

Identity and parentage of two alpine grape cultivars from Switzerland (*Vitis vinifera* L. Lafnetscha and Himbertscha)

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Summary

Four closely related white grape cultivars from the Western Alps (Switzerland) - Humagne Blanc, Completer, Lafnetscha and Himbertscha - and three putative relatives or synonyms - Gouais Blanc, Plantscher and Bordeaux Blanc - were analyzed with up to 50 microsatellite markers. Humagne Blanc and Completer are ancient cultivars from the Haut-Valais and Graubünden regions, respectively. Lafnetscha and Himbertscha are lesser-known cultivars scarcely cultivated in Haut-Valais. Lafnetscha is frequently considered as synonym of Completer. Himbertscha might be related to Gouais Blanc, one of the parents of Chardonnay, Gamay, etc. Plantscher, a putative synonym of Lafnetscha, is scarcely cultivated in Haut-Valais (Switzerland) and Bordeaux Blanc (or Gros Bourgogne) is a cultivar of unknown origin (despite its names) cultivated in Switzerland. Our results allowed us to determine the true-to-type Lafnetscha and confirmed that Lafnetscha is not a synonym of Completer. Plantscher is not a synonym of Lafnetscha but a synonym of Bordeaux Blanc (or Gros Bourgogne) and is a likely parent or progeny of the Hungarian Furmint. Himbertscha is not related to Gouais Blanc and shares at least one allele at each locus with Humagne Blanc, providing strong evidence of a parent/progeny relationship. Given that Humagne Blanc is an older cultivar, we propose that it is the parent of Himbertscha. Alleles at 49 out of 50 microsatellite loci are consistent with Lafnetscha being the progeny of Completer and Humagne Blanc. The exception is a 10-base pair discrepancy at one locus (VVMD 36), most likely due to the occurrence of a null allele, since this parentage is supported at other markers by very high likelihood ratios. With Lafnetscha = Completer x Humagne Blanc, we present the second grape cultivar parentage showing a multiple repeat discrepancy at one locus. This study emphasizes that one multiple repeat unit discrepancy is not sufficient to reject a parentage, and that the greater is the number of loci, the greater are the chances to encounter null alleles or clonal mutations.

Key words: grape cultivar, microsatellite, null allele, parentage, *Vitis vinifera*.

Introduction

Several indigenous grape cultivars have been maintained in ancient wine-growing regions of the Western Alps. While

studying the genetic relationships among some autochthonous alpine cultivars, we observed that 4 white cultivars from Switzerland seemed to be closely related: Humagne Blanc, Completer, Lafnetscha and Himbertscha. Humagne Blanc is an ancient cultivar, regarded as indigenous to the Haut-Valais region (BERGET 1904 b). It was first mentioned in 1313 as *Humagny* in a manuscript relating the purchase of a vineyard (CARRUZZO 1991). Completer (synonyms: Malans-traube, Lindauer, and sometimes Lafnetscha) is regarded as an indigenous cultivar from Graubünden (Eastern Switzerland) and has never been mentioned in Haut-Valais (Southwestern Switzerland). Some authors claim that Completer was already mentioned in Malans (Graubünden) in 926 (*e.g.* BELLASI *et al.* 1993). This would represent one of the oldest references of a grape cultivar name. However, neither a detailed study of the archives concerning ancient vineyards in Malans (JENNY 1938), nor other local studies (DURNWALDER 1983; SEELIGER 1988) or personal communications confirmed the date 926. Vineyards were indeed already known around Malans in 765 (KOBLET 1994), but the first mention of the name Completer for a cultivar dates back to 1321 ('... *quoad vinea vini completorii sisa in Malans...*') "only" (JENNY 1938; DURNWALDER 1983). Lafnetscha and Himbertscha are scarcely cultivated in Haut-Valais. Lafnetscha is frequently considered as a synonym of Completer in literature (AMBROSI *et al.* 1997; GALET 2000). According to CHANTON (1972), Himbertscha is morphologically close to Gouais Blanc and could have been introduced from Liguria (Italy). This hypothesis is interesting since SCHNEIDER *et al.* (2001) recently showed that Liseiret from Piedmont (north of Liguria) is in fact Gouais Blanc. Lafnetscha and Himbertscha might not be as old cultivars as Humagne Blanc. The first document mentioning cultivars from Haut-Valais dates back to 1586 (NICOLLIER 1970) and speaks about "heyda, göchschen, gwäss, blantschier...", corresponding to the modern cultivars "Heida, ?, Gouais Blanc, Blanchier...". Heida is the local synonym for Savagnin blanc (syn. Traminer). The name "göchschen" does not correspond to any known cultivar. Gouais Blanc (syn. Heunisch) was widespread all over Europe in the Middle Ages and is the direct parent of several important cultivars such as Chardonnay and Gamay (BOWERS *et al.* 1999). Blanchier, according to BERGET (1904 a) and CHANTON (1972), is a synonym of Lafnetscha. This would mean that Lafnetscha is an ancient cultivar. However, BIFFIGER (1972) considers Blanchier as a synonym of Plantscher (or Plantschier, Plantier), a scarcely cultivated white wine grape from Haut-Valais that seemed to be possibly related to Bor-

deaux blanc, cultivars of unknown origin (despite its names) cultivated in Switzerland). In this case, if Blanchier is not a synonym of Lafnetscha, then neither Lafnetscha nor Himbertscha were cited in the 1586 document and their first mention would be in STEBLER (1901).

There are 8,000 to 10,000 grape cultivars existing worldwide today (ALLEWELDT 1997), and the origin of most of them is generally wrapped in legends. Since the development of microsatellite markers for grape cultivars (THOMAS and SCOTT 1993), the origin and parentage of economically important cultivars such as Cabernet Sauvignon (BOWERS and MEREDITH 1997), Chardonnay (BOWERS *et al.* 1999) and Syrah (BOWERS *et al.* 2000) have been uncovered. The high polymorphism of microsatellites (or SSR - simple sequence repeats), a special class of tandem repeat loci involving a base motif of 1-6 bp of DNA repeated up to 100 times (TAUTZ 1993), allows cultivar identification (THOMAS *et al.* 1994; BOTTA *et al.* 1995; SEFC *et al.* 1998a) and their co-dominant Mendelian inheritance allows the reconstruction of crosses (SEFC *et al.* 1998b; PILJAC *et al.* 2002; VOULLAMOZ *et al.* 2003). Interestingly, hundreds of cultivars have now been genotyped by different researchers, but the number of parent/offspring relationships discovered among the total number of cultivars is low. This is probably because the parents of most modern cultivars have now likely disappeared. For a better understanding of viticultural history and migrations, it is therefore also interesting to document the parentage of autochthonous cultivars (SEFC *et al.* 1998 b; MEREDITH *et al.* 1999; PILJAC *et al.* 2002; VOULLAMOZ *et al.* 2003). In this work, true-to-typeness and parentage among closely or supposedly related alpine cultivars from Switzerland (Humagne Blanc, Lafnetscha, Himbertscha, Completer, Gouais Blanc, Plantscher and Bordeaux blanc) were analysed with up to 50 microsatellite markers.

Material and Methods

Materials and methods are similar to VOULLAMOZ *et al.* (2003). DNA was extracted with the Qiagen DNEasy Plant

Mini Kit from young leaves of different accessions of grapevine cultivars (Tab. 1) sampled from Agroscope RAC Changins, Centre viticole du Caudoz, Pully (n=6) and from the Oskar Chanton Kellerei vineyards (Visp, Switzerland) (n=3). Blanchier was not included in the sampling because there is no accession under this name in grape germplasms or vineyards in Switzerland. 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. Nebbiolo Lampia from the collection of CNR (Consiglio Nazionale delle Ricerche, Unità Viticoltura, Torino, Italy) at Grinzane Cavour (Piedmont) was included in this study as a well-known reference variety for allele size. The genotypes were compared to more than 1,600 known genotypes from various references in literature and from different databases (University of California, Davis; Greek Vitis Database, University of Crete, Heraklion, Greece, <http://www.biology.uoc.gr/gvd/>; Grape Microsatellite Collection, IASMA, Italy, <http://relay.ismaa.it:2164/genetic/gmc.html>) at all the available loci and to other accessions of Lafnetscha and Completer (BAUMGARTNER *et al.* 2000) at 5 loci (VVS 1, VVS 2, VVS 4, VVMD 5, VrZAG 21, VrZAG 62). Comparisons were made possible after harmonizing the genotype data from different databases or sources by confronting genotypes of standard well-known cultivars.

A total of 50 microsatellite markers were used for parentage analysis (Tab. 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, unpubl.). 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, we then used the Identity program version 1.0 (WAGNER and SEFC 1999) 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) with the relative allelic frequencies at 31 microsatellite markers (the discrepancy locus was omitted) from the geno-

Table 1

Grapevine cultivars included in this study. 9 accessions of 7 cultivars were selected in Switzerland for genetic relationship and parentage analysis

Cultivar	Origin	Collection site ^a
Humagne Blanc	Valais	RAC
Lafnetscha	Valais	RAC; Chanton, Schlüssel-Achra vineyard
Himbertscha	Valais	RAC; Chanton, Varen vineyard
Completer	Graubünden	RAC
Plantscher	Valais	Chanton, Schlüssel-Achra vineyard
Bordeaux Blanc	Switzerland	RAC
Gouais Blanc	Europe	RAC

^a RAC = Agroscope RAC Changins, Centre Viticole du Caudoz, Pully (Switzerland); Chanton = Oskar Chanton Kellerei vineyards (Visp, Switzerland), Josef-Marie Chanton.

types of up to 400 cultivars (the upper limit number of cultivars accepted in the Identity program). The number of genotypes available in the database of the University of California, Davis at 31 microsatellite markers ranges between 95 and 400 (see Tab. 2 for details). In order to compensate for sampling error for loci with a smaller number of cultivars, the cumulative likelihood ratios were also calculated with the 95 % upper confidence limits of the allele frequencies. Observed (H_o) and expected (H_e) levels of heterozygosity were calculated by standard methods with the software Identity 1.0 (WAGNER and SEFC 1999). The frequency of null alleles was calculated as $(H_e - H_o)/(1 + H_e)$ according to BROOKFIELD (1996) at the first 32 markers. The remaining 18 markers (Tab. 2) were not included in the likelihood analysis and in the estimation of the frequency of null alleles because they were only genotyped for a geographically narrow group of 8 to 20 interrelated cultivars.

Results

Identity and parentage: All the accessions of the grape cvs Humagne Blanc, Completer, Lafnetscha, Himbertscha, Gouais Blanc, Plantscher and Bordeaux Blanc (Tab. 1) were firstly genotyped at 34 microsatellite loci (Tab. 2), which is far more than required for identification purposes (see SEFC *et al.* 2001). It showed that a) Humagne Blanc, Completer, Lafnetscha and Himbertscha did not have any unexpected synonym among over 1,600 already genotyped cultivars, b) Plantscher, which was different from both Lafnetscha accessions, turned out to be a synonym of Bordeaux Blanc from RAC and Gros Bourgogne from the database of the University of California, Davis (data not shown) c) both accessions of Himbertscha were identical, d) Himbertscha was not related to Gouais Blanc (data not shown) and e) both accessions of Lafnetscha were different. The accession Lafnetscha from RAC had the same genotype as Completer from RAC, as suggested by morphological examination, whereas the accession Lafnetscha from Chanton showed a distinct and unique genotype. The accession Completer from RAC is consistent with the first ampelographic description of Completer (KÖHLER 1869) and matches the genotypes of 8 other independent accessions of Completer in Switzerland analysed at 5 loci (BAUMGARTNER *et al.* 2000). The analysis of 16 additional loci for a total of 50 microsatellite markers (Tab. 2) showed that Himbertscha shared at least one allele at each locus with Humagne Blanc and that alleles at 49 out of 50 loci were consistent with Lafnetscha being the progeny of Completer and Humagne Blanc. The only exception was a 10-base pair discrepancy at locus VVMD 36.

Likelihood ratios: Locus VVMD 36 was not included in the likelihood ratio calculation since it showed a discrepancy in the parentage (Tab. 2). Computation was made with 400 cultivars (present in the database of the University of California, Davis) from different geographical origins with a very low total probability of identity ($PI = 4.76 \times 10^{-25}$). Likelihood ratios of the proposed parentage 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 (Tab. 3). The likelihood ratios of the probability of the proposed parentage Lafnetscha = Completer x Humagne Blanc vs. two random cultivars is extremely high: $>10^{24}$ ($>10^{19}$ with 95 % upper confidence limits of allele frequencies). The ratio of probability of this parentage versus a cross between one of the parents and a relative of the other parent is over 10,000 (over 5,000 with 95 % upper confidence limits of allele frequencies).

Null allele frequency: The frequency of null alleles (Tab. 2) was estimated with expected (H_e) and observed (H_o) heterozygosities (data not shown) at the first 32 markers. The average frequency of null alleles over all 32 markers was -0.01 (min. = -0.059, max. = 0.134). Only 7 out of 32 markers showed positive frequencies (Tab. 2) and ranged from 0.002 (VVMD 17) to 0.134 (VVMD 8). The discrepancy locus (VVMD 36) showed a positive null allele frequency of 0.016.

Discussion

The analysis of 50 microsatellites allowed us to determine true-to-type cultivars and parentage within the accessions of 7 alpine grape cultivars from Switzerland (Humagne Blanc, Completer, Lafnetscha, Himbertscha, Gouais Blanc, Plantscher and Bordeaux Blanc). Completer and Lafnetscha are often considered as synonyms in literature (AMBROSI *et al.* 1997; GALET 2000). The genotype of Completer from RAC (Tab. 1) is identical at 5 loci to that of 8 independent accessions of Completer in Switzerland reported in BAUMGARTNER *et al.* (2000). It can be therefore considered the true-to-type Completer. The accession Lafnetscha from RAC was consequently misidentified, as suggested by recent morphological examination, since it is in fact Completer. Misidentification of other grape cultivars under the name Lafnetscha is not unique: BAUMGARTNER *et al.* (2000) showed with 5 microsatellite markers that three independent accessions labelled Lafnetscha in different collections in Switzerland matched the genotypes of Gouais Blanc, Heida (synonym of Savagnin) and Completer, respectively. Is Lafnetscha a synonym of Blanchier as suggested by BERGET (1904 a) and CHANTON (1972)? Unfortunately, there is no accession of Blanchier available in collections or vineyards of Switzerland, so no direct comparison would be possible. The term Blanchier comes from “blanc” (“white”) and is therefore equivocal. Blanchier was supposedly already mentioned in 1586 (NICOLLIER 1970) under the name “blantschier”. According to BIFFIGER (1972), Blanchier is different from Lafnetscha and could be identical to Plantscher. Microsatellite analysis confirmed that Plantscher is different from Lafnetscha and revealed that Plantscher is actually a synonym of Bordeaux Blanc from RAC (or Gros Bourgogne in the database of the University of California, Davis), an ancient cultivar of unknown origin (GALET 2000). Moreover, Plantscher is in fact the likely parent or progeny of the well-known Hungarian cv. Furmint (from the database of the University of California, Davis), since they share at least

Table 2

Genotypes at 50 microsatellite markers. The proposed parentage Lafnetscha = Completer x Humagne Blanc is consistent for 49 out of 50 markers. The only discrepancy is a 10-base pair difference at VVMD 36 (bold). Himbertscha shares at least one allele at each locus with Humagne Blanc and is most likely the progeny of the more ancient Humagne Blanc, the other parent remaining unknown. Plantscher is a synonym of Bordeaux Blanc (or Gros Bourgogne) and shares at least one allele at each locus with Furmint (not shown). The estimated frequency of null allele (*r*) and the number of cultivars included in the likelihood ratios analysis (*n*) are given for each locus. Nebbiolo Lampia was included as a reference cultivar

Locus	<i>r</i>	<i>n</i>	Parent 1	Progeny	Parent 2	Progeny of Humagne B.		Reference cultivar
			Completer	Lafnetscha	Humagne B.	Himbertscha	Plantscher	Nebbiolo
VVMD 5	-0.013	400	232-228	236-228	236-226	236-226	240-232	236-232
VVMD 6	-0.037	400	214-205	212-205	212-211	211-211	214-194	214-212
VVMD 7	-0.011	400	239-233	253-239	253-243	243-233	249-239	249-247
VVMD 8	+0.134	111	143-141	143-143	143-143	143-141	143-143	167-141
VVMD 17	+0.002	191	212-212	222-212	222-220	222-212	222-222	222-212
VVMD 21	-0.007	400	256-243	256-249	256-249	266-249	258-243	249-249
VVMD 24	-0.024	400	214-214	214-208	210-208	212-210	210-210	214-210
VVMD 25	-0.032	400	245-243	259-245	259-253	259-245	245-245	245-243
VVMD 26	-0.027	391	255-249	249-249	251-249	249-249	249-249	251-249
VVMD 27	-0.033	400	185-179	185-179	189-185	189-185	194-183	189-185
VVMD 28	-0.022	400	237-231	247-237	261-247	261-247	251-231	271-239
VVMD 31	-0.011	400	216-196	216-212	212-204	212-212	214-212	212-212
VVMD 32	-0.013	400	263-241	263-257	263-257	263-241	273-265	263-241
VVMD 34	-0.013	400	240-240	240-240	240-240	240-240	248-240	242-240
VVMD 36	+0.016	-	254-254	264 -264	276-264	276-272	276-264	264-264
VVS 1	-0.026	125	190-190	190-162	190-162	187-162	190-181	190-187
VVS 2	-0.004	400	155-139	155-133	151-133	133-133	153-145	155-155
VVS 4	-0.034	170	169-168	175-168	175-167	175-175	175-167	169-168
VVS 29	-0.024	400	179-171	181-179	181-171	181-171	179-171	179-171
VrZAG 62	-0.025	377	197-195	201-195	201-189	197-189	205-197	201-195
VrZAG 64	-0.053	114	165-161	165-141	145-141	161-141	165-161	151-143
VrZAG 79	-0.007	377	259-245	259-239	247-239	251-239	259-237	251-243
VrZAG 83	-0.032	385	197-191	197-197	203-197	203-173	191-191	203-197
VrZAG 93	+0.076	353	189-189	197-189	199-197	199-189	199-189	189-189
VMC 2A5	-0.016	340	189-157	189-173	173-157	179-157	157-157	157-157
VMC 2B3	-	-	186-180	186-166	182-166	186-182	166-166	190-186
VMC 2C3	+0.006	365	192-192	192-165	195-165	195-170	195-179	192-170
VMC 2H4	-0.019	340	218-218	238-218	238-202	228-202	224-224	218-218
VMC 5A1	-0.059	95	167-167	167-165	171-165	171-167	171-161	167-167
VMC 5C1	-	-	147-147	172-147	172-147	147-147	147-147	147-147
VMC 5G1.1	-0.022	95	239-140	239-130	239-130	134-130	130-130	130-130
VMC 5G6.1	-0.019	340	155-133	142-133	142-139	151-138	139-139	133-129
VMC 5H2	+0.008	95	195-195	195-195	195-195	195-194	213-194	209-209
VMC 5H5	+0.017	95	186-168	176-168	176-176	176-168	194-178	188-176
For the following 16 loci, the actual allele size in base pairs was not determined; alleles are indicated by letters, A being the smallest ^a :								
VMC 1E8	-	-	E-B	E-D	D-C	D-A	-	C-B
VMC 2B1.1	-	-	C-A	C-A	C-A	C-A	-	C-A
VMC 2B5	-	-	C-A	C-C	C-C	C-B	-	C-A
VMC 2E7	-	-	D-C	E-D	E-E	E-D	-	E-C
VMC 2E8	-	-	B-A	D-A	D-C	C-A	-	B-A
VMC 2F10	-	-	C-B	D-B	D-B	B-B	-	D-B
VMC 2H10	-	-	D-C	F-D	F-C	F-E	-	E-C
VMC 3D12	-	-	C-C	C-B	C-B	B-B	-	C-A
VMC 4C6	-	-	C-B	C-B	C-B	C-B	-	A-A
VMC 5A10	-	-	D-C	D-A	D-A	D-D	-	D-D
VMC 5C5	-	-	A-A	A-A	B-A	B-A	-	C-B
VMC 5G8	-	-	A-A	A-A	C-A	C-A	-	C-A
VMC 8G6	-	-	F-D	F-F	F-B	B-A	-	E-B
VMC 8G9	-	-	F-C	F-F	F-A	E-A	-	I-G
VMC 9A3.1	-	-	E-E	E-A	E-A	D-A	-	F-B
VMC 16F3	-	-	D-B	D-A	E-A	E-B	-	D-B

^a 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.

one allele at each 34 microsatellite markers analysed (data not shown). Then, we suggest that the cultivar mentioned under the name “blantschier” in 1586 was probably not Lafnetscha but Plantscher, which might be a relic of an ancient Hungarian introduction. So what is Lafnetscha? Unfortunately, there is no ampelographical description of Lafnetscha and there is no true-to-type Lafnetscha accession that could be used as control. Since Lafnetscha from Chanton has a distinct and unique genotype and is cultivated today by several producers in Haut-Valais where it was first mentioned (STEBLER 1901), we will consider this one as the true-to-type Lafnetscha. Himbertscha is not closely related to Gouais Blanc as supposed by CHANTON (1972) but it shares at least one allele at each of the 50 loci with Humagne Blanc, providing strong evidence for a parent/offspring relationship (Tab. 2). When only two cultivars of a parentage are known, it is impossible to infer the direction of the parent/progeny relationship from nuclear microsatellite data. However, Humagne Blanc was already cultivated in Haut-Valais in 1313 (CARRUZZO 1991), whereas Himbertscha was first mentioned much later (STEBLER 1901). It is then logical to assume that Humagne Blanc is one of the parents of Himbertscha. The other parent was found neither in other cultivars (from Switzerland, France and North Italy) also sampled (VOUILLAMOZ *et al.* in prep.), nor in the different international databases investigated. This suggests that the other parent has likely disappeared.

The parentage Lafnetscha = Completer x Humagne Blanc is strongly supported by high likelihood ratio values (Tab. 3): it is over 10^{24} ($>10^{19}$ with 95 % upper confidence limits of allele frequencies) times more likely that Lafnetscha is the progeny of Completer and Humagne Blanc than any two other random cultivars. The likelihood ratios are still high if one of the suggested parents is assumed and the other parent is a close relative to the second suggested parent ($>10,000$), even with the 95 % upper confidence limits for the allele frequencies ($>5,000$).

Completer has never been mentioned in Haut-Valais, the only place where its progeny Lafnetscha is cultivated, and is regarded as indigenous to Graubünden, an ancient wine-

growing region in Eastern Switzerland (VIALA and VERMOREL 1901-1910; BELLASI *et al.* 1993; AMBROSI *et al.* 1997; GALET 2000). So how could Lafnetscha originate from Completer? The frequent confusion between Lafnetscha and Completer and the parentage of Lafnetscha presented in this study led us to hypothesize that Completer does (or did) exist in Haut-Valais but it is (or was) mistaken for Lafnetscha. This hypothesis was recently confirmed in autumn 2002 when Michel Pont (Office Cantonal de la Viticulture, Sion, Valais, Switzerland) and one of us (Dominique Maigre) discovered a few individuals of Completer within vine arbors of Lafnetscha growing near Visp (Haut-Valais). Locals called them “Grosse Lafnetscha” (big Lafnetscha), thus confirming the frequent confusion between these two cultivars. After this discovery, we hypothesized another identity for the so-called Blanchier described in BERGET (1904 a). We have seen that Blanchier is a supposed synonym of Lafnetscha (BERGET 1904a; CHANTON 1972) or Plantscher (BIFFIGER 1972) and was already cited in 1586. We compared the first ampelographical descriptions of both Blanchier (BERGET 1904 a) and Completer (KÖHLER 1869). Their significant similarity and the evidence for the presence of Completer in Haut-Valais let us suggest that BERGET (1904 a) actually described under the name Blanchier the cultivar Completer.

The proposed parentage for Lafnetscha is supported by high likelihood ratios and historical data, but it shows a 10-base pair discrepancy at locus VVMD 36 (Tab. 2). Some recent parentage studies showed the occurrence of a 2-base pair discrepancy between parents and progeny (BOWERS *et al.* 1999; PILJAC *et al.* 2002). This is consistent with the stepwise mutation model proposed for microsatellites evolution (see JARNE and LAGODA 1996) and it was simply explained by a somatic mutation in either one parent or the offspring. Recently, we reported a 10-base pair discrepancy in a grape parentage analysis that was inconsistent with the stepwise mutation model and was justified by a single somatic mutation in one of the parents (VOUILLAMOZ *et al.* 2003). At locus VVMD 36, the genotype of Completer is 254-254 and the genotype of its progeny is 264-264 (Tab. 2). In this case, instead of a somatic mutation of 10-base pair as in

Table 3

Likelihood-ratio values. Likelihood-ratio values based on relative allele frequencies are given for the parentage Completer x Humagne Blanc = Lafnetscha *versus* other possibilities. Relative allele frequencies (data not shown) were calculated from up to 400 cultivars (total probability of identity: $PI = 4.76 \times 10^{-25}$) 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 proposed parents ^a of Lafnetscha: Completer (C) x Humagne Blanc (HB)				
<i>versus</i>				
X x Y ^b	C x X ^c	C x HB relative ^d	HB x X ^c	HB x C relative ^d
1.36 x 10 ²⁴ (2.53 x 10 ¹⁹)	1.31 x 10 ¹⁷ (2.43 x 10 ¹⁴)	8.97 x 10 ⁴ (2.94 x 10 ⁴)	3.74 x 10 ¹² (4.60 x 10 ¹⁰)	1.85 x 10 ⁴ (5.64 x 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.

VOUILLAMOZ *et al.* (2003), we suggest that Completer has a null allele at VVMD 36 (a non-amplified allele due to mutations in the PCR primer site). Individuals with a null allele at a given locus would typically be scored as homozygous. This error in genotypic assignment, known to occur in grape cultivars (THOMAS *et al.* 1994; SEFC *et al.* 1999), can have important implications for parentage studies (PEMBERTON *et al.* 1995). The frequency of null alleles (r) for the discrepancy locus VVMD 36 is 0.016 (Tab. 2), which is above the average (-0.01) among 32 microsatellite markers. However, for the calculation of r values, BROOKFIELD (1996) stated panmixia and the absence of population subdivision in the analyzed sample. This is obviously not valid for grapevine cultivars. Therefore, a positive value for r does not necessarily imply the presence of null alleles, it only indicates the possible presence of null alleles. Thus this positive r value provides support for our hypothesis that Completer has a null allele at VVMD 36.

Conclusion

Lafnetscha and Completer are often confused in collections and literature. According to our results, they are different cultivars: the true-to-type Completer is the cultivar sampled in RAC, and the true-to-type Lafnetscha is the cultivar sampled in Chanton's vineyard (Tab. 1). Himbertscha is not closely related to Gouais Blanc. Plantscher is not a synonym of Lafnetscha but a synonym of Bordeaux Blanc or Gros Bourgogne, and it most likely has a parent/progeny relationship with Furmint and might be therefore considered as a relic of an ancient Hungarian introduction. The analysis of 50 microsatellite markers provided evidence for the following parentages: Lafnetscha = Completer x Humagne Blanc; Himbertscha = Humagne Blanc x?

Humagne Blanc has been cultivated in the same area as its progeny Lafnetscha and Himbertscha for a long time, but Completer was not mentioned in Haut-Valais until 2002, probably because it was mistaken for its progeny Lafnetscha. The parentage Lafnetscha = Completer x Humagne Blanc is the second grape parentage analysis showing a multiple repeat discrepancy at one locus. Rather than a somatic mutation as in VOUILLAMOZ *et al.* (2003), the 10-bp discrepancy observed in this study can be explained by the occurrence of a null allele in one of the parents, underlining the potential effect of null alleles on parentage analysis (PEMBERTON *et al.* 1995; JONES and ARDREN 2003). This study confirms that a sole multiple repeat unit discrepancy is not sufficient to reject a parentage and that the greater are the number of loci analysed, the greater are the chances to encounter null alleles or clonal mutations.

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