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Microsatellite analysis of ancient alpine grape cultivars: pedigree reconstruction of *Vitis vinifera* L. 'Cornalin du Valais'

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Abstract Ancient and closely related grape cultivars from the Alps were analyzed with 50 microsatellite markers: 'Cornalin', 'Humagne Rouge' and 'Goron' from Valais (Switzerland); 'Cornalin', 'Petit Rouge' and 'Mayolet' from the Aosta Valley (Italy). Our results confirmed previous studies showing that the 'Cornalin' cultivars from Switzerland and Italy are distinct, and that 'Humagne Rouge' is identical to 'Cornalin' from the Aosta Valley. We propose the nomenclature 'Cornalin du Valais' and 'Cornalin d'Aoste' in order to prevent further confusion. At each locus, 'Goron', 'Petit Rouge', 'Mayolet' and 'Cornalin d'Aoste' all share at least one allele with 'Cornalin du Valais', strongly suggesting parent/offspring relationships. Alleles at 49 out of 50 microsatellite loci are consistent with 'Cornalin du Valais' being the progeny of 'Petit Rouge' and 'Mayolet'. The exception is a 10-base pair discrepancy at one locus, most likely the result of somatic mutation in one of the parents, since this parentage is supported by high likelihood ratios and historical data. We hypothesize that 'Cornalin du Valais' originated in the Aosta Valley through a natural cross and was then introduced into Valais centuries ago, probably via the Great St. Bernard Pass. Furthermore, 'Cornalin du Valais' is likely to be one of the parents of both 'Goron' and 'Cornalin d'Aoste', the respective second parents remaining unknown. This pedigree provides a convincing explanation for the allele-sharing patterns and is strongly supported by historical data. The present work is the first grapevine parentage study to deal with a multiple repeat unit discrepancy at a microsatellite locus. We suggest that the use of increasingly large numbers of loci in making parentage determinations leads to a corresponding increase in the probability of encountering a locus with intra-cultivar variability during the analysis. We therefore assume that a sole multiple repeat unit discrepancy is not sufficient to discard a parentage hypothesis.

Keywords *Vitis vinifera* · Microsatellite · Parentage · Mutation · Pedigree reconstruction

Introduction

Valais (Switzerland) and the Aosta Valley (Italy) are adjacent wine-growing regions in the western Alps where several indigenous and ancient grape cultivars have been maintained. Previous ampelographic (Berget 1903) and molecular (Labra et al. 2002) studies pointed out some similarities between their cultivars. Within the sampling of a larger study of alpine cultivars (Vouillamoz et al., in preparation), we have selected a subset of closely related red varieties that are still cultivated to some extent in Valais and in the Aosta Valley (Table 1), and we have focused on their genetic relationships determined by microsatellite analysis.

'Cornalin' has been cultivated in Valais for a long time under the name 'Rouge du Pays' or 'Rouge du Valais'. A manuscript written in 1313 relates the purchase of a vineyard containing the variety *Neyrum* (from the Latin *nigrum* = black) that probably corresponds to 'Rouge du Pays' (Carruzzo 1991). Nicollier (1972) suggested chang-

Table 1 Grape cultivars included in this study. Six closely related cultivars were selected in Valais (Switzerland) and the Aosta Valley (Italy) for genetic relationship and parentage analysis

Valais	Aosta Valley
'Cornalin'	'Cornalin'
'Humagne Rouge'	'Petit rouge'
'Goron'	'Mayolet'

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ing the name ‘Rouge du Pays’ into ‘Cornalin’, borrowing this name from an ancient cultivar from the Aosta Valley. This Italian ‘Cornalin’, already described by Gatta (1837), was once relatively widespread but is now very rare in the Aosta Valley (Moriondo 1999). On the contrary, ‘Rouge du Pays’ in Valais was close to extinction a few decades ago but has now regained interest under this new name (Maigre et al. 2000). Therefore, there are now two different cultivars sharing the name ‘Cornalin’. ‘Petit Rouge’ is the most widespread indigenous cultivar in the Aosta Valley and was already described by Gatta (1837). It is a polymorphic cultivar for which Moriondo (1999) describes four different biotypes (clones). The name ‘Humagne Rouge’ was first mentioned in 1896 on a manuscript (Nicollier 1985, note 3) and the cultivar was probably introduced into Valais in the late XIXth century[1]. Nicollier (1972) believed that ‘Humagne Rouge’ was identical to ‘Petit Rouge’. However, recent morphological and molecular studies (Moriondo 1999; Maigre 2000; Labra et al. 2002) showed that ‘Humagne Rouge’ is in fact identical to ‘Cornalin’ from the Aosta Valley. As a consequence, both ‘Cornalin’ from the Aosta Valley and Valais are actually present in Valais under different names. ‘Mayolet’, a cultivar probably indigenous to the Aosta Valley (Berget 1904a), was first mentioned in a cellar notebook in 1788 (Tognan 2002) and was first described by Gatta (1837). Although it almost disappeared (Messiez 1998), Mayolet is now occasionally cultivated in the Aosta Valley in association with other cultivars (Moriondo 1999). ‘Goron’ is considered indigenous to Valais but is absent from the local literature until 1870, according to Nicollier (1985) who suggests that this cultivar was probably introduced into Valais from Italy, most likely from the Aosta Valley. The name Goron is probably derived from the indo-european root “gor” meaning red (Desfayes 1969), and it was sometimes confusingly applied to some undetermined red cultivars in Valais (Pulliat 1885; Berget 1903). Today ‘Goron’ is no longer cultivated but kept in grapevine collections only in Switzerland, and surprisingly not in the Aosta Valley. Besides its ampelographical meaning, Goron is also the name applied since 1959 in Valais (Nicollier 1974) to a red wine blend of ‘Pinot Noir’ and ‘Gamay’.

Microsatellites (or SSR – simple sequence repeats) are short tandem repeats of 1 to 6 bp of DNA distributed throughout the genome of the Eukaryotes. These molecular markers have recently proven to be useful in DNA fingerprinting and parentage analysis of grape cultivars (for a review see Sefc et al. 2001). Indeed, their high rate of polymorphism provides unique genotypes for every distinct cultivar (Thomas et al. 1994) and their co-dominant Mendelian inheritance allows the reconstruction of crosses (Bowers and Meredith 1997; Sefc et al. 1998; Bowers et al. 1999). In this study, microsatellite analysis has been carried out in order to determine genetic relationships and possible parent-offspring pairs in a group of closely related cultivars (Table 1). A new nomenclature for both ‘Cornalin’ from Valais and the Aosta Valley has been proposed. A total of 50 microsatellite markers were necessary to identify the parents of ‘Cornalin’ cultivated in Valais. Despite a multiple repeat unit discrepancy at one locus, the proposed parentage is strongly supported by both likelihood analysis and historical data.

Materials and method

DNA was extracted with the Qiagen DNEasy Plant Mini Kit from young leaves of grapevine cultivars (Table 1) sampled from Valais, Switzerland (n = 3; Station fédérale de Changins, Centre viticole du Caudoz, Pully) and the Aosta Valley, Italy (n = 3; IAR, Institut Agricole Régional, Aosta). Microsatellite loci were amplified and visualized as previously described (Bowers et al. 1996). Allele sizes were determined by comparison with known genotypes of standard cultivars. A total of 50 microsatellite markers were used for parentage analysis (Table 2). On the basis of their SSR profiles, the cultivars were probed for possible parent-offspring groups using a computer program (“DNA-data”, B. H. Prins, unpublished). This program offers the option of a user-defined level of discrepancy, in order to ascertain possible parentages despite the presence of a few allelic mismatches. After discarding the mismatching loci, the Identity program version 1.0 (Wagner and Sefc 1999) was then used to calculate the cumulative likelihood ratios for the proposed parentage. Likelihood ratios and their 95% upper confidence limits were calculated as described elsewhere (Bowers and Meredith 1997; Sefc et al. 1997) with the relative allelic frequencies at 31 microsatellite markers (one of the first 32 loci in Table 2 showed a discrepancy and was omitted) from the genotypes of up to 400 cultivars [2]. The remaining 18 markers in Table 2 were not included in the likelihood analysis because they were only genotyped for a geographically narrow group of 8 to 20 interrelated cultivars.

Results

Identity and parentage

The analysis of 32 microsatellite loci (Table 2) confirmed recent molecular studies (Moriondo 1999; Labra et al. 2002) showing that ‘Cornalin’ from Valais and ‘Cornalin’ from the Aosta Valley are distinct cultivars and that ‘Humagne Rouge’ is in fact identical to the ‘Cornalin’ from the Aosta Valley[3]. In order to prevent confusion, we propose the names ‘Cornalin du Valais’ and ‘Cornalin d’Aoste’ to distinguish the two cultivars. ‘Cornalin d’Aoste’, ‘Petit Rouge’, ‘Mayolet’ and ‘Goron’ all share

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[1] This name ‘Humagne Rouge’ is absent from the first ampelographic studies (Pulliat 1885; Berget 1903) and from early local surveys (Catalogue des différentes variétés de raisins exposés au concours agricole de Lucerne par la Société d’Agriculture de Sion, Imprimerie L. Schmid, Sion, 1881; Catalogue des raisins et des vins du Valais, Imprimerie P. Pfefferlé, Sion, 1903)

[2] The upper limit number of cultivars accepted in the Identity program is 400. The number of genotypes available in the database of the University of California, Davis, at 31 microsatellite markers ranges between 95 to 400 (see Table 2 for details)

[3] The only exception is at locus VVMD 8 where ‘Humagne Rouge’ shows the genotype 257–241 instead of 241–241 for ‘Cornalin d’Aoste’ (Table 2). This clonal variation is probably due to a non-amplified null allele in ‘Cornalin d’Aoste’

Table 2 Genotypes at 50 microsatellite markers. The proposed parentage ‘Cornalin du Valais’ = ‘Mayolet’ × ‘Petit Rouge’ is consistent for 49 out of 50 markers. The only discrepancy is a 10-base pair difference at VVMD 28 (bold). The number of cultivars included in the likelihood ratios (LR) analysis are given for each locus. ‘Cornalin d’Aoste’ and ‘Goron’ share at least one allele at each locus with ‘Cornalin du Valais’ and are most likely progenies of ‘Cornalin du Valais’, the other respective parents remaining unknown

Locus[a]	cvs in LR analysis	Parent 1 ‘Mayolet’	Progeny ‘Cornalin du Valais’	Parent 2 ‘Petit Rouge’	Progeny of ‘Cornalin du Valais’	
					‘Cornalin d’Aoste’	‘Goron’
VVMD 5	*400*	238–226	228–226	228–226	228–226	228–228
VVMD 6	*400*	214–211	211–211	214–211	211–205	211–205
VVMD 7	*400*	263–247	263–247	247–247	257–247	247–239
VVMD 24	*400*	214–208	214–214	219–214	214–210	214–210
VVMD 25	*400*	245–243	245–243	245–243	243–243	267–245
VVMD 27	*400*	189–185	189–185	189–189	189–185	189–189
VVMD 28	–	261–247	**271**–247	261–247	271–251	247–231
VVMD 31	*400*	216–212	212–210	216–210	216–212	216–210
VVMD 32	*400*	253–253	263–253	263–253	253–253	263–241
VVMD 34	*400*	240–240	240–240	240–240	240–240	240–240
VVMD 36	*400*	264–252	264–252	264–252	264–252	295–252
VVS 2	*400*	139–133	139–133	139–133	139–133	151–133
VVS 29	*400*	171–171	171–171	171–171	171–171	171–171
SsrVrZAG 62	*377*	205–195	195–195	205–195	195–195	195–195
SsrVrZAG 79	*377*	251–239	247–239	247–245	245–239	259–247
SsrVrZAG 83	*385*	197–193	197–193	197–193	197–193	197–193
SsrVrZAG 93	*353*	197–189	189–189	191–189	197–189	189–189
VVMD 21	*400*	256–249	256–249	249–249	256–249	256–249
VMC 2C3	*365*	192–170	198–192	198–165	198–165	198–198
VMC 5G6.1	*340*	139–138	139–138	153–139	142–138	151–139
VMC 2H4	*340*	224–202	224–202	224–202	224–210	218–202
VMC 2A5	*340*	173–157	157–157	171–157	157–157	157–157
VVMD 26	*391*	251–249	249–249	251–249	255–249	251–249
VVS 1	*125*	182–181	181–181	181–181	181–162	190–181
VVMD 17	*191*	220–212	220–212	221–212	220–220	212–212
VVS 4	*170*	175–169	175–169	175–168	169–167	175–168
SsrVrZAG 64	*114*	165–143	165–143	143–141	165–165	143–143
VMC 5A1	*95*	171–157	171–167	171–167	171–167	167–167
VMC 5G1.1	*95*	259–239	239–130	259–130	239–130	239–140
VMC 5H2	*95*	209–194	209–194	201–194	209–195	194–194
VMC 5H5	*95*	188–168	180–168	188–180	168–168	180–178
VVMD 8	*111*	141–141	143–141	143–141	141–141*	141–141
VMC 2B3	–	182–182	182–182	182–166	182–182	182–182
VMC 5C1	–	172–147	147–147	172–147	147–147	147–147
For the following 16 loci, the actual allele size in base pairs was not determined; alleles are indicated by letters, A being the smallest [b]						
VMC 1E8	–	B-A	C-B	C-B	C-A	D-B
VMC 2B1.1	–	B-A	A-A	B-A	A-A	A-A
VMC 2B5	–	B-A	D-B	D-C	B-A	E-D
VMC 2E7	–	D-A	B-A	E-B	C-A	D-A
VMC 2E8	–	E-A	E-A	B-A	E-C	C-A
VMC 2F10	–	B-A	B-A	B-B	B-A	C-A
VMC 2H10	–	C-B	B-A	E-A	E-A	D-B
VMC 3D12	–	C-B	D-B	D-B	D-A	B-A
VMC 4C6	–	A-A	A-A	B-A	B-A	A-A
VMC 5A10	–	C-A	C-C	C-A	C-C	C-B
VMC 5C5	–	C-B	D-B	D-C	B-A	B-B
VMC 5G8	–	D-B	B-A	B-A	B-A	C-B
VMC 8G6	–	C-B	C-B	C-B	C-A	D-B
VMC 8G9	–	A-A	C-A	C-A	B-A	C-C
VMC 9A3.1	–	A-A	A-A	A-A	A-A	B-A
VMC 16F3	–	C-A	A-A	C-A	B-A	B-A

[a] Loci are given in the chronological order of their analysis

[b] Allele sizes range between 100–230 bp. The number of bp between two subsequent letters is not linear within a marker and not equivalent over the 16 markers (e.g.: at the locus VMC 1E8, the number of bp between alleles A and B is 4, between B and C is 24, etc; at the locus VMC 2B1.1, the number of bp between alleles A and B is 10, between B and C is 4, etc)

* The accession ‘Humagne Rouge’, synonym of ‘Cornalin d’Aoste’, showed the genotype 157–141

Table 3 Potential pairs of parents for 'Cornalin du Valais' with 32 and then 50 microsatellite markers. After the analysis of a first set of 32 markers, and taking into account the possibility of 1 to 3 discrepancies, four different pairs of parents could not be excluded. An additional set of 18 markers (50 microsatellite loci in total) was necessary to isolate the pair 'Mayolet' and 'Petit Rouge', showing only one discrepancy at VVMD 28

Putative pairs of parents	First set of 32 markers[a]		Second set of 18 markers[b]		Total
	Mismatching loci	Discrepancies	Mismatching loci	Discrepancies	
'Goron' × 'Mayolet'	VVMD 8, 28	2	VMC 2H10, 2E7, 3D12, 5C5, 5G8	5	7
'Goron' × 'Cornalin d'Aoste'	VVMD 7, 8, VMC 2C3	3	VMC 2E7, 5C5	2	5
'Petit Rouge' × 'Cornalin d'Aoste'	VVMD 7, VMC 2C3	2	VMC 2H10	1	3
'Mayolet' × 'Petit Rouge'	VVMD 28	1		0	1

[a] First 32 markers in Table 2
[b] Last 18 markers in Table 2

Table 4 Likelihood-ratio values. Likelihood-ratio values based on relative allele frequencies are given for the parentage 'Mayolet' × 'Petit Rouge' = 'Cornalin du Valais' versus other possibilities. Relative allele frequencies (data not shown) were calculated from up to 400 cultivars (total probability of identity: PI = 1.07×10^{-24}) at 31 microsatellite markers. Values in parentheses are the cumulative likelihood ratios calculated with the 95% upper confidence limits for the allele frequencies

Cumulative likelihood ratios of the suggested parents[a] of 'Cornalin du Valais': 'Mayolet' × 'Petit Rouge' (2) *versus*				
X × Y[b]	(1) × X[c]	(1) × (2) relative[d]	(2) × X[c]	(2) × (1) relative[d]
2.04×10^{19} (2.84×10^{15})	8.39×10^{9} (1.05×10^{8})	2.38×10^{3} (6.63×10^{2})	2.88×10^{10} (4.54×10^{8})	5.27×10^{3} (1.48×10^{3})

[a] The order of the parents does not indicate the actual direction of the cross
[b] X and Y are random unrelated cultivars
[c] The identity of one of the suggested parents is assumed and the other parent is unknown
[d] The identity of one of the suggested parents is assumed and the other parent is a close relative to the second suggested parent

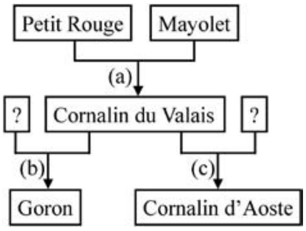

Fig. 1 Pedigree reconstruction of 'Cornalin du Valais'. Alleles at 49/50 microsatellite loci are consitent with 'Cornalin du Valais' being the progeny of a cross between 'Mayolet' and 'Petit Rouge' (*a*). Alleles at 50/50 microsatellite loci are consistent with 'Cornalin du Valais' being one of the parents of 'Goron' (*b*) and 'Cornalin d'Aoste' (*c*), the other respective parents remaining unknown (extinct?)

at least one allele at each locus with 'Cornalin du Valais'. Within this group, and taking into account the possibility of discrepancies at some loci, four possible pairs of parents for 'Cornalin du Valais' could not be excluded (Table 3). After the analysis of 18 additional markers (Table 2), 'Cornalin du Valais' still shared at least one allele at each locus with the other four cultivars, however only one possible parental relationship remained (Fig. 1): alleles at 49 out of 50 loci are consistent with 'Cornalin du Valais' being the progeny of 'Petit Rouge' and 'Mayolet'. The only exception is a 10-base pair discrepancy at locus VVMD 28 (Table 2).

Likelihood ratios

Because of the discrepancy, locus VVMD 28 was omitted from the likelihood analysis. The total probability of identity among the 400 cultivars used for the likelihood analysis is very low (PI = 1.07×10^{-24}). Likelihood ratios compare the probability of the observed genotype if the alleles came from the proposed parents with the probability of the genotype if the alleles came from two random parents or from close relatives of the proposed parents (Table 4). The likelihood ratios of the probability of the proposed parentage 'Cornalin du Valais' = 'Mayolet' × 'Petit Rouge' versus two random cultivars is extremely high: $>10^{19}$ ($>10^{15}$ with 95% upper confidence limits of allele frequencies). The ratios of probability of this parentage versus a cross between one of the parents and a relative of the other parent are much lower, however they are still over 2,000 (over 600 with 95% upper confidence limits of allele frequencies).

Discussion

Nomenclature

The name ‘Cornalin’ is applied to two different cultivars (Table 1): one in the Aosta Valley and one in Valais, where it was formerly named ‘Rouge du Pays’ or ‘Rouge du Valais’ until Nicollier (1972) suggested changing the name from ‘Rouge du Pays’ to ‘Cornalin’, borrowing this name from the Aosta Valley cultivar. This name change was in disagreement with Article 26.2 of the International Code of Nomenclature for Cultivated Plants (Trehane et al. 1995) concerning the rules for the re-use of a cultivar epithet. According to the principle of precedence in the Code, the correct names should be ‘Rouge du Valais’ for the Valais cultivar, first described in Berget (1904d), and ‘Cornalin’ for the Aosta Valley cultivar, first described in Gatta (1837). However, in order to maintain the current use and to avoid undesirable changes in nomenclature (according to Article 14.2 of the Code), we propose here the following new names (the complete botanical names are given):

(1) *Vitis vinifera* L. ‘Cornalin du Valais’ should be applied to ‘Rouge du Valais’, first described in Berget (1904d), also called ‘Rouge du Pays’ and lately named ‘Cornalin’ by Nicollier (1972). The standard is in the collection at Centre viticole du Caudoz, Pully, Switzerland;
(2) *Vitis vinifera* L. ‘Cornalin d’Aoste’ should be applied to ‘Cornalin’ first described by Gatta (1837), called ‘Humagne Rouge’ in Valais and sometimes ‘Cornallin’ or ‘Corniola’ in the Aosta Valley. The standard is in the collection at the Institut Agricole Régional d’Aoste (IAR).

These simple changes in nomenclature should prevent further confusion, preserve the common usage and give valuable information for both the consumers and the producers of Cornalin wines.

Parentage analysis

The parentage ‘Cornalin du Valais’ = ‘Mayolet’ × ‘Petit Rouge’ is strongly supported by high likelihood ratio values (Table 4): it is over 10^{19} times more likely that ‘Cornalin du Valais’ is the progeny of ‘Mayolet’ and ‘Petit Rouge’ than any two random cultivars. Allelic frequencies were calculated at 31 microsatellite markers with a range of 95 to 400 cultivars (see Table 2 for details). In order to compensate sampling errors for loci with a smaller number of cultivars, the cumulative likelihood ratios were also calculated with the 95% upper confidence limits for the allele frequencies: the proposed parentage is still highly supported ($>10^{15}$) versus any two random cultivars. The values become lower when one of the suggested parents is assumed and the other parent is a close relative to the second suggested parent because close relatives share many alleles with the putative parents.

This parentage is also well supported by historical data. Viticulture is supposed to be approximately two thousand years old in the Aosta Valley (Moriondo 1999), and at least one thousand years old in Valais (Nicollier 1985). In both areas, numerous barbaric invasions led to the abandonment of viticulture, sometimes for more than a century. During these periods, grapevines had returned to a more or less feral state, thus making very likely the loss of some ancient cultivars. It is from this indigenous resource that the local vine growers had to reconstitute their vineyards (Gatta 1837; Berget 1904b; Moriondo 1999). This may explain why many cultivars are genetically so closely related. The vestiges of old Roman roads joining Valais and the Aosta Valley over the mountains testify that these valleys have been connected for at least two thousand years. A monastery on top of the Great St. Bernard Pass has been controlling the links between the two valleys for about one thousand years (Donnet 1950). According to Berget (1904b, c), the church canons were probably responsible for the exchange of grapevines from one valley to another (for example, the white ‘Prié’ from Aosta is known in Valais under the name ‘Bernarde’, probably deriving from the name of the pass). This could explain how ‘Cornalin du Valais’, born in the Aosta Valley, reached Valais several centuries ago. Another historical fact supports this parentage: ‘Petit Rouge’ and ‘Mayolet’ have been grown together in the same vineyards for at least two centuries to produce the red wine blend “Torrette” that was already famous at the beginning of the XIXth century (Gatta 1837). Together with the long viticultural history of both valleys, this strongly supports the parentage of ‘Cornalin du Valais’. So, why did ‘Cornalin du Valais’ disappear from the Aosta Valley? The variety ‘Petit Rouge de Châtillon’ may be the answer: Berget (1904b) notes that this cultivar is different from the other forms of ‘Petit Rouge’ in the Aosta valley because its leaves turn red during berry maturation. This is actually one of the main characteristics of ‘Cornalin du Valais’. Berget (op. cit.) proposed that ‘Cornalin du Valais’ is in fact this ‘Petit Rouge de Châtillon’ imported in Valais centuries ago, an opinion shared by Nicollier (1974). Unfortunately, ‘Petit Rouge de Châtillon’ has now most likely disappeared from the Aosta Valley (G. Moriondo, personal communication) so no molecular confirmation of this identity will be possible.

Although grapevines are mainly self-pollinated due to cleistogamic flower morphology, wind and insects can occasionally be responsible for cross-pollination (Mullins et al. 1992). In 1694, Camerarius was the first to assert that plants, like animals, are sexually differentiated, and the first recorded deliberate cross between plants was made with carnations in 1718 (North 1979). Given that ‘Cornalin du Valais’ was already cultivated before its first mention in 1313 (Carruzzo 1991), and because cleistogamy in grapes makes man-made cross-pollination difficult, we believe that ‘Cornalin du Valais’ is the result of a natural cross between ‘Mayolet’ and ‘Petit Rouge’.

The suggested parentage is supported by high likelihood ratios and historical data, but it shows a 10-base pair discrepancy at locus VVMD 28 (Table 2). In grapevine genotyping studies, 2-base pair discrepancies have already been observed between parents and progeny (Bowers et al. 1999; Piljac et al. 2002), and they were simply explained by a somatic mutation in either a parent or the offspring. However, a 10-base pair discrepancy has never been reported in grapevine parentage analysis. Can this discrepancy still be justified by a simple somatic mutation? In our VVMD 28 discrepancy case, both putative parents ‘Mayolet’ and ‘Petit Rouge’ have the allele 261 whereas ‘Cornalin du Valais’ has the allele 271 (Table 2), which is an increase of five repeat units (VVMD 28 is a di-nucleotide microsatellite). In two recent studies on grapevine microsatellite clonal variation (Franks et al. 2002; Riaz et al. 2002), increases of more than one or two repeats have been reported to occur as a chimeric third allele intermediate in size to the two standard alleles of the cultivar. Moreover, Riaz et al. (2002, Tables 2 and 3) observed several clonal variations within two cultivars (‘Pinot Noir’ and ‘Chardonnay’) where one allele of the standard genotype of the cultivar was replaced by a variant allele exhibiting a size difference as large as 18-base pairs. ‘Petit Rouge’, ‘Mayolet’ and ‘Cornalin du Valais’ are of comparable age to ‘Pinot Noir’ or ‘Chardonnay’ and we therefore suggest that clonal variation is also responsible for the 10-base pair difference between ‘Cornalin du Valais’ and its parents. Does the clonal variation exist in the parents or in the progeny? We have genotyped a different clone of ‘Cornalin du Valais’ growing in Ardon (Valais) as an uncultivated vine supposed to be around 250 years old (M. Pont, personal communication) and both clones showed the same genotype. We therefore suggest that this 271-bp allele at VVMD 28 comes from clonal variation within one of the parents. Since ‘Mayolet’ is rather homogeneous and ‘Petit Rouge’ is phenotypically very variable (Moriondo 1999), the clonal variation most likely exists in ‘Petit Rouge’.

If we hypothesize that the VVMD 28 allele of 271 base pairs in ‘Cornalin du Valais’ did not originate as a clonal variant of one of the parents, then it might have been supplied by another cultivar. Among the sampling of our larger study (Vouillamoz et al., in preparation), as well as in the different databases investigated (University of California, Davis; Grape Microsatellite Collection, IASMA, Italy[4]), there are only two cultivars (other than the putative parents) that share at least one allele with ‘Cornalin du Valais’ at each locus: ‘Goron’ and ‘Cornalin d’Aoste’. Between the two, only ‘Cornalin d’Aoste’ has the particular allele 271 at VVMD 28 (Table 2). For this 10-base pair discrepancy, we could therefore suggest an alternative explanation that is consistent at all 50 loci: ‘Cornalin du Valais’ = (‘Mayolet’ × ‘Petit Rouge’) × ‘Cornalin d’Aoste’[5]. Every available cultivar from the Aosta Valley was sampled (Vouillamoz et al., in preparation), so the theoretical offspring-cultivar (‘Mayolet’ × ‘Petit Rouge’) might represent an extinct cultivar. However, if this alternative hypothesis were correct, ‘Mayolet’ and ‘Petit Rouge’ would be the grandparents of ‘Cornalin du Valais’ and each of them should share (on average) 1/4 of its alleles with ‘Cornalin du Valais’. On the contrary, our results showed that both ‘Mayolet’ and ‘Petit Rouge’ actually share at least 1/2 of their alleles with ‘Cornalin du Valais’ (Table 2), which is the basic condition for parent/offspring relationships. For this reason, and despite the 10-base pair discrepancy at one marker, we strongly favor the first parentage hypothesis (Fig. 1a) where ‘Cornalin du Valais’ is the direct progeny of ‘Mayolet’ and ‘Petit Rouge’, rather than the second parentage hypothesis where ‘Cornalin du Valais’ would be the grandchild of ‘Mayolet’ and ‘Petit Rouge’.

Pedigree reconstruction

‘Cornalin du Valais’ being the progeny of ‘Mayolet’ and ‘Petit Rouge’ does not explain why ‘Goron’ and ‘Cornalin d’Aoste’ also share at least one allele at each locus with ‘Cornalin du Valais’ (Table 2). This can be justified by introducing two additional parentages (Fig. 1b and c): ‘Cornalin du Valais’ is one of the parents of both ‘Goron’ and ‘Cornalin d’Aoste’. The other respective parents are two different unknown cultivars, possibly one of the extinct red cultivars from the Aosta Valley (‘Persagn’, ‘Pertenzi’, ‘Cugnet’, ‘Eperon’) mentioned by Moriondo (1999). It is not surprising to see ‘Cornalin du Valais’ as ancestral to the other two cultivars, since the first mention of ‘Cornalin du Valais’ most likely dates back to 1313 (Carruzzo 1991), while ‘Cornalin d’Aoste’ and ‘Goron’ were mentioned much later (Gatta 1837; Nicollier 1985). Furthermore, this pedigree has the advantage of explaining why the particular allele 271 at VVMD 28 in ‘Cornalin du Valais’ is also present in ‘Cornalin d’Aoste’ (Table 2).

Conclusion

The complete pedigree reconstruction of ‘Cornalin du Valais’ can be summarized as follows (Fig. 1):

(1) ‘Cornalin du Valais’ = ‘Mayolet’ × ‘Petit Rouge’,
(2) ‘Goron’ = ‘Cornalin du Valais’ × ‘?’,
(3) ‘Cornalin d’Aoste’ (syn.: ‘Humagne Rouge’) = ‘Cornalin du Valais’ × ‘?’.

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[4] http://www.ismaa.it/areabioav/gmc.html

[5] The following relationships are also consistent at all 50 loci: ‘Cornalin du Valais’ = (‘Petit Rouge’ × ‘Cornalin d’Aoste’) × ‘Mayolet’, and ‘Cornalin du Valais’ = (‘Cornalin d’Aoste’ × ‘Mayolet’) × ‘Petit Rouge’

This group of cultivars constitute a closely related genetic pool originating in the Aosta Valley: ‘Petit Rouge’ and ‘Mayolet’ are the parents of ‘Cornalin du Valais’ and the grandparents of both ‘Goron’ and ‘Cornalin d’Aoste’. The present work is the first microsatellite grapevine parentage study to deal with a multiple repeat unit discrepancy. Since the parentage ‘Cornalin du Valais’ = ‘Mayolet‘ × ‘Petit Rouge‘ is supported by high likelihood ratio values and historical data, this shows that a sole multiple repeat unit discrepancy is not sufficient to reject a parentage. The majority of parent-offspring relationships recently published for grapevine used 25 to 32 microsatellite markers (Bowers and Meredith 1997; Sefc et al. 1997, 1998; Bowers et al. 1999; Piljac et al. 2002). We had to analyse 50 microsatellite markers in order to clarify the pedigree of this group of closely related cultivars. By increasing the number of loci, we also increased the chances to encounter a clonal mutation, especially with such ancient cultivars. We therefore suggest that, when possible, several clones of the cultivars should be analysed to prevent clonal variation from hiding actual parentages.

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