining strategy has revealed 17 variants at the powdery mildew resistance locus *Pm3* in wheat landraces (Bhullar et al. 2010), and ~50 functional alleles of the *RPP13* locus could be isolated in ecotypes of *Arabidopsis thaliana* resistant to downy mildew (Hall et al. 2009). More than 30 alleles, paralogues, and orthologues have been identified at the *Mla* locus in barley and wheat, conferring resistance to host specific strains of the powdery mildew fungus *Blumeria graminis* (Jordan et al. 2011, Seeholzer et al. 2010). This abundance of allelic diversity in NB-LRR genes is generated by a variety of mechanisms: single nucleotide polymorphisms mostly in the LRR encoding region (Yahiaoui et al. 2006), chimeric arrangements of gene segments, reshuffled recombination, gene conversion between alleles/paralogues (Yahiaoui et al. 2009), or recruitment of an ectopic promoter that resumes expression of a silent gene copy (Hayashi et al. 2010).

Selection of favourable *Rpv3* haplotypes in North American grapevines and perspectives for conventional breeding

The *Rpv3* haplotypes that are associated with today's resistant breeding lines originated in different North American species, among them *V. rupestris*, *V. labrusca*, and *V. riparia*, which are distributed across vast geographic regions and diverse environmental conditions. Novel resistance specificities arise by chance and are retained in restricted populations when they confer the ability to withstand local populations of pathogen variants (Cadle-Davidson 2008). Thus, haplotypes originating from geographically unrelated species are expected to complement each other in protecting the plant against a broader range of pathogenic forms, when they are combined in a single host genome. A number of hybrid seedlings have already been produced from all possible pairwise combinations of the parents that carry the different *Rpv3* haplotypes reported in this paper (Supplementary Material S4 and S6). The fact that many favourable haplotypes at a single locus are present in natural populations makes it difficult to exploit all of them by combining more than two haplotypes into a diploid individual through conventional breeding. The lower arm of chromosome 18 contains more than a hundred NB-LRR genes over a region spanning more than 7 million nucleotides, which are all potential factors for disease resistance. The possibility that *Rpv3* haplotypes in diverse descent groups contain tightly linked functional paralogues rather than allelic variants of the same gene is not excluded. However, the persistent association between alleles of the diagnostic markers and the resistance phenotype across all lines and generations analysed in this study suggest that this is not a highly

recombinogenic region. The pyramidisation of such tightly linked paralogues by recombination would require innumerable meioses. The proliferation of resistance genes within small portions of the genome is common in plant evolution and particularly true for grapevine. For the species, this organisation facilitates the rapid adaptation of the innate immune system to the changing pathogen population. The limitations that this organisation places on conventional breeding are compounded by the fact that resistance to other pathogens, such as the grape powdery mildew *Erysiphe necator*, that breeders seek to further pyramidise, is controlled by genes located in the same chromosomal region in unrelated grapevine species which originated in the Asian continent (Riaz et al. 2011).

Breeding founder effects, genetic bottlenecks, and diversity in the wild

Breeding for DM resistance in grapevine has produced more powerful effects in the selection of particular *Rpv3* haplotypes than natural selective pressure. Grape breeders have recurrently selected a few North American haplotypes, introduced from a small set of founders initially identified in the wilderness during the mid 1800s, while natural populations have maintained a highly diverse collection of *Rpv3* haplotypes. We have shown that during the intricate history of grape breeding the favourable *Rpv3* haplotypes associated with DM resistance must have provided outstanding phenotypes that were consistently evident in field screening throughout the decades and across very different environments. However, other valuable resistance haplotypes could exist in nature awaiting discovery. In the past, grapevine improvement was restricted to conventional methods of hybridisation, which limited the choice of resistant parents to those native accessions that also secured suitable fruit flavours. This constraint has favoured breeders' preference for Midwestern US grapes such as *V. rupestris* and *V. lincecumii* over the East Coast natives such as *V. labrusca*, *V. aestivalis,* and *V. riparia*. Within each species, collectors and breeders forced themselves to discard accessions that imparted unsuitable characteristics to the wine, even if they excelled at withstanding diseases. In Munson's tribute to the meticulous selection done by Hermann Jaeger, it was reported that from the tens of thousands of vines Jaeger examined in the wilderness and the hundreds moved into vineyards, he utilised less than half a dozen to lay the foundation of his breeding program (Munson 1909).

This severe selection of native accessions caused an initial bottleneck in the number of North American founders actually used for breeding (Supplementary Material S2 and S6). For instance, three lineages account for more than a thousand registered varieties. The descent group of 'Munson', including 'America', 'Vivarais', 'Plantet', 'Seibel 2', and 'Aramon du Gard' collectively accounted for the generation of ~ 700 varieties that were created in Europe during the last decades of the nineteenth century. The descent group of 'Noah', including 'Gaillard' and 'Seibel 5163', was bred at the same time and consisted of ~300 recorded selections. The descent group of 'Seibel 4614' expanded mostly in the twentieth century when descendants like 'Seibel 6468', 'Villard blanc', 'Pierrelle', 'Muscat de Saint-Vallier blanc', 'Dattier de Saint-Vallier', 'Varousset', and 'Seibel 13666' gave rise to ~270 recorded selections. In the period between the World Wars, French breeders diversified the schemes of hybridisation and, intentionally or inadvertently, they crossed lines that today we know to descend from different kinship groups and carry different *Rpv3* haplotypes. The offspring they selected most frequently retained the combination of two wild haplotypes at the *Rpv3* locus, as it occurred in lines such as 'Villard noir', 'Chambourcin', 'Chancellor', and 'Seyval', which became widely used DM resistant varieties and popular parents for contemporary breeders.

After World War II, another bottleneck occurred when breeders shifted their focus to wine quality and improvement for varietal aromas, and thus practiced repeated backcrossing of resistant lines to noble cultivars of *V. vinifera*. This reverted the trend to combine two complementary wild *Rpv3* haplotypes and favoured the selection in the offspring of those single *Rpv3* haplotypes that conferred superior resistance. The major descent groups were created from 'Villard blanc', 'Rayon d'or', 'Seyval', and 'Chancellor', with any new variety retaining only one resistant haplotype among $Rpv3^{299-279}$, $Rpv3^{null-271}$, $Rpv3^{null-297}$, and $Rpv3^{321-312}$. Consequently, this contemporary breeding scheme has raised concerns over the risk of disseminating strains of pathogens that may evade recognition provided by a single resistance haplotype, amidst the cultivation of varieties that all harbour that particular haplotype.

In the future, it may be possible to engineer multiple resistance alleles into a recipient *V. vinifera* genome, once they are identified. This would remove the constraints brought about by the colocalisation of paralogous resistance genes and the negative fruit characteristics of valuable resistant wild grapes. This study clearly illustrates a need to resume the search for undiscovered genetic resources and to diversify the combination of resistance genes currently present in different breeding lineages.

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