

Large-scale parentage analysis in an extended set of grapevine cultivars (*Vitis vinifera* L.)

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Abstract Inheritance of nuclear microsatellite markers (nSSR) has been proved to be a powerful tool to verify or uncover the parentage of grapevine cultivars. The aim of the present study was to undertake an extended parentage analysis using a large sample of *Vitis vinifera* cultivars held in the INRA “Domaine de Vassal” Grape Germplasm Repository (France). A dataset of 2,344 unique genotypes (i.e. cultivars without synonyms, clones or mutants) identified using 20 nSSR was analysed with FAMOZ software. Parentages showing a logarithm of odds score higher than 18 were validated in relation to the historical data available. The analysis first revealed the full parentage of 828 cultivars resulting in: (1) 315 original full parentages uncovered for traditional

cultivars, (2) 100 full parentages confirming results established with molecular markers in prior papers and 32 full parentages that invalidated prior results, (3) 255 full parentages confirming pedigrees as disclosed by the breeders and (4) 126 full parentages that invalidated breeders’ data. Second, incomplete parentages were determined in 1,087 cultivars due to the absence of complementary parents in our cultivar sample. Last, a group of 276 genotypes showed no direct relationship with any other cultivar in the collection. Compiling these results from the largest set of parentage data published so far both enlarges and clarifies our knowledge of the genetic constitution of cultivated *V. vinifera* germplasm. It also allows the identification of the main genitors involved in varietal assortment evolution and grapevine breeding.

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This work is dedicated to the memory of Dr Alain Bouquet, visionary breeder who was curious about traditional grape genealogy.

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Introduction

Parentage analysis in plants enabled important progress for characterizing pollination patterns and gene flow rates (Ashley 2010), detecting quantitative trait loci (QTL) through association and linkage methods (Jannink et al. 2001), estimating components of agronomic traits variance (Weir et al. 2006) and tracing back the origin of alleles of interest (Di Gaspero et al. 2012). In addition, for perennial crops, when large germplasm collections exist with genotypes maintained over a long period through vegetative propagation (e.g. *Citrus*, *Malus*, *Prunus* or *Vitis*), it is possible to retrace cultivar genealogy and therefore to learn more about domestication and artificial selection processes. Parentage assignment (i.e. paternity testing and relationships inference) was permitted by the development in the 1990s of microsatellite markers (Blouin 2003; Ashley 2010), appropriate statistical methods and software packages for molecular-based kinship analysis (Blouin 2003; Weir et al. 2006; Jones et al. 2010).

Since domestication, grape breeding has combined hybridization and selection of somatic mutations (Levadoux 1956; Olmo 1996) leading to the existence of 5,000–10,000 cultivars of *Vitis vinifera* L. (Alleweldt and Possingham 1988; Galet 2000). Open pollinations that generated ancient grape cultivars are obviously not documented and these cultivars will hereafter be termed traditional. The first artificial crosses in *Vitis* species were performed in the eastern USA during the early nineteenth century (Di Gaspero et al. 2012; Maul et al. 2012), the mother plant being the only parent selected most of the time. From the late nineteenth century onwards, thousands of *Vitis* interspecific crosses were performed to create cultivars including rootstocks resistant to phylloxera and pathogens introduced in Europe (This et al. 2006; Di Gaspero et al. 2012). In *V. vinifera*, the first crossbreds documented (choosing both father and mother plants) seem to be those created by L. Bouschet de Bernard in 1828 in southern France, with the objective of reinforcing the colour of red wines (Galet 2000). From this time onwards, several private grape breeders undertook hybridization works to improve different traits of agronomical interest such as precocity, berry size, yield, muscat flavour or resistance (Huglin and Schneider 1998). Cultivars derived from these recent works will hereafter be termed modern. After World War II, most breeding programmes were undertaken by public agronomical institutes, using mating designs based on a deeper genetic knowledge (Bouquet et al. 1981; Huglin and Schneider 1998). Hermaphroditism, self-fertility and easy out-crossing appeared preponderant in cultivated *V. vinifera* (This et al. 2006) which was generally affected by inbreeding depression when self-pollinated (Alleweldt and Possingham 1988; Olmo 1996), in relation to its high heterozygosity (This et al. 2006; Laucou et al. 2011).

The putative parentage of modern grape cultivars is known through historical breeders' data [see the *Vitis* International Variety Catalogue (Maul et al. 2012) for a review] even though these records can contain some inaccuracies. Additionally, since the pioneering work of Thomas et al. (1994), many studies based on the inheritance of highly polymorphic nuclear microsatellite (nSSR) markers have been performed to clarify the parentage of several cultivars [see Sefc et al. (2009), Maul et al. (2012) for a review]. More recently, single nucleotide polymorphisms (SNP) have also been used for this purpose (Myles et al. 2011). The results of these studies permitted: (1) confirming breeders' information (Ibanez et al. 2009), (2) invalidating some breeders' data (Dettweiler et al. 2000) and (3) uncovering parentages for traditional cultivars (Regner et al. 1998a; Sefc et al. 1998; Bowers et al. 1999; Boursiquot et al. 2009). Given the maternal inheritance of chloroplasts in grape (Strefeler et al. 1992) and despite their low polymorphism (Arroyo-Garcia et al. 2006),

chloroplast microsatellite (cpSSR) markers have sometimes been used (Boursiquot et al. 2009) to identify the maternal parent in the cross.

Generally, the number of nSSR analysed is about 20 (Sefc et al. 1997; García-Muñoz et al. 2011), from approximately 10 for verifying reported pedigrees (Grando and Frisinghelli 1998; Lopes et al. 1999) to 50 or more for investigation without prior hypothesis (Vouillamoz et al. 2003).

Most previous studies tried to resolve the genealogy of *V. vinifera* cultivars belonging to a specific geographic area (Tapia et al. 2007), a historical period (Lacombe et al. 2007) or a specific kin group (Crespan and Milani 2001) analysing usually less than 100 cultivars and revealing or validating 1–50 parentages. Only Cipriani et al. (2010) and Myles et al. (2011) analysed larger samples of international cultivars (745 and 583, respectively) leading to the documentation of 74 and 83 parentages (including 28 and 39 validations of prior knowledge, respectively).

The objective of this study was to undertake a large-scale analysis in a sample of 2,344 *V. vinifera* cultivars to uncover or confirm their parentages. Besides improving our knowledge of cultivar pedigrees, this work could help us to better understand the general characteristics of grapevine breeding through history. The identified parentages would also provide valuable information to define samples for linkage disequilibrium and association genetics studies and could help today's breeders to choose appropriate genitors in new mating designs.

Materials and methods

Cultivar dataset and microsatellite analysis

We studied the 20 nSSR dataset obtained by Laucou et al. (2011) from 2,344 *V. vinifera* cultivars maintained in the INRA Grape Germplasm Repository at "Domaine de Vassal" (34340 Marseillan-Plage, France; <http://www.1.montpellier.inra.fr/vassal>). The list of cultivars and passport data are provided in Online Resource 1. Additional information (e.g. synonymy) is available on the French Network of Grapevine Repositories Database (http://bioweb.supagro.inra.fr/collections_vigne). Based on the previous characterization performed by Laucou et al. (2011), all duplicates and interspecific hybrids were eliminated and our dataset corresponds to 2,344 unique genotypes of *V. vinifera* (i.e. cultivars having different nSSR profiles) without synonyms, clones or mutants. Sample composition in terms of cultivar geographical origin, use of grapes, berry skin colour, berry flavour, presence of seeds and flower sex is presented in Table 1. Pairwise genetic distance (GD) was calculated on the 20 nSSR data with GENALEX 6.41 program (Peakall and Smouse 2006).

Table 1 Sample composition in terms of geographical origin, historical status, use of grapes, berry skin colour, berry flavour, presence of seeds and sex for the 2,344 *Vitis vinifera* cultivars studied from the INRA Domaine de Vassal grapevine repository and numbers of full parentages uncovered or validated in the present study using 20 nSSR

Code	Characteristic	Cultivars analysed	Full parentages identified in this study
Geographical origin (countries^a)			
MAGH	Maghreb (DZA, MAR, TUN)	117	21 (18 %)
IBER	Iberian Peninsula (ESP, PRT)	270	79 (29 %)
WEUR	Western Europe (BEL, FRA, GBR, NLD)	597	293 (49 %)
CEUR	Central Europe (AUT, DEU, CHE, CZE, SVK, TCH)	127	74 (58 %)
ITAP	Italian Peninsula (ITA)	333	76 (23 %)
EEUR	Eastern Europe (BGR, HUN, ROU)	218	110 (50 %)
BALP	Balkan Peninsula (BIH, CYP, GRC, HRV, SCG, YUG)	177	24 (14 %)
RUUK	Russia and Ukraine (MDA, RUS, UKR, URS)	102	36 (35 %)
CAUC	Caucasus and Turkey (ARM, AZE, GEO, TUR)	108	16 (15 %)
NEAS	Near East (EGY, ISR, LBN, SYR)	54	9 (17 %)
MEAS	Middle East (IRN, YEM)	26	1 (4 %)
FEAS	Central Asia and Far East (AFG, CHN, IND, JPN, KAZ, TJK, TKM, UZB)	60	9 (15 %)
NEWO	New World Vineyards (ARG, AUS, CHL, MEX, PER, USA, ZAF)	116	62 (53 %)
ND	Non determined	39	18 (46 %)
Historical status			
Trad	Traditional cultivars	1,771	380 (21 %)
Mod	Modern cultivars (created by breeders)	573	447 (78 %)
ND	Non determined	–	–
Use of grapes			
W	Wine grape	1,375	453 (33 %)
T	Table grape	711	315 (44 %)
WT	Wine and table grape	188	54 (29 %)
ND	Non determined	70	5 (7 %)
Berry skin colour			
Nt	Black with coloured pulp	25	21 (84 %)
N	Black	875	265 (30 %)
NR	Black-red	40	11 (28 %)
Rg	Red	68	18 (26 %)
G	Grey	5	3 (60 %)
Rs	Rose	118	52 (44 %)
B	White	1,126	444 (39 %)
ND	Non determined	87	13 (15 %)
Presence of seeds			
pips	Seeded	2,197	784 (36 %)
sdl	Seedless	67	31 (46 %)
ND	Non determined	80	12 (15 %)
Sex			
H	Hermaphrodite	1,915	731 (38 %)
F	Female	187	54 (29 %)
ND	Non determined	242	42 (17 %)

^a Country codes according to the ISO 3166-1 alpha 3 standard

Parentage analysis

Parentage analysis was performed on the nSSR dataset of unique genotypes using FAMOZ software (Gerber et al. 2003) adapted to grapevine (Di Vecchi-Staraz et al. 2005) and already known as a programme of choice for this purpose (Jones et al. 2010). A discrepancy at one nSSR locus was authorized to allow for possible genotyping errors, presence of null alleles or mutations as previously proposed (Di Vecchi-Staraz et al. 2007; Boursiquot et al. 2009; Cipriani et al. 2010). Logarithm of odds (LOD) scores were assigned by FAMOZ to each possible parent and parent pair. The LOD score is the likelihood for an individual to be the parent of a given offspring divided by the likelihood for both individuals to be unrelated (Gerber et al. 2003). LOD score threshold was determined empirically through the validation of known pedigrees. The final parental assignment took into account historical data when available in the ampelographic literature [see Maul et al. (2012), Galet (2000) for a review] as well as some main phenotypic traits governed by major genes (i.e. berry skin colour, seedlessness, muscat flavour) likely to reveal mistakes in doubtful cases. Comparisons with previous published parentages based on molecular markers were also realized [see Sefc et al. (2009), Maul et al. (2012) for a review]. Pedigrees for validated full parentages were drawn using PEDIGRAPH software (Garbe and Da 2004) and the half-kinships network was designed with IGRAPH package in R (Csajrdi and Nepusz 2009).

Results

A general overview of results is shown in Fig. 1 with the number of full parentages identified both for traditional and modern cultivars, in relation to breeders' data and prior molecular works.

Assignment of full parentages

Parentage analysis on the 2,344 dataset using FAMOZ software enabled detecting 1,515 *likely parent pairs*, 805 without any nSSR mismatch and 710 with mismatch at one nSSR locus. For these putative parentages, LOD scores extended from 10.92 to 62.37 (mean 31.57). The confirmation in our sample of known pedigrees (i.e. attested by breeders' data and/or prior published works based on nSSR or SNP markers) enabled us to empirically determine an LOD score threshold value (>18) for hypothesis validation. This also justified the allowance for the discrepancy at one nSSR locus adopted in our method. Under this threshold value, no *likely parent pair* was validated due to too many

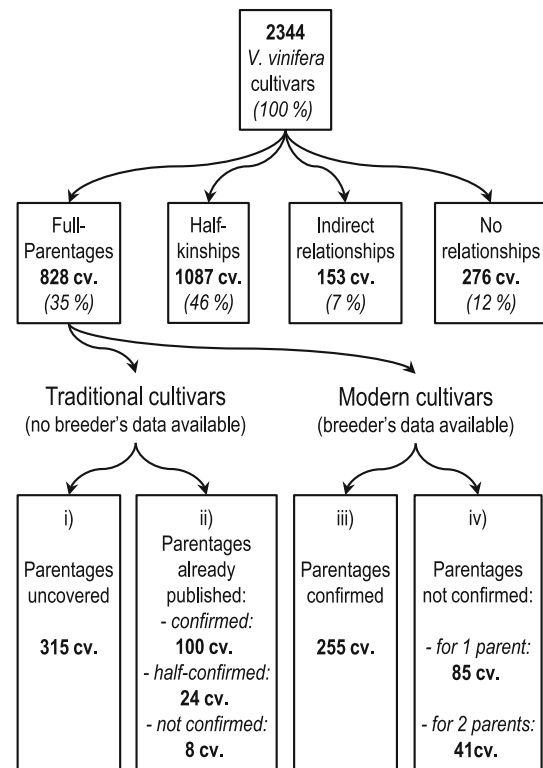


Fig. 1 Overview of parentage analysis results from all 2,344 *Vitis vinifera* cultivars using 20 nSSR

nSSR discrepancies or to obvious anachronisms between parents and offsprings.

Historical and phenotypic data allowed validating the full parentage of 828 cultivars (35 %) involving 592 parent pairs and 434 genitors. Some international examples of full parentages are shown in Table 2 and Fig. 2; a complete list and pedigree picture are provided in Online Resource 2 and 3. The 20 nSSR data on all parents and offsprings are provided in Online Resource 4. Linking the detected parentages to breeders' data and other published molecular studies led to sort them into four categories (Fig. 1):

1. 315 original full parentages uncovered in this work for traditional cultivars;
2. 100 full parentages that confirmed results established on the basis of molecular markers in prior published studies and 32 full parentages that invalidated prior results;
3. 255 full parentages that confirmed the pedigrees as disclosed by the breeders;
4. 126 full parentages that invalidated breeders' data. When breeder's stated pedigree was wrong, the father plant used in the cross was rejected in 58 cases, the mother plant in 27 cases and both parents in 41 cases.

For 12 traditional cultivars (i.e. no bibliography available on pedigrees), we had to consider more than one full

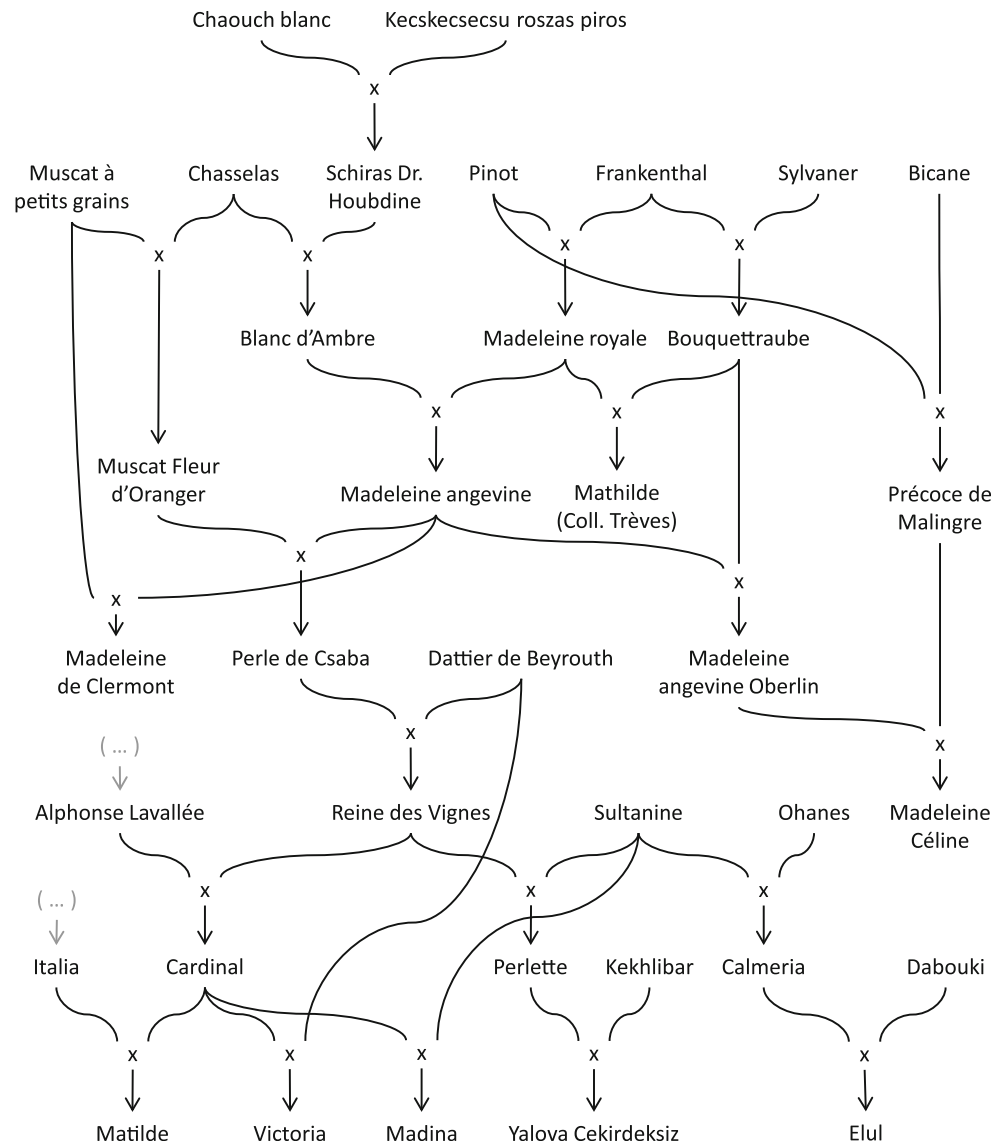
Table 2 Examples of full parentages identified within a set of 2,344 *Vitis vinifera* cultivars from the INRA Domaine de Vassal grapevine repository using 20 nSSR

Progeny (#ID code)	Parent 1 (#ID code)	Parent 2 (#ID code)	Parent pair LOD score
Aïn el Bouma (#1901)	Olivette blanche (#667)	Ugni blanc = Trebbiano toscano (#74)	32.60
Aït Hamama (#3700)	Amokrane (#2030)	Arekak (#3702)	42.32
Arinto do Dão (#1527)	Gibi = Hebén (#45)	Alfrocheiro preto (#50)	34.67
Blanc de Rhafsai (#2002)	Taferielt (#1905)	Pardina = Jaen blanco (#1963)	38.38
Boal cachudo (#3022)	Arinto do Dão (#1527)	Pardina = Jaen blanco (#1963)	37.68
Cainho branco (#1499)	Amaral = Caiño longo (#1459)	Alvarinho (#55)	41.38
Castelão = Periquita (#1521)	Pardina = Jaen blanco (#1963)	Alfrocheiro preto (#50)	32.05
Coarna rosie (#1188)	Rosa menna di vacca (#1662)	Tsitsa Kaprei (#2471)	42.12
Crimposie (#2243)	Beala debela (#2237)	Zemoasa = Timpurie (#1668)	43.47
Daphni (#263)	Ferral = Prunesta rosso violacea (#2234)	Assouad karech (#564)	41.17
Dimiat (#1666)	Coarna alba (#749)	Gouais blanc = Heunisch weiss (#211)	34.68
Efremovsky (#3155)	Coarna alba (#749)	Plavaï (#1665)	33.79
Goher (#2244)	Alba imputotato (#44)	Lizstes feher (#2808)	39.70
Gouveio (Verdelho) (#296)	Savagnin = Traminer (#257)	Castellana blanca (#3797)	43.68
Greco nero (#1274)	Terrano (#1293)	Grenache = Garnacha (#34)	39.20
Gros Colman (#774)	Furjmony feher (#2134)	Kadarka (#1610)	39.07
Icod de los Vinos (#2905)	Negra mole = Mollar (#1471)	Listan = Palomino fino (#28)	43.09
Kékmedoc (#2878)	Chasselas (#585)	Muscat d'Eisenstadt (#687)	37.59
Kizil Yakdona (#3432)	Tagobi (#2659)	Khoussainé blanc (#1227)	57.07
Kurtelaska (#1640)	Bombino bianco (#1341)	Mostosa = Empibotte bianco (#2054)	37.92
Lacrima di Maria (#1967)	Graeco (#3224)	Heptakilo (#743)	43.60
Lignan (#646)	Bermestia bianca (#1252)	Madeleine Salomon = Agostenga blanc (#649)	46.00
Louali (#2165)	Oul b'Ouzgueur (#1896)	Chikki (#460)	40.64
Malvar (#1371)	Gibi = Hebén (#45)	Tortozon (#1362)	40.46
Maren (#3182)	Negrara trentina (#1290)	Blank Blauer = Vulpea (#1631)	33.26
Merseguera (#1360)	Gibi = Hebén (#45)	Tortozon (#1362)	38.63
Mouchketny (#2739)	Bekalny (#3150)	Plavaï (#1665)	36.76
Muscat d'Alexandrie (#308)	Muscat à petits grains (#555)	Heptakilo (#743)	45.30
Muscat d'Istamboul (#398)	Muscat d'Alexandrie (#308)	Valenci blanco = Beba (#660)	45.24
Orlovi nokti (#2461)	Rosa menna di vacca (#1662)	Teta de Vaca (#1367)	47.46
Papaskarasi (#2109)	Alba imputotato (#44)	Prokupac (#1630)	41.05
Planta nova (#1476)	Royal (#628)	Heptakilo (#743)	45.13
Plavaï (#1665)	Beala debela (#2237)	Zemoasa = Timpurie (#1668)	41.13
Rassegui (#1956)	Mayorquin = Farana (#1900)	Heptakilo (#743)	37.87
Ruzevina (#1653)	Bombino bianco (#1341)	Lasina (#1642)	38.37
Savvatiano (#1781)	Rhoditis (#1779)	Karystino (#364)	50.20
Sykiotis (#2124)	Kontegalo (#3396)	Vradyano (#3393)	36.57
Turki (#1957)	Karystino (#364)	Heptakilo (#743)	53.17
Vékonyhéju (#2620)	Blank Blauer = Vulpea (#1631)	Gouais blanc = Heunisch weiss (#211)	34.21
Vital (#2103)	Arinto do Dão (#1527)	Rabigato (#2496)	40.73

parentage hypothesis showing high LOD scores and no nSSR mismatch, without any objective reason to conclude (e.g. cv. Olasz Kadarka).

The 828 full parentages assigned allowed ranking the genitors among the 434 total parents engaged: 34 main genitors generated ten or more offsprings (Table 3); 190

Fig. 2 Example of grape pedigrees focused on early ripening *Vitis vinifera* cultivars



secondary genitors generated 2–9 offsprings and 208 occasional genitors were involved in one cross only (not shown). The latter are mostly traditional cultivars (85 %), mainly used as wine grapes (67 %) and originating from most European countries (72 %).

As most grape cultivars are hermaphrodite, and except when a female-flowered parent was involved (242 cases), we could not determine, using only nuclear SSR, which parent was involved as the mother or father plant.

The number of generations in a given genealogy was rarely higher than three to four, exceptionally reaching seven for modern early ripening cultivars (Fig. 2).

The genetic distance between two parents involved in the 828 full parentages ranged from 0 to 43 (mean 28.48), while GD for the whole dataset ranged from 0 to 62 (mean 31.33) (Fig. 3). Only 19 occurrences (2 %) of

self-pollination were detected (e.g. cv. Picolin), eight being experimental, while two reported self-pollinations were infirmed (cv. Autofécondation Chasselas rose no. 18 and Kosovska rana). Full-sibs were detected in 244 cases (e.g. cv. Camarate, Cornifesto and Moreto).

The comparison of the geographical origin and fruit use of parents for a given progeny (Table 4) showed a majority of crosses using parents from the same geographical area and with the same use of grapes.

Last, a general overview of results according to sample composition (Table 1) showed a higher percentage of resolved parentages for western versus eastern cultivars, for modern versus traditional cultivars, for table versus wine grape cultivars, for white versus black cultivars, for seedless versus seeded cultivars and for hermaphrodite versus female cultivars.

Table 3 Main grapevine cultivars genitors, based on the 828 full parentages identified within a set of 2,344 *Vitis vinifera* cultivars from the INRA Domaine de Vassal grapevine repository using 20 nSSR

ID code	Genitor	Passport data							Offspring number
		Berry colour	Use	Flavour	Seeds	Sex	Country ^a	Historical status	
#211	Gouais blanc = Heunisch weiss	B	W	None	Sd	H	ND	Trad	63
#308	Muscat d'Alexandrie	B	WT	Muscat	Sd	H	ND	Trad	60
#585	Chasselas	B	WT	None	Sd	H	FRA	Trad	50
#193	Pinot	N	W	None	Sd	H	FRA	Trad	49
#766	Frankenthal = Schiava grossa	N	WT	None	Sd	H	ND	Trad	39
#1566	Sultanine	B	T	None	Sdless	H	GRC	Trad	35
#584	Muscat de Hambourg	N	T	Muscat	Sd	H	ND	Trad	28
#45	Gibi = Hebéen	B	W	None	Sd	F	ESP	Trad	28
#274	Riesling	B	W	None	Sd	H	FRA	Trad	27
#74	Ugni blanc = Trebbiano toscano	B	WT	None	Sd	H	ITA	Trad	25
#696	Bicane	B	T	None	Sd	F	ND	Trad	24
#1077	Mathiasz Janosne = Mathiasz 210	Rs	T	Muscat	Sd	H	HUN	Mod	21
#627	Alphonse Lavallée	N	T	None	Sd	H	FRA	Mod	21
#634	Dattier de Beyrouth = Afuz Ali	B	T	None	Sd	H	TUR	Trad	19
#257	Savagnin = Traminer	B	W	None	Sd	H	ND	Trad	18
#652	Madeleine angevine	B	T	None	Sd	F	FRA	Mod	17
#34	Grenache = Garnacha	N	W	None	Sd	H	ESP	Trad	17
#757	Black Morocco	N	T	None	Sd	F	MAR	Trad	17
#749	Coarna alba	B	T	None	Sd	F	ROU	Trad	15
#555	Muscat à petits grains	B	W	Muscat	Sd	H	GRC	Trad	15
#656	Précoce de Malingre	B	WT	None	Sd	H	FRA	Mod	14
#1069	Perle de Csaba	B	T	Muscat	Sd	H	HUN	Mod	14
#1078	Reine des Vignes	B	T	Muscat	Sd	H	HUN	Mod	14
#322	Cabernet-Sauvignon	N	W	None	Sd	H	FRA	Trad	13
#479	Petit Bouschet	Nt	W	None	Sd	H	FRA	Mod	13
#774	Gros Colman	N	T	None	Sd	H	RUS	Trad	13
#926	Italia = Pirovano 65	B	T	Muscat	Sd	H	ITA	Mod	12
#1095	Cardinal = G 10–30	Rg	T	None	Sd	H	USA	Mod	12
#270	Sylvaner	B	W	None	Sd	H	AUT	Trad	12
#290	Müller-Thurgau	B	W	None	Sd	H	FRA	Mod	12
#680	Muscat Saint-Laurent	B	T	Muscat	Sd	H	FRA	Mod	11
#1963	Pardina = Jaen blanco	B	W	None	Sd	H	ESP	Trad	10
#1827	Mission = Pais	N	W	None	Sd	H	MEX	Trad	10
#743	Heptakilo	N	T	None	Sd	H	GRC	Trad	10

Nt black with coloured pulp, N black, NR black-red, Rg red, G grey, Rs rose, B white, W wine, T table and/or raisin, Sd seeded, Sdless seedless, H hermaphrodite, F female, Trad, traditional, Mod modern, ND non determined

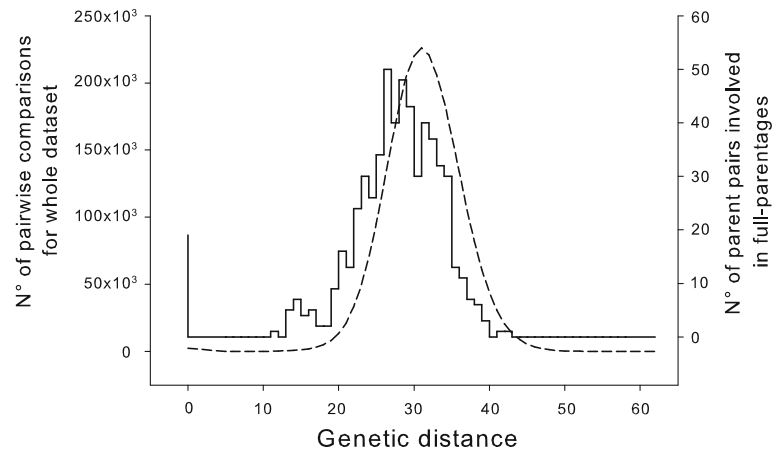
^a Codes according to the ISO 3166-1 alpha 3 standard

Half kinships, indirect relationships and unrelated cultivars

Among the 1,516 cultivars for which no full parentage was found, 1,087 (72 %) had possible half kinships, 153 (10 %) showed only indirect relationships and 276 (18 %) were unrelated (Fig. 1). These categories represented 46, 7 and

12 % of the initial dataset, respectively. Half kinships were represented by cultivars sharing half of their alleles with another cultivar at each of the 20 nSSR loci. When considering all 2,344 cultivars, a total of 3,603 half kinships were revealed among which: (1) 1,656 were parent–offspring relationships for the 828 full parentages validated above, (2) 158 corresponded to full-sib relationships, (3)

Fig. 3 Distribution of genetic distances between two parents involved in the 828 full parentages identified within 2,344 *Vitis vinifera* cultivars using 20 nSSR (*continuous line*), compared to pairwise genetic distances for the whole dataset (*dashed line*)



272 corresponded to half-sib or putative half-sib relationships and (4) 201 were not possible given their respective parentages or due to anachronism.

Some examples are shown in Table 5 and the complete list is provided in Online Resource 5. Except for full-sibs, half-sibs and cases chronologically impossible, these

represent putative direct (first degree) relationships with no possibility, based on our nSSR data only, to determine whether a cultivar is either a parent, an offspring or a full-sibling of the second cultivar. This category of genotypes designs different kin groups of variable size (2–52 cultivars), the largest being the kin groups of cv. Gouais blanc,

Table 4 Number of progenies according to parental geographical origin and fruit use of the two parents, based on the 828 full parentages detected within a set of 2,344 *Vitis vinifera* cultivars from the INRA Domaine de Vassal grapevine repository using 20 nSSR

	Geographical origin ^a															Use of grapes			
	Parent 2	MAGH	IBER	WEUR	CEUR	ITAP	EEUR	BALP	RUUK	CAUC	NEAS	MEAS	FEAS	NEWO	nd	Wine	Table	Wine/Table	nd
Parent 1																			
MAGH		5																	
IBER		3	27																
WEUR		11	26	286															
CEUR		-	1	28	2														
ITAP		3	2	15	3	23													
EEUR		3	2	21	1	5	47												
BALP		3	6	16	1	6	14	11											
RUUK		-	-	3	-	1	3	2	7										
CAUC		1	2	6	-	4	13	2	-	5									
NEAS		-	1	5	1	-	-	-	-	-	1								
MEAS		-	-	1	-	-	-	4	-	-	1	-							
FEAS		-	-	2	-	-	2	5	5	2	-	-	1						
NEWO		-	3	11	-	4	4	3	2	2	1	-	1	4					
nd		13	12	34	6	19	10	12	13	8	4	-	2	12	13				
Use	Wine															299			
Table																120	181		
Wine/Table																102	102	36	
nd																2	6	1	-

^a Region codes, region names (with country codes according to the ISO 3166-3 alpha standard) are MAGH, Maghreb (DZA, MAR, TUN); IBER, Iberian Peninsula (ESP, PRT); WEUR, Western Europe (BEL, FRA, GBR, NLD); CEUR, Central Europe (AUT, DEU, CHE, CZE, SVK, TCH); ITAP, Italian Peninsula (ITA); EEUR, Eastern Europe (BGR, HUN, ROU); BALP, Balkan Peninsula (BIH, CYP, GRC, HRV, SCG, YUG); RUUK, Russia and Ukraine (MDA, RUS, UKR, URS); CAUC, Caucasus and Turkey (ARM, AZE, GEO, TUR); NEAS, Near East (EGY, ISR, LBN, SYR); MEAS, Middle East (IRN, YEM); FEAS, Central Asia and Far East (AFG, CHN, IND, JPN, KAZ, TJK, TKM, UZB); NEWO, New World Vineyards (ARG, AUS, CHL, MEX, PER, USA, ZAF); nd, non determined

Table 5 Examples of grape cultivars sharing half of their alleles at each of the 20 nSSR markers analysed on 2,344 *Vitis vinifera* cultivars from the INRA Domaine de Vassal grapevine repository

Cultivar 1 (#ID code)	Cultivar 2 (#ID code)
Adissi (#2114)	Voskeat (#2511)
Aghiorgitiko (#1816)	Corinthe noir (#1802)
Airén = El Biod (#1423)	Gibi = Hebén (#45)
Aledo (#1477)	Valenci blanco = Beba (#660)
Assyrtico (#1798)	Gaidouria (#337)
Barbera (#1239)	Pattaresco (#1291)
Blank Blauer = Vulpea (#1631)	Prosecco (#1285)
Bratkovina bijela (#1634)	Posip bijeli (#1654)
Canaiolo (#1246)	Colorino (#1353)
Feteasca alba = Leanyka (#1661)	Argant (#261)
Fileri (#1817)	Koritsanos (#2031)
Forcallat blanca (#3146)	Ohanes (#1365)
Freisa (#1245)	Nebbiolo (#1240)
Hadari = Gounaia (#1893)	Dedo de Dama (#2630)
Henab turki = Fraoula kokkini (#1677)	Hunisa (#2903)
Hibou noir = Avana (#171)	Rèze (#1838)
Istchak rouge (#3272)	Khoussainé blanc (#1227)
Karystino (#364)	Avgoulato (#1761)
Kontegalo (#3396)	Aetonychi lefko (#2281)
Korithi mavro (#1767)	Roussaïtis (#2312)
Mandilaria (#1782)	Vaftra = Vapsa (#1790)
Manseng noir (#420)	Tannat (#401)
Marsanne (#148)	Roussanne (#147)
Montepulciano (#1284)	Bombino bianco (#1341)
Nocera (#1319)	Prunesta (#2409)
Picadillo = Turrutes (#1411)	Tempranillo (#1369)
Primitivo = Zinfandel (#1277)	Vranac crni (#1845)
Rabo de Ovelha = Rabigato (#1543)	Amaral = Caiño longo (#1459)
Rèze (#1838)	Poulsard (#252)
Ribolla gialla (#1294)	Refosco nostrano (#2039)
Roussaïtis (#2312)	Debina (#267)
Sabalkanskoï (#1731)	Dattier de Beyrouth = Afuz Ali (#634)
Sylvaner (#270)	Portugais bleu (#450)
Tempranillo (#1369)	Picadillo = Turrutes (#1411)
Tinta Amarela = Trincadeira (#1485)	Siria = Codega (#2833)
Tinto Cao (#1488)	Malvarisco (#2131)
Touriga franca (#1491)	Touriga nacional (#1493)
Treixadura (#1424)	Batoca (#42)
Vernaccia nera (#1234)	Aglianico (#1259)

Gibi (syn. Hebén), Savagnin (syn. Traminer), Pardina (syn. Jaén blanco), Chasselas and Sultanine (Online Resource 6).

The 153 cultivars sharing less than 18 alleles with another one (out of the 20 nSSR loci, i.e. more than the discrepancy of two loci allowed), whatever the LOD scores, were classified as having indirect relationships (not shown).

Last, the FAMOZ analysis showed a significant proportion (12 %) of cultivars having neither *likely parent pair* nor *likely parent*, that is to say unrelated within the whole sample (e.g. cv. Arbane, Dolcetto, Tsolikouri; full list is provided in Online Resource 7).

Discussion

Number of nSSR markers

Our work demonstrated that the analysis of 20 SSR markers (well scattered on the genome) on an extended set of grape cultivars enabled uncovering a large number of new parentage relationships and confirmed many relationships already known.

Even if many parentage analysis works analysed about 20 nSSR (Crespan and Milani 2001; Aguero et al. 2003; Cabezas et al. 2003; Maletic et al. 2004; Ibanez et al. 2009), this number could be considered in some cases as insufficient given the level of coancestry typical of grape. This could notably be the case, if samples are too limited in size thereby reducing the number of testable possible parents. Some authors thus suggested increasing the number of nSSR loci beyond 30 to strengthen the statistical power of pedigree reconstruction (Sefc et al. 2009; Cipriani et al. 2010) and more than 50 markers were sometimes used (Vouillamoz et al. 2003). Obviously, analysing additional nSSR or even SNP markers could be helpful to solve the 12 litigious cases (e.g. cv. Olasz Kadarka) or to confirm the 315 new pedigrees uncovered. However, not only the number of supplementary markers, but also their information content (number of alleles) would then have to be considered. The choice of supplementary nSSR could be done among those more commonly used in prior works on grape genealogy (see additional bibliography in Online Resource 2, 4), whereas SNP markers could be chosen among the 48 proposed by Cabezas et al. (2011).

However, it is noteworthy that our results based on 20 selected nSSR allowed blind validation of numerous publications based on different (and sometimes much larger) sets of nSSR and SNP markers (see additional bibliography in Online Resource 2, 4). A similar blind validation was widely observed for breeder's records. All these confirmations (355 in total) provided effective validation of the methodology we followed, and the 315 new full parentages can thus be considered as strongly conclusive. In the same way, even if other first-degree relationships revealed here

would have to be confirmed using additional markers in the future, they already have to be regarded as strong indications of close relationships between the cultivars involved.

Pedigree of grape cultivars

A total of 828 full parentages were found in our dataset of 2,344 *V. vinifera* cultivars. Among them, the 315 full parentages uncovered and the 158 parentages corrected represent a major and original contribution to the improved understanding of grape cultivars origin, past distribution and historical importance. They also provide keys for interpreting their agronomical potential with respect to their genitors. The 355 confirmations of both breeders' prior data (many for the first time) and previously published molecular works also provided a major contribution to the field of grape genealogy. On the other hand, it is worth noting that 33 % of the pedigrees as stated by the breeders were found to be partially or totally false. These errors involved all the breeders, whatever their country or the time period. Pollen pollution during crossing seems to be the first reason for the majority of cases corresponding to a false identity of fathers. Incorrect identification or synonymy in genitors' names is obviously a second involuntary reason. The willingness of some breeders to conceal their mating design (trade secret) or to usurp the fame of a given genitor seems to be a third reason. Last, we have also corrected 32 molecular marker-based parentages previously published, but erroneous due to (1) cultivar mislabelling (e.g. cv. Robin noir), (2) absence of one parent in the dataset (e.g. cv. Saint-Côme) or (3) insufficient number and polymorphism of nSSR (e.g. cv. Feteasca regala) used by the authors.

The parentages found or confirmed are more numerous on western versus eastern cultivars, in relation with the geographical representativeness of the grape collection studied. The better resolution of modern cultivar pedigrees compared to traditional ones can be related to the genetic erosion over time that leads to the absence of ancient cultivars in today's repositories. Quantifying in our conditions this genetic erosion was only conceivable for the French present gene pool (556 cultivars in the dataset), which is supposed to be entirely represented in the Vassal collection (the official reference in France): full parentage of 270 cultivars, half kinship for 116 cultivars and 26 unrelated cultivars were found, leading to a maximum of 168 missing ancestors, possibly not all of French origin [=116 + (26 × 2), i.e. a 30 % genetic erosion]. However, this number is certainly overestimated, with one genitor often having several progenies.

Whenever full parentage could not be resolved, first-degree relationships are of interest to understand the kin groups of cultivars (Vouillamoz et al. 2003) and the 1,087

putative half kinships identified here provide such precious information, even if confirmation with additional markers is advisable. For a given pair of directly related cultivars, one was either a parent, an offspring or a full-sibling of the second one, the third contributor to the cross being absent from the collection. Adding supplementary genotypes to the dataset would be the only way leading to the detection of full parentages for these cultivars. However, the present scarcity of some key genitors due to genetic erosion [e.g. cv. Gouais blanc and Magdeleine noire des Charentes reported by Bowers et al. (1999), Boursiquot et al. (2009), respectively] suggests that many *missing links*, including hypothetical wild ancestors, would not be recovered in the future. Interestingly, one can note that former ampelographic works based on morphological data only had already suspected several of these half-kinships (Levadoux 1956).

These kin groups could be extended with the 153 cultivars showing indirect relationships. They can be interpreted as half-sibling, cousins, uncle versus nephew or grandparent versus grandchild. These intricate relationships are further complicated by the possibility of generation overlap and would only be solved by analysing additional nSSR markers.

Interestingly, 12 % of the cultivars analysed were unrelated to any individual within the complete sample as no parent, offspring or sibling could be found. Absence of relationship could be due to the biased representativeness of the Vassal collection (under-representation of Middle and Far East germplasm). However, many unrelated cultivars do originate from well-represented areas (e.g. France, Italy, Spain). The historical signification of this group of cultivars is obscure as both reputed (e.g. cv. Dolcetto) or anciently grown cultivars (e.g. cv. Aïdani mavro) were found unrelated to any individual. In this category, we found only eight recent crossbreds (e.g. cv. Red Globe) created from unreleased parents and never used as genitors until now. If they show high genetic recombination ability, these unrelated genotypes could be of great interest for breeding programmes, because of their potential originality and therefore have to be preserved in collections as a priority. It would also be interesting to include them in global diversity studies to help interpret the origin of gene pools.

Our results also illustrated three main characteristics of grapevine breeding throughout history. The first one is the possibility of generation overlaps due to the combination between sexual reproduction and vegetative propagation and reinforced by the hermaphroditism of the majority of cultivars. Our results showed that overlaps were frequent, some occurring over centuries (e.g. cv. Madina) and others over continents (e.g. cv. Tarrango). Thus, the number of hybridization events that separate modern cultivars from

ancestral forms can differ widely, with a notable contrast between wine and table grapes, the latter group having been subjected to more intensive breeding. The second characteristic is the low number of generations found in pedigrees with a maximum of seven generations resolved on the longest branch. This may be due to long-term genetic erosion preventing access to putative genitors. Another hypothesis would be the actually small number of generations since grape domestication (Arroyo-Garcia et al. 2006), which is consistent with the extent of linkage disequilibrium (LD) between nSSR observed in cultivated grapes, maintained by vegetative propagation, compared to the smaller LD in wild progenitors (Barnaud et al. 2010). However, this does not fit the present diffusion of Gret1 (a retro transposon involved in the arising of white colour in berries) into almost the whole gene pool of cultivated grapevine (Fournier-Level et al. 2010). The third characteristic is the very low number of cultivars obtained through self-pollination, most probably due to inbreeding depression as already described in grapevine (Alleweldt and Possingham 1988; Olmo 1996).

Genitors

Starting from the early nineteenth century, grape breeders intentionally used genitors displaying one or several major traits of interest for growers. Thus, the success of a cultivar as a parent can first be explained by its reputed high yield, wine quality, muscat flavour, phenology and bunch aspect or seedlessness (for table grapes). However, cultivars displaying the same attractive phenotype do not all show the same recombination ability in a cross and the success of a genitor can thus also be explained by its higher genetic potential. Our results clearly confirm the narrow genetic basis of many modern cultivars created during the nineteenth and twentieth centuries, especially for table grapes, because of the extensive use of a few famed genitors, for example cv. Muscat de Hambourg, Bicane, Alphonse Lavallée, Madeleine angevine, Perle de Csaba, Reine des Vignes, Gros Colman and Cardinal.

Out of the stated breeders' works, the success of a genitor can evidently be related not only to its traits of interest (e.g. cv. Chasselas, Muscat d'Alexandrie, Sultanine), but also to its antiquity. Most of the main genitors of traditional cultivars are indeed ancient ones and consequently have been involved in open pollination events for centuries. The fact that some key genitors are ancient has already been underlined for cv. Gouais blanc and Pinot (Bowers et al. 1999), cv. Savagnin (Regner et al. 1998b; Myles et al. 2011), cv. Cabernet franc and Magdeleine noire des Charentes (Boursiquot et al. 2009), cv. Muscat à petits grains (Crespan and Milani 2001) or cv. Mission (syn. Pais) (Tapia et al. 2007). According to the

ampelographic literature, this also seems to be the case for other main genitors revealed in the present study such as cv. Black Morocco, Coarna alba, Gibi, Grenache (syn. Garnacha), Dattier de Beyrouth (syn. Afuz Ali), Frankenthal, Mayorquin (syn. Farana), Sylvaner and Ugni blanc (syn. Trebbiano toscano).

It could also be noted that several key genitors are female flowered (e.g. cv. Chaouch blanc, Rosa menna di vacca, Shiras Dr. Houbdine), which is considered as an ancestral trait in *V. vinifera* evolution (Levadoux 1956; This et al. 2006). Most hermaphrodite cultivars are heterozygous at this locus (Huglin and Schneider 1998), which can be explained by the long-time involvement of homozygous female cultivars as parents or by the recent and prompt diffusion of the domesticated hermaphroditic allele, as described for Gret1 involved in the white berry trait (Fournier-Level et al. 2010). Another hypothesis could be the putative benefit of heterozygosity for this fertility trait, which would then have been selected. On the contrary, 30 female cultivars displayed no relationship at all, also confirming their antiquity. Except when a genitor is a female cultivar, we cannot identify the mother plant in the cross using only nuclear SSR data. Phenological data sometimes allow excluding some hypothesis for a given offspring: when flowering times are very different, the mother is most probably the latest blooming cultivar receiving the pollen already available in the environment or stored by the breeder (e.g. ascendance of cv. Tempranilla blanca).

Parentages of traditional cultivars were proportionally less strictly directed concerning the use of grapes with more wine/table mating and more double-use genitors involved than for modern cultivars. Even if this specialization has been in existence for a long time, it has rapidly increased since the nineteenth century, as modern breeders preferably select genitors with similar use of grapes (wine vs. table).

The GD between parent pairs in the 828 validated full parentages was slightly lower than for pairwise comparison in all 2,344 cultivars. The small difference may be due to the close geographical origin and aptitude of genitors. This distribution showed that, whatever the GD between two genitors and excepting self-pollinations, *V. vinifera* breeders could obtain valuable offsprings without strong limitation from inbreeding effect.

Geographical aspects

Here again, modern and traditional cultivars have to be considered separately. Breeders usually worked with geographically diversified genitors gathered in a collection, while spontaneous crosses involved only parents grown in a given region. Most of our results are consistent with this

general hypothesis, but various counterexamples provide interesting information to trace back the historical movements of a given genitor. For instance, the involvement of cv. Savagnin in the genealogy of cultivars from northern and central Europe has already been underlined as well as its ancestral status (Regner et al. 1998b), but its extended kin group, revealed in our study, also covers unsuspected regions such as western France, Spain and Portugal. Conversely, the geographical origin of an offspring can be revised when its parents are known (Tapia et al. 2007). For example, the French genetic origin of cv. Glacière, Mandrègue and Servant was totally invalidated by our analysis.

When considering a particular region, parentages and half kinships highlight the main genitors that contributed to the local gene pool structure despite the periodical exchanges of material with distant regions. In the Iberian Peninsula, the white female cv. Gibi (syn. Heben) proved to be a key genitor. This cultivar seems to originate from North Africa (Galet 2000) which would be consistent with the relationships between Spanish and North African grape gene pools (El Oualkadi et al. 2011; Bacilieri et al. in preparation). Other main Iberian genitors are cv. Pardina (syn. Jaen blanco), Caiño longo, Alfrocheiro preto, Arinto do Dão and Mourisco tinto. In addition to cv. Pinot noir, Gouais blanc, Cabernet franc and Magdeleine noire des Charentes, the French gene pool also originates from other secondary genitors such as cv. Cahours, Fer, Grec rouge, Pougayen and Terret. In Italy, in addition to the already reported cv. Sangiovese (syn. Nielluccio) and Garganega (Di Vecchi-Staraz et al. 2007), some key genitors are actually wine cultivars mentioned for centuries such as cv. Bombino bianco and Bermestia bianca. The referenced Greek cv. Heptakilo (syn. Bouresla in Tunisia, Centorotolli in Sicily and, according to Cipriani et al. (2010), Axina de tres bias in Sardinia) proved to be the unsuspected genitor of ten offsprings including the famous cv. Muscat d'Alexandrie. The central and eastern Europe gene pool is characterized by the genitors cv. Blank Blauer, Bouquettraube, Coarna alba, Harslevelu, Kadarka and Kövidinka. For the Middle and Far East gene pool, in addition to cv. Sultanine, only cv. Khoussaïné blanc and Katta-kourgan appeared as secondary genitors. None of our six *Assouad* cultivars corresponded to the *Aswad* key genitor revealed by Myles et al. (2011). In North Africa, the role of cv. Abouhou and Dedo de Dama was confirmed. Overall, the origin of western wine grape cultivars is better resolved than the origin of eastern traditional table grapes. This can be explained by the biased composition of our sample containing more western genotypes and also by the traditionally intense breeding practices in the east (Fournier-Level et al. 2010) that led to shorter generations and more indirect relationships. In the past, cultivated grapevine

propagation by seeds was indeed a usual process in eastern regions (Ibn Al Awwâm \approx 1190), whereas western people preferably used cuttings (Huglin and Schneider 1998). Thus, the number of sexual cycles that accumulated in the two gene pools may be different.

On a wider geographical scale, kin groups represent a new illustration of the separation of cultivated grapevine into two main groups, wine versus table grape, being also western versus eastern genetic pools. The good correspondence between the origin of parents and offsprings is also consistent with the structuring of grapevine diversity based on eco-morphology and molecular markers (Aradhya et al. 2003). Kin groups can thus be considered as the highest level for explaining diversity structuring.

Conclusions

Identifying the parents of a grape cultivar requires a large sample of referenced genotypes, efficient molecular markers and powerful methods to analyse the resulting data.

Obviously, in order to be detected, both parents of an offspring must be included in the sample studied, and therefore the cultivar sample has to be as large as possible. Large grapevine repositories, such as the INRA Vassal collection, constitute indeed precious investigation tools, but no single collection is exhaustive. Only a network of national collections with well-identified genetic resources could provide such an optimal sample. So far, only the European Vitis Database (<http://www.eu-vitis.de>) corresponds to such an initiative. The acquisition of complementary germplasm, the assignment of a reliable prime name to each cultivar, the identification of duplicates and stated synonyms, and the disclosure of validated nSSR profiles must remain the goals of this kind of network, providing the basis for further successful parentage findings.

Nuclear microsatellite markers (nSSR) have long proved to be powerful tools for parentage investigation in cultivated grapevine. The nSSR set used here is appropriate to resolve most of the parentages whenever both parents belong to the sample. The use of chloroplast SSR (cpSSR) markers would not increase the power of resolution due to their low polymorphism, but would provide information on the direction of the crosses.

FAMAZ again proved to be a programme of choice (Jones et al. 2010), especially for grape parentage assignments, despite its limitations: lack of conviviality, maximum sample size studied (about 5,000 genotypes in our conditions) and results on parsing. Future improvements of FAMAZ and other software packages could focus on these points (Jones et al. 2010), as well as the simultaneous treatment of cultivar passport data.

Nevertheless, even with ideal materials and methods (sample, molecular characterization and software), not all parentages of traditional grape cultivars will be uncovered, since too many links have disappeared over the years. First-degree parentages only capture the most recent period in cultivated grape history and other methodologies are required to investigate the earlier steps of grapevine evolution under human selection.

Despite these limitations, we have demonstrated here the interest of parental relationship analysis from a large germplasm repository to understand the involvement, intentional or not, of genitors during the breeding history of a perennial crop. As expected, we were able to resolve more grape parentages on modern versus traditional cultivars and on western versus eastern cultivars probably due to genetic erosion and to collection representativeness, respectively. We showed that very few self-pollination events were selected. Rather short genealogies were revealed which may be the result either of genetic loss through history or of an actually limited number of crosses during the evolution of cultivated grape, in accordance with the existence of overlapping generations.

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