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ORIGINAL PAPER

Microsatellite analysis of Alpine grape cultivars (*Vitis vinifera* L.): alleged descendants of Pliny the Elder's *Raetica* are genetically related

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Abstract According to Pliny the Elder and other Greco-Roman geoponics, *Raetica* was a famous white grape as well as a white wine produced in *Raetia*, a Province of the Roman Empire. Does *Raetica* grape have modern descendants? Etymologically and geographically, the white 'Rèze' from Valais (Switzerland) would be the best candidate. Using available microsatellite data, we searched for relatives of 'Rèze' in our database containing over 1,700 genotypes of grape cultivars from all over the world. Twelve cultivars showing putative first-degree (parent–offspring or full-siblings) or second-degree (grandparent–grandoffspring, uncle–nephew or half-siblings) relationships with 'Rèze' were then analysed at 60 microsatellite markers. Calculation of allele sharing and likelihood ratios between competing relationship categories revealed that four cultivars had parent–offspring relationship with 'Rèze': 'Cascarolo Bianco' (Piedmont, Italy), 'Arvine Grande' (Valais, Switzerland), 'Groppello di Revò' and 'Nosiola' (Trentino, Italy). Given that some of these are also said to be *Raetica* descendants, we may well be on the tracks of Pliny the Elder's *Raetica* grape. However, there is no evidence about the identity of *Raetica*. Analysis of ancient DNA of grape pips excavated from archaeological sites of the Roman times might provide key information. Our first attempts were unsuccessful, but analysis of additional samples and optimisation of the method could provide groundbreaking results about the identity of the grapes cultivated in classical antiquity.

Keywords Grape cultivar · Microsatellite · Parentage · Likelihood ratios · *Vitis vinifera*

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Introduction

Raetica (or *Rhaetica*) was mentioned by the Greco-Roman authors as one of the most widespread white wine and white grape (Vitis vinifera L.) in *Raetia*, a Province of the Roman Empire corresponding to modern Graubünden (Switzerland), Vorarlberg (western Austria), Tirol (Austria and northern Italy) and part of Lombardy (northern Italy). Cato (234–149 BC) provided in *De Re Rustica* the first written mention of *Raetica*

wine. Virgil (70–19 BC) stated in *Georgica* II that only *Falernum*, the renowned wine of Rome, surpassed *Raetica*. At the same time, the Greek Strabo considered in *Geographika* the wine of the *Reti*, the inhabitants of *Raetia*, as good as the most famous wines of Rome. In *Re Medica*, Celsi suggested to drink warm *Raetica* against sterility, as well as other resinated wines such as *Allobrogica* (Buchi 1996). This property was probably related to the good fertility of the *Raetica* grape, as mentioned by Columella (1st century AD) in *De Re Rustica* III (Tchernia 1986). Pliny the Elder (23–79 AD) provided the first mention of *Raetica* as a grape. In his *Historiae Naturalis* XIV, he considered the region of Verona as the homeland of *Raetica*. Yet, he did distinguish a *Raetica* from the Maritime Alps, producing a poor wine, from a *Raetica* growing in the territory of Verona, producing one of the best wines of that time.

Today, it is problematical to pinpoint which modern varieties might be descendants of *Raetica*. The Austrian 'Veltliner', supposedly originating from Valtelline Valley (Lombardy, northern Italy), is a putative candidate (Buchi 1996). Indeed, the Swiss botanist Bauhin (1650) used the name *Veltolinas* for *Uvae Rheticae*, a group of grapes cultivated in Valtelline. In addition, Jean Hardouin, an erudite Jesuit who edited Pliny's *Historiae Naturalis* in 1685, annotated that *Raetica* grapes were still growing in Valtelline and were remarkable for their excellence (Crane 2005). However, this hypothesis is doubtful because today the name 'Veltliner' is used for several distinct and partially unrelated grape cultivars (Sefc et al. 1998) and because none of them has ever been mentioned in Valtelline. The most serious candidate as a descendant of *Raetica* is 'Rèze', a white grape cultivar confined to Valais (Switzerland), an Alpine region bordering the former land of the *Raeti*. Etymologically, Aebischer (1937) hypothesized that the name *Raetica* has outlived in the modern 'Rèze' that was already mentioned in 1313. Furthermore, 'Rèze' is considered the sole modern etymological relict of the *Raetica* grape (André and Levadoux 1964). Nevertheless, Berget (1903) suggested that 'Rèze' was a descendant of 'Prié', a white cultivar from the neighbouring Aosta Valley (Italy), and Desfayes (2002) proposed that the name 'Rèze' derived from Retz region in Austria rather than *Raeti* or *Raetica*.

In the present study, we used microsatellites, the undisputed markers of choice for grape identification and parentage analysis (Sefc et al. 2001), to follow the tracks of *Raetica* by searching for possible parents and relatives of 'Rèze' in our database. This database contains microsatellite data of more than 1,700 grape cultivars from all over the world, with particular emphasis on traditional cultivars from the land of the *Raeti*. Using a probabilistic approach recently applied successfully for the first time to grape cultivars (Vouillamoz and Grando in press), we present here several unexpected parent–offspring pairs and other genetic relationships with 'Rèze'.

Materials and methods

Plant material and microsatellite analysis

In order to select the cultivars to be analysed, we compared at every already available microsatellite markers the genotype of 'Rèze' (sampled from RAC: the collection at Agroscope RAC Changins, Centre Viticole du Caudoz, Pully, Switzerland) to the genotypes of more than 1,700 grape cultivars from all over the world, put together and standardized from different sources: SSR database of the University of California, Davis (Carole Meredith, personal communication); Grape Microsatellite Collection, IASMA, Italy[1]; Greek Vitis Database, University of Crete, Heraklion, Greece[2]; Bulgarian Grape nSSR Database[3] ; various references in literature; personal unpublished data. In particular, we thoroughly investigated 151 traditional grape cultivars (Table 1) from the land of the *Raeti* and surrounding areas (Austria, Germany, northern Italy and Switzerland) for possible genetic relationship with 'Rèze'. Using the computer program LOCI (A. Schneider and P. Bussa, CNR-IVV unpublished) to detect pairs sharing at

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

[2] http://www.biology.uoc.gr/gvd/

[3] http://www.bulgenom.abi.bg/Grape%20nSSR%20Database.html

Table 1 Grapevine cultivars included in this study

Austria	*Northern Italy*	*Northern Italy*	*Northern Italy*
Blauer Portugieser	Lombardy	Piedmont	Veneto
Brauner Veltliner	Croatina	Neiret	Cabrusina
Frühroter Veltliner	Groppello di S. Stefano	Neretta Cuneese	Corvina Veronese
Grüner Veltliner	Groppellone	Neretto di Marengo	Corvinone
Österreichisch weiβ	Moscato di Scanzo	Neretto Duro	Dindarella
Rotgipfler	Piedmont	Neretto Gentile	Durella
St. Laurent	Arneis	Pelaverga	Garganega
Zierfandler Rot	Avanà	Pignolo Spano	Mollinara
Germany	Avarengo	Quagliano	Oseleta
Affenthaler	Baratuciat	Rastajola	Pelara
Elbling	Barbacarlo	Ruché	Raboso del Piave
Hansen Rot	Barbassese	Tadone	Rondinella
Limberger	Barbera	Timorasso	Rosetta di Montagna
Putzscheere	Barbrassa	Uva Rara	Rossola
Riesling	Bian ver	Uvalino	Rossoletta
Sylvaner	Blanchet	Varenzasca	Trebbiano di Soave
Wildbacher Blau	Bonarda Piemontese	Vespolina	Vespaiola
Northern Italy	Bonardina	Zanello	*Switzerland*
Aosta Valley	Brachetto d'Acqui	Northern Italy	Graubünden
Bonda	Brunetta di Rivoli	Trentino-Alto Adige	Completer
Cornalin d'Aoste	Bubbierasco	Biancaccia	Ticino
Crovassa	Buzzetto	Enantio	Bondola
Fumin	Carcairone Gravere	Groppello di Revò	Valais
Mayolet	Cardin	Lagarino Bianco	Amigne
Ner d'Ala	Cascarolo Bianco	Lagrein	Arvine
Petit Rouge	Cellerina	Lambrusco Casetta	Arvine Grande
Premëtta	Cortese	Marzemino	Cornalin du Valais
Prié	Crovin	Montagna	Durize
Roussin	Dolcetto	Negrara	Eyholzer Roter
Roussin de Morgex	Erbaluce	Nera dei Baisi	Goron
Vien de Nus	Freisa	Nosiola	Himbertscha
Vuillermin	Grignolino	Schiava Grossa	Lafnetscha
Emilia-Romagna	Grisa nera	Schiava Media	Humagne Blanc
Albana	Lambrusca di Alessandria	Teroldego	Rèze
Lambrusco di Sorbara	Lambrusca Vittona	Valderbara	Zurich
Friuli	Lambruschetto	Verdealbara	Hitzkircher
Refosco dal Pedunculo Rosso	Luglienga	Vernaccia Trentina	Räuschling Blanc
Riesling Italico	Malvasia Bianca	Visentina	
Schioppettino	Malvasia Casorzo	Valtellina	
Tocai Friulano	Malvasia Nera Lunga	Chiavennasca Bianca	
Verduzzo Friulano	Malvasia Schierano	Negrera	
Liguria	Moscato Nero d'Acqui	Rossera	
Albarola	Nascetta	Rossolino Nero	
Bosco	Nebbiolo		
	Neirera		

A total of 151 traditional cultivars from the ancient land of the *Raeti* and surrounding areas were selected

least one allele at each locus and the program Microsat (Minch et al. 1995) to calculate pairwise genetic distance (*Das*, proportion of shared alleles), we isolated the cultivars showing putative relationships with 'Rèze'. For further analysis, we obtained samples of these cultivars from the collections at Agroscope RAC Changins, Centre Viticole du Caudoz, Pully, Switzerland (RAC), at Istituto Agrario di San Michele all'Adige, Trento, Italy (IASMA) and at Unità di Viticoltura dell'Istituto di Virologia Vegetale, Consiglio Nazionale delle Ricerche, Torino, Italy (CNR-IVV). DNA was extracted with the Qiagen DNEasy Plant Mini Kit and all cultivars were genotyped at 60 microsatellite loci (Table 2), including the six microsatellites chosen as a core set for grape

Table 2 Genotypes at 60 microsatellite markers

Loci	Rèze	Arvine Grande	Cascarolo Bianco	Groppello di Revò	Nosiola	Freisa	Cornalin du Valais	Roussin de Morgex	Petit Rouge	Cornalin d'Aoste	Goron	Vuillermin	Mayolet	Pinot
VVMD5	228-226	238-226	228-228	238-228	228-226	232-226	228-226	238-232	228-226	228-226	228-228	228-226	238-226	238-228
VVMD6	214-212	214-205	212-212	212-205	214-214	214-212	211-211	212-212	214-211	211-205	211-205	214-211	214-211	205-205
VVMD7	249-247	257-247	253-249	249-239	247-247	249-247	263-247	247-233	247-247	257-247	247-239	263-247	263-247	243-239
VVMD8	143-143	143-143	143-143	167-143	143-143	141-141	143-141	141-141	143-141	141-141	141-141	141-141	141-141	143-141
VVMD17	221-212	220-212	212-212	221-220	221-221	212-212	220-212	224-221	222-212	221-221	212-212	212-212	220-212	221-212
VVMD21	256-249	256-249	266-256	256-249	256-256	256-249	256-249	256-256	249-249	256-249	256-249	256-249	256-249	249-249
VVMD24	214-210	218-214	214-210	210-208	214-210	214-214	214-214	214-210	219-214	214-210	214-210	214-210	214-208	218-216
VVMD25	245-243	245-243	271-243	259-245	261-245	245-243	245-243	253-243	245-243	243-243	267-245	243-243	245-243	253-243
VVMD26	251-249	251-249	251-251	255-251	253-249	251-249	249-249	249-249	251-249	255-249	251-249	249-249	251-249	255-249
VVMD27	189-185	189-185	189-185	185-179	185-185	189-185	189-185	185-185	189-189	189-185	189-189	189-189	189-185	189-185
VVMD28	271-247	247-237	271-221	271-231	271-261	271-247	271-247	271-247	261-247	271-251	247-231	259-235	261-247	239-221
VVMD31	216-212	216-216	212-212	216-212	216-216	216-212	212-210	210-196	216-210	216-212	216-210	216-212	216-212	216-216
VVMD32	263-241	241-241	263-241	273-263	263-251	273-241	263-251	273-263	263-253	253-251	263-241	253-251	253-251	273-241
VVMD34	240-240	240-240	240-240	240-240	240-240	242-240	240-240	240-240	240-240	240-240	240-240	240-240	240-240	240-240
VVMD36	264-252	252-248	295-264	264-254	264-264	272-264	264-252	264-252	264-252	264-252	295-252	264-262	264-252	254-254
VMC1B11	184-182	194-182	184-174	184-182	184-172	184-168	184-182	184-182	184-166	182-166	184-174	184-182	184-182	172-166
VMC1C10	168-156	156-156	156-156	168-156	168-156	174-156	162-156	156-136	168-156	168-156	162-156	170-156	162-156	156-156
VMC1E8	222-218	230-218	230-222	218-208	222-208	222-208	222-218	218-208	222-218	222-208	228-218	218-208	218-208	230-226
VMC2A5	157-157	189-157	157-157	173-157	157-157	157-157	157-157	157-157	171-157	157-157	157-157	173-157	173-157	189-189
VMC2B11	182-182	182-180	184-182	184-182	182-176	182-176	182-176	182-180	182-168	182-176	182-182	180-168	180-176	182-180
VMC2B3	186-182	186-164	182-166	186-180	182-170	190-166	182-180	182-182	182-166	182-182	182-182	182-182	182-180	170-164
VMC2B5	188-184	188-184	192-188	192-188	188-184	188-184	192-188	192-190	188-186	192-190	188-186	204-188	192-188	192-188
VMC2C3	198-198	198-170	198-165	198-165	198-192	198-192	198-192	198-170	198-165	198-165	198-198	198-170	192-170	198-170
VMC2E7	170-152	170-156	160-152	158-152	170-152	160-152	156-144	170-144	170-156	158-144	160-144	170-160	160-144	158-152
VMC2F10	107-89	93-89	107-99	93-89	107-99	103-89	93-89	89-89	93-93	93-89	115-89	93-89	93-89	115-93
VMC2H4	224-210	218-210	210-202	224-202	224-202	218-210	224-202	224-204	224-202	224-210	218-202	204-202	224-202	204-204
VMC3D12	205-198	205-205	205-205	198-198	215-205	205-196	222-205	205-198	222-205	222-198	205-198	205-205	217-205	222-205
VMC4C6	175-163	175-153	163-163	175-163	163-157	165-157	163-163	163-157	165-163	165-163	163-163	163-163	163-163	163-163
VMC4G6	124-120	122-120	134-120	122-120	124-120	122-120	138-120	120-120	138-138	122-120	122-120	138-120	138-120	122-122
VMC5A1	171-167	171-157	167-167	167-157	171-167	171-167	171-167	167-157	171-167	171-167	167-167	171-167	171-157	167-157
VMC5C1	147-147	147-147	172-147	147-147	147-147	147-147	147-147	147-147	172-147	147-147	147-147	175-147	172-147	166-147
VMC5C5	124-118	118-116	124-122	118-116	124-122	118-118	124-118	124-124	124-120	118-116	118-118	124-120	120-118	118-116
VMC5E9	205-199	213-205	217-205	222-205	205-199	222-207	213-191	207-193	213-199	215-213	222-213	213-213	207-191	222-217
VMC5G1.1	239-130	239-130	239-239	239-130	239-130	239-130	239-130	239-239	259-130	239-130	239-140	239-130	259-239	140-130
VMC5G8	317-311	317-317	317-311	317-317	317-311	317-303	311-303	321-317	311-303	311-303	317-311	321-303	321-311	317-313
VMC5H2	209-194	209-209	194-194	209-194	194-194	209-194	209-194	209-194	201-194	209-194	194-194	209-194	209-194	209-194
VMC5H5	188-176	188-168	176-176	188-176	188-188	188-176	180-168	186-170	188-180	168-168	180-178	188-176	188-168	178-168
VMC6E1	151-141	151-151	165-151	161-141	151-139	151-139	161-151	151-151	151-139	151-151	165-151	151-139	151-151	165-151
VMC6E10	117-93	117-107	109-93	103-93	93-93	117-109	109-93	117-107	93-93	107-93	115-109	117-93	117-93	109-107
VMC6G1	188-178	178-178	178-170	188-178	188-178	198-188	188-178	188-170	178-170	188-188	192-188	192-188	188-188	178-170

Table 2 continued

Loci	Rèze	Arvine Grande	Cascarolo Bianco	Groppello di Revò	Nosiola	Freisa	Cornalin du Valais	Roussin de Morgex	Petit Rouge	Cornalin d'Aoste	Goron	Vuillermin	Mayolet	Pinot
VMC7F2	201-199	199-199	201-199	199-197	201-201	199-199	201-199	201-199	201-201	201-199	203-201	201-199	201-199	201-199
VMC8D1	215-211	215-193	211-195	215-209	211-199	219-195	211-197	211-211	211-195	211-211	211-209	211-211	211-211	199-193
VMC8D11	132-132	132-132	132-126	136-132	132-124	136-132	140-124	138-132	140-124	144-124	140-140	140-132	140-132	132-122
VMC8F10	197-197	213-197	233-197	197-195	221-197	233-197	199-197	197-197	221-197	221-197	233-199	233-221	233-197	197-195
VMC8G6	169-155	169-155	169-165	169-155	155-147	165-155	149-147	149-147	149-147	149-137	155-147	147-147	149-147	169-147
VMC8G9	167-161	173-161	161-157	161-161	167-161	173-167	183-167	183-167	183-167	171-167	207-183	177-171	167-167	217-183
VMC9B5	262-242	262-248	242-242	246-242	244-242	262-242	244-242	262-244	244-244	246-242	244-242	262-244	262-242	248-244
VMC16F3	181-175	177-175	181-173	181-177	183-175	181-175	175-175	175-175	183-175	177-175	177-175	183-175	183-175	183-177
VrZAG21	202-190	190-190	202-202	206-202	202-190	206-190	202-200	206-200	202-200	214-200	204-200	206-202	200-192	206-200
VrZAG29	112-112	112-112	112-112	116-112	112-112	114-112	112-112	112-112	112-112	112-112	112-112	112-112	112-112	116-112
VrZAG62	201-195	195-195	201-201	201-195	205-195	201-195	195-195	197-193	205-195	195-195	195-195	205-195	205-195	195-189
VrZAG64	165-143	165-139	143-141	165-143	165-143	165-151	165-143	141-139	143-141	165-165	143-141	165-139	165-143	165-141
VrZAG67	153-126	150-126	126-126	126-126	153-126	153-126	150-126	155-126	155-126	150-132	126-126	153-150	150-126	153-126
VrZAG79	251-247	251-251	247-239	251-251	251-239	251-251	247-239	251-239	247-245	245-239	259-247	251-245	251-239	245-239
VrZAG83	203-197	203-197	197-193	197-191	197-197	203-191	197-193	197-193	197-193	197-193	197-193	197-197	197-193	203-191
VrZAG93	197-189	189-189	199-197	189-189	189-189	189-189	189-189	197-189	191-189	197-189	189-189	197-189	197-189	189-189
VrZAG112	263-241	263-241	263-235	243-241	243-241	263-243	263-243	263-241	263-235	263-235	263-241	243-241	243-241	243-241
VVS2	133-133	133-133	151-133	143-133	155-133	155-133	139-133	155-139	139-133	139-133	151-133	151-139	139-133	151-137
VVS4	175-169	173-169	169-168	169-168	175-168	169-168	175-169	175-169	175-168	169-167	175-168	175-168	175-169	173-168
VVS29	179-171	179-171	179-171	179-179	179-171	179-171	171-171	175-171	171-171	171-171	171-171	171-171	171-171	179-171

cultivars identification by the GENRES#81 European research project (This et al. 2004). Primer pairs for most of the VMC microsatellite markers are unpublished (except VMC7F2 in Pellerone et al. 2001) and belong to the Vitis Microsatellite Consortium. Primer pairs for VVMD microsatellites were published in Bowers et al. (1996, 1999); for VrZAG in Sefc et al. (1999); for VVS in Thomas and Scott (1993) and Thomas et al. (1998). The PCR mix was prepared in 10-µl volumes containing 0.2–3.0 ng of template DNA, 2–4 pmol of each forward and reverse primers, 1 × PCR buffer, 2 mM $MgCl_2$, 0.2 mM dNTPs and 0.5 U of HotStarTaq polymerase. Three different fluorescent dyes (6-FAM, HEX and NED) were used to label the forward primers. All PCR reagents were supplied with the Qiagen HotStarTaq DNA polymerase kit, with the exception of dNTPs (Promega). PCR amplifications were performed in Biometra Tgradient Thermocycler with the following conditions for all markers: 15′ at 95° (HotStarTaq activation step) followed by 35 cycles consisting of 60″ at 94°C (denaturation), 30″ at 52°C/56°C (annealing), 90″ at 72°C (extension). In the last cycle, extension time at 72°C was increased to 10′. Every individual was amplified at least twice to correct possible mistyping or amplification errors. PCR products were size-separated by capillary electrophoresis performed on a genetic analyser (ABI Prism 3100; Applied Biosystems, Inc.) using Performance Optimised Polymer 4 (POP 4, Applied Biosystems, Inc.). Samples were prepared with 9.6 µl of deionised Formamide, 0.1 µl of GeneScan 500 ROX size standard (Applied Biosystems, Inc.), and 0.3 µl of 10 × diluted PCR product. Mixture was heat denaturated (95°C for 3′) and placed 5′ on ice prior to injection in the ABI 3100. Alleles were then separated at 15,000 V for approximately 45′ with a running temperature of 60°C. Resulting data were analysed with Genescan 3.7 (Applied Biosystems, Inc.) for internal standard and fragment size determination. Allelic designations were ascertained using Genotyper 3.7 (Applied Biosystems, Inc.). Relative allele sizes were determined by comparison with known genotypes of standard cultivars. 'Pinot' from the collection at RAC was included in this study as a well-known reference. Parent–offspring pairs that were confirmed with 60 microsatellites were assessed in the program KINGROUP v. 1.0 (Konovalov et al. 2004) following Goodnight and Queller's (1999) algorithm to reconstruct pedigree relationships with the relative allelic frequencies of 89 cultivars from western Europe (data not shown). For each putative relationship, pairwise likelihood ratios were calculated between the likelihood of a primary hypothesis (e.g. parent–offspring) and the likelihood (through 3,000 simulations) of the next competing relationship category (e.g. full-siblings).

Results and discussion

According to literature and etymology, 'Rèze' is the best candidate to represent a modern descendant of *Raetica*. In search for relatives of 'Rèze' that could be putative descendants of *Raetica* as well, we screened more than 1,700 microsatellite grape genotypes from all over the world with particular emphasis on 151 cultivars (Table 1) from the former land of the *Raetian* people and surrounding areas (Austria, Germany, northern Italy and Switzerland). Based on Dps (proportion of shared alleles) pairwise genetic distance, we found 12 grape cultivars having Dps values < 0.5 with respect to 'Rèze' (data not shown) for further analysis: 'Arvine Grande', 'Cornalin du Valais' and 'Goron' from Valais (Switzerland); 'Cornalin d'Aoste', 'Mayolet', 'Petit Rouge', 'Roussin de Morgex' and 'Vuillermin' from Aosta Valley (Italy); 'Cascarolo Bianco' and 'Freisa' from Piedmont (Italy); 'Groppello di Revò' and 'Nosiola' from Trentino (Italy). Unsurprisingly, they all belong to the 151 cultivars selected in former *Raetia* (Table 1). As we recently showed that at least 57 microsatellite markers are necessary to establish parent–offspring relationships between two cultivars without knowing the other parent (Vouillamoz and Grando in press), 'Rèze' and its putative relatives were subsequently analysed at a total of 60 microsatellite markers (Table 2). Finally, only four cultivars turned out to share at least one allele at each locus with 'Rèze', thus strongly suggesting parent–offspring relationships: 'Cascarolo

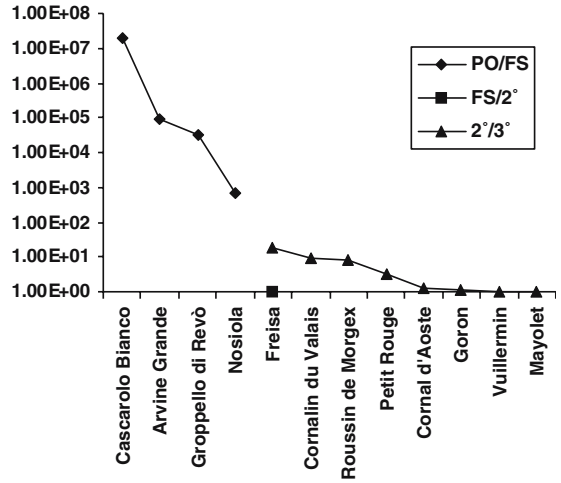

Fig. 1 Likelihood ratios analysis of the relationships with 'Rèze'. The relationship categories considered here are parent–offspring (PO), full-siblings (FS), second-degree (2°) and third-degree (3°) relatives. The ratios (e.g. PO/FS) are calculated between the likelihood of the primary hypothesis (e.g. the cultivar and 'Rèze' are PO) and the likelihood (through simulation) of the next competing relationship category as null hypothesis (e.g. the cultivar and 'Rèze' are FS). As it was almost equally likely that 'Rèze' and 'Freisa' have these genotypes because they are full-siblings instead of second-degree relatives (LRs of FS/2° = 1.06), we also calculated the LR of 2°/3° for this pair

Bianco', 'Arvine Grande', 'Groppello di Revò' and 'Nosiola'. These putative parent–offspring pairs were then assessed by likelihood ratio analysis (Fig. 1). The ratios between the likelihood of the primary hypothesis (*'Rèze' has parent–offspring relationships with 'Cascarolo Bianco', 'Arvine Grande', 'Groppello di Revò' and 'Nosiola'*) and the likelihood (through simulation) of the next competing relationship category as null hypothesis (*Rèze' has full-siblings relationships with 'Cascarolo Bianco', 'Arvine Grande', 'Groppello di Revò' and 'Nosiola'*) ranged from 2×10^7 ('Rèze'–'Cascarolo Bianco') to 659 ('Rèze'–'Nosiola'). In other words, it is 2×10^7 times more likely that 'Rèze' and 'Cascarolo Bianco' have these genotypes because they are parent–offsprings instead of full-siblings. Ratios were obviously much higher using ''unrelated'' as null hypothesis (data not shown; e.g. ratios were 1×10^{12} and 2×10^7 for 'Rèze'–'Cascarolo Bianco' and 'Rèze'–'Nosiola', respectively). Therefore, all four pairs were significantly supported as parent–offspring. Yet, none of these cultivars had ever been suspected to be related to 'Rèze'. 'Cascarolo Bianco' is much widespread in Piedmont (northern Italy), especially at the foot of the Alps where it was already mentioned by Croce (1606). The white 'Arvine Grande' is no longer cultivated in Valais where it is only kept in grape collections. It is supposed to be related to the more widespread 'Petite Arvine', but we found here that it is more related to 'Rèze' than to 'Petite Arvine' (data not shown). The black-berried 'Groppello di Revò' (also locally named 'Gropel', 'Gropel Nones') is only cultivated in the Non Valley in Trentino (northern Italy). It is genetically different from 'Groppello Gentile' and 'Groppello di Santo Stefano' grown in Lombardy (Costantini et al. 2001), contrary to Galet's (2000) opinion. 'Nosiola' is the only autochthonous white grape in Trentino, also locally named 'Groppello Bianco' or 'Nosellara', and it is also scarcely cultivated in Alto Adige under the name 'Spargelen'. Interestingly, both 'Nosiola' and 'Groppello di Revò' are traditionally considered putative descendants of *Raetica*. In grape parentages, when only two cultivars are involved in a parent–offspring relationship and the second parent is missing, the direction of the relationship (i.e. which is the parent and which is the offspring) cannot be inferred from molecular data. Thus, these four cultivars could be either parents or offspring of 'Rèze'. However, our data reject the possibility of a father–mother–progeny trio among these cultivars. Therefore, only one of them could theoretically be a parent of 'Rèze', the other ones being necessarily progenies of 'Rèze'. Given that 'Rèze' was mentioned much earlier than the other four cultivars, it is more likely that 'Rèze' could be their parent by way of four distinct crosses at different times and places (Table 3).

The remaining eight cultivars could be full-siblings, second-degree (grandparent–grandoffspring, uncle–nephew or half-siblings) or more distant relatives of 'Rèze'. In order to assign these pairs to the most likely relationship category, we tested the likelihood ratios using full-sibling as primary hypothesis and second-degree relative as the next competing category. Only 'Rèze'-'Freisa' turned out to have likelihood ratio higher than 1 (Fig. 1), though with very low value (1.06). In

Table 3 Grape cultivars closely related to 'Rèze', the most likely descendant of *Raetica*

Category of relationship to 'Rèze'	
Parent–offspring	*Second-degree relatives*
Cascarolo Bianco	Freisa
Arvine Grande	Cornalin du Valais
Nosiola	Roussin de Morgex
Groppello di Revò	Petit Rouge

Four parent–offspring relationships were detected in Switzerland (Valais) and northern Italy (Piedmont and Trentino). As 'Rèze' was mentioned much earlier, we suppose that they are progenies of four distinct crosses between 'Rèze' and unknown cultivars. Furthermore, four second-degree relatives of 'Rèze' were found in Switzerland (Valais) and northern Italy (Piedmont and Aosta Valley)

other words, it is almost equally likely that 'Rèze' and 'Freisa' have these genotypes because they are full-siblings instead of second-degree relatives. This pair was therefore included in the likelihood ratio analysis using second-degree relatives as primary hypothesis and third-degree relatives as the next competing category. 'Freisa', 'Cornalin du Valais', 'Roussin de Morgex' and 'Petit Rouge' were supported as second-degree relatives of 'Rèze' (Table 3), with likelihood ratios ranging from 19.1 for 'Rèze'–'Freisa' to 3.33 for 'Rèze'–'Petit Rouge' (Fig. 1). 'Freisa' is an ancient cultivar from Piedmont where it was first mentioned in 1517 under the name *fresearum* (Nada Patrone 1987). 'Cornalin du Valais' is only cultivated in Valais, but Vouillamoz et al. (2003) showed that it is a progeny of 'Petit Rouge' and 'Mayolet' from the Aosta Valley. Moreover, 'Roussin de Morgex' from the Aosta Valley is most likely a progeny of 'Cornalin du Valais' and an unknown cultivar (Vouillamoz et al. in prep.). 'Cornalin d'Aoste', 'Mayolet' and 'Vuillermin' from the Aosta Valley as well as 'Goron' from Valais were almost equally likely either second-degree or third-degree relatives of 'Rèze' (Fig. 1). This is consistent with the pedigree of Vouillamoz et al. (2003) showing that 'Cornalin d'Aoste' and 'Goron' are progenies of 'Cornalin du Valais', since the latter has a likely second-degree relationship with 'Rèze'. As mentioned by Blouin (2003) and verified in Vouillamoz and Grando (in press), likelihood ratios between competing categories reach some limitations above second-degree relatives, so that we did not analysed putative third-degree relatives of 'Rèze'.

Though formerly cultivated throughout Central Europe as table grapes, modern 'Veltliner' cultivars are now mainly restricted to eastern Austria. None of the four 'Veltliner' cultivars genotyped in Sefc et al. (1998) showed any possible relationship with 'Rèze'. Given their genetic diversity and their modern distribution, the 'Veltliner' group has not been investigated any further as putative descendant of *Raetica*. We also tested Berget's (1903) hypothesis that 'Rèze' could be a seedling of 'Prié' from the Aosta Valley. Our data rejected a parent–offspring relationship because 'Rèze' and 'Prié' had one allele in common at only 51 out of the 60 microsatellite loci analysed. However, they certainly are somehow related. Likelihood ratios analysis of these cultivars being second-degree relatives versus unrelated was very low (1.76), and the ratios of these cultivars being second-degree relatives versus third-degree relationship was even lower (0.47), so that it is equally likely that they have a second-degree or third-degree relationship.

Conclusion

Will we ever know the identity of *Raetica*? In this work we detected eight cultivars having parent–offspring or second-degree relatives relationship with 'Rèze', the most likely descendant of *Raetica*. Still, it is for now impossible to prove that 'Rèze' or any other cultivar actually is a descendant of *Raetica*. This might be achieved with ancient DNA analysis. Excavations from archaeological sites of the Roman period in the ancient land of the *Raeti* and surrounding areas yielded

plenty of grape remains, mainly grape pips (see Ciurletti 1996 for Trentino-Alto Adige, Mermod 2005 for Valais). DNA extraction and amplification have already been successfully carried out at three microsatellite markers with grape pips from Hungary and France (Manen et al. 2003). Increasing the number of markers to at least six microsatellites would allow the comparison of archaeological grape remains with modern cultivars. We have tested Manen et al.'s method on grape pips dating form the Roman period excavated in Trento, northern Italy (G. Ciurletti personal communication) and in Gamsen, Valais (O. Mermod personal communication) but we were not able to amplify any microsatellite (data not shown), probably because of poor DNA conservation. In the future, other attempts with archaeological material from the *Raetian* regions should be tested and improved. If *Raetian* grape remains were proven to be close or identical to some modern cultivars, this would represent an invaluable and groundbreaking discovery for ancient history and viticulture.

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