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Reconstruction of a grapevine pedigree by microsatellite analysis

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Abstract Microsatellites are ideal markers for revealing genetic relationships between individuals because of their co-dominant inheritance. In this study we determined the genetic profiles of 52 grapevine cultivars using 32 microsatellite markers. We were able to define the complex genetic relationship among nine European grapevine cultivars. None of these parent-offspring combinations were anticipated beforehand. The ancient cultivar Silvaner is shown to be an offspring from Traminer and Österreichisch Weiß. Rotgipfler originates from a cross between Traminer and Roter Veltliner, while Frühroter Veltliner originates from Roter Veltliner × Silvaner and Frühroter Veltliner × Portugieser gave rise to Jubiläumsrebe. A pedigree illustrating the putative crosses was reconstructed.

Key words Grapevine · *Vitis* · Parentage analysis · Microsatellites · Simple sequence repeats

Introduction

Viticulture is one of the oldest agricultural activities of mankind. Grapes were harvested by Egyptian farmers about 4700 B.C., and evidence of wine production in Mesopotamia dates back until 3000 B.C. (Olmo 1976; Ambrosi et al. 1994; Jackson 1994). About 2000 years later, the cultivation of grapevines was introduced to Greece and from there spread over Europe (Olmo 1976). Domesticated varieties were imported from the Near East, while at the same time endogenous wild vines were used for grape and wine production (Ambrosi et al. 1994). Roman authors emphasized the broad range of grapevine cultivars as well as the difficulties of their differentiation. In the Middle Ages, the discrimination between grapevine cultivars was of minor importance. The only differentiation made was between *vinum hunicum* (huntsch), a wine of poor quality, and *vinum francicum* (frentsch), a high-quality wine (Bassermann-Jordan 1975). Only from the 14th and 15th century onwards did the names of grapevine cultivars appear again. Among the earliest to be mentioned were Traminer (1349), Ruländer (1375), Rheinriesling (1435) and Gutedel (1523). The term "Frentsch" changed from the designation of high-quality wine to the cultivar name "Franken", which is one of the synonyms of Silvaner (Bassermann-Jordan 1975).

Considering the age and the variety of the grapevine cultivars, it is not surprising that the reconstruction of the events which led to the appearance of certain cultivars is difficult. The possible sources of European grapevines are the in situ domestication of wild vines, dissemination from the Near East, and crosses of existing cultivars. In most cases, neither the geographic nor the genetic origin of a cultivar is recorded.

In order to determine the genetic relationships between varieties, DNA-based microsatellite markers (simple sequence repeats SSRs) have already been successfully applied (Thomas et al. 1994; Bowers and Meredith 1996; Sefc et al. 1997). Microsatellite markers provide the advantages that: (1) they are highly polymorphic, which allows the creation of unique genetic profiles for all grapevine cultivars (Sefc et al. 1998), and (2) they are inherited in a co-dominant Mendelian manner, thus allowing the reconstruction of crosses. SSR analysis has revealed that Müller Thurgau is not, as assumed, an offspring of Riesling and Silvaner, but descends from a cross between Riesling and Chasselas

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de Courtillier (Regner et al. 1996; Sefc et al. 1997). For some cultivars, however, not even speculations about possible origins exist and textbooks just refer to the variety as a 'very old' one. This term has, for example, often been applied to Cabernet Sauvignon. Recently, SSR analysis discovered its origin from the cultivars Sauvignon blanc and Cabernet franc (Bowers and Meredith 1997; Sefc et al. 1997).

In the present paper, we have genotyped 52 grapevine cultivars at 32 microsatellite loci and searched our data for possible parent-offspring combinations. The genetic relationship among nine cultivars was discovered and a (possible) pedigree reconstructed.

Materials and methods

Plant material was obtained from the collections of the HBLA u. BA Klosterneuburg, Austria (Table 1). DNA was extracted from leaf tissue following the procedure described by Thomas et al. 1993.

The microsatellite loci investigated are: VVS1, VVS2, VVS3, VVS4 (Thomas and Scott 1993), VVS29 (Thomas, personal communication; CSIRO Division of Horticulture, Adelaide, Australia), VVMD5, VVMD7 (Bowers et al. 1996), VVMD6, VVMD8, VVMD17, VVMD21, VVMD24, VVMD25, VVMD26, VVMD27, VVMD28, VVMD31, VVMD32 and VVMD36 (Bowers and Meredith, personal communication; Department of Viticulture and Enology, UC Davis, California, USA), and microsatellite loci recently developed in our laboratory from a library of *Vitis riparia* (publication submitted): namely, ssrVrZAG 7, ssrVrZAG 15, ssrVrZAG 21, ssrVrZAG 25, ssrVrZAG 29, ssrVrZAG 30, ssrVrZAG 47, ssrVrZAG 62, ssrVrZAG 64, ssrVrZAG 67, ssrVrZAG 79, ssrVrZAG 83 and ssrVrZAG 112. PCR and electrophoresis with the ALFexpress sequencer (Pharmacia Biotech, Vienna, Austria) were performed as described by Sefc et al. (1997).

On the basis of their SSR profiles, the cultivars were searched for possible parent-offspring groups using a software written by H. W. Wagner. Cumulative likelihood ratios for the proposed parentages were calculated from the relative allelic frequencies in the 52 cultivars and their 95% upper confidence limits (Bowers and Meredith 1997; Sefc et al. 1997).

Results and discussion

Fifty two grapevine cultivars (Table 1) distinguishable by microsatellite analysis at 32 loci were included in this study. A comparison of the genetic profiles of these cultivars revealed close genetic relationships between a group of nine European cultivars (Österreichisch Weiß, Traminer, Roter Veltliner, Silvaner, Rotgipfler, Neuburger, Frühroter Veltliner, Grauer Portugieser, Jubiläumsrebe).

A pedigree illustrating the relationship between these varieties was constructed (Fig. 1). Genetic profiles show that Silvaner is the offspring of Traminer and Österreichisch Weiß. As Silvaner has been cultivated since medieval times, the cross must have taken place more than 500 years ago and therefore very likely occurred spontaneously. Traminer was mentioned first in 1349 (Gollmick et al. 1991) and is widely distributed, whereas

Table 1 Grapevine cultivars included in this study

Cultivars	
Aligote	Müller Thurgau
Andre	Muskat à petits grains
Bianca	Muskat Ottonel
Blauburger	Neuburger
Blauer Muskat	Österreichisch Weiß
Blauer Portugieser	Perle von Csaba
Blauer Wildbacher	Pinot noir
Blaufränkisch	Rathay
Bouvier	Rheinriesling
Brauner Veltliner	Rösler
Cabernet franc	Roter Veltliner
Cabernet Sauvignon	Rotgipfler
Chardonnay	Sangiovese
Chasselas de Courtillier	Sauvignon blanc
Frühroter Veltliner	Scheibenkern
Gelber Muskateller	Schwarzer Riesling
Goldburger	Semillon
Grenache	St. Laurent
Grüner Silvaner	Steinschiller
Grüner Veltliner	Sultanina
Heunisch	Traminer
Jubiläumsrebe	Trollinger
Königin der Weingärten	Weißer Gutedel
Lambrusco di Sorbara	Welschriesling
Merlot	Zierfandler
Meunier	Zweigelt

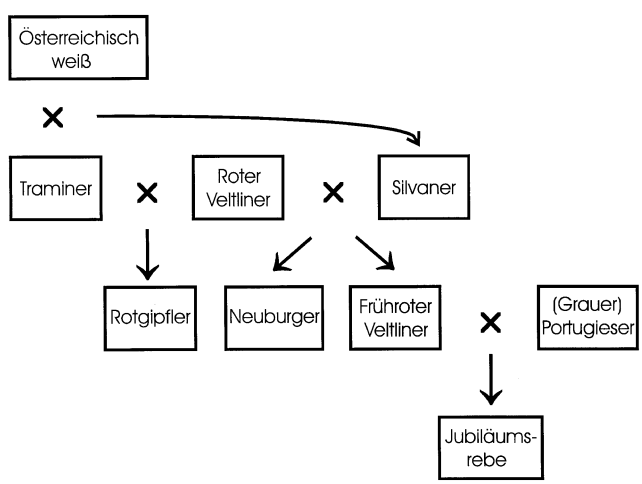

Fig. 1 Pedigree illustrating the genetic relationship between nine grapevine cultivars as reconstructed from microsatellite data. SSR data do not discriminate the Portugieser cultivars, but the participation of Grauer Portugieser is inferred from breeding reports

Österreichisch Weiß is an ancient variety cultivated mainly in Austria in the region around Vienna. Until the 1950s, Österreichisch Weiß was used for the production of sparkling wine, but has no economic importance today. Since Österreichisch Weiß was rather restricted to the Eastern part of Austria, the descent of Silvaner also indicates its geographic origin from that region. The synonym 'Österreicher' for Silvaner (Bassermann-Jordan 1975) would thus be

justified. However, our results reject the hypothesis that Silvaner was selected from a wild vine on the banks of the river Danube (Bassermann-Jordan 1975).

Furthermore, our SSR data show that a cross between Traminer and Roter Veltliner led to the Austrian variety Rotgipfler. In former times, Roter Veltliner was a wide spread variety for the production of table grapes and wine, while nowadays it is mainly grown in eastern Austria. Rotgipfler is cultivated in the Gumpoldskirchen region in eastern Austria. For the production of a high quality wine specialty, it is often blended with Zierfandler. In contrast to previous assumptions (Ambrosi et al. 1994), no close genetic relationship between Rotgipfler and Zierfandler could be detected.

The origin of Neuburger from Roter Veltliner and Silvaner has already been reported (Regner et al. 1996; Sefc et al. 1997). Surprisingly, the same parents gave rise to another cultivar, Frühroter Veltliner, which is also known under the name Malvasier. This cultivar was once a common table grape in Central Europe and is now grown for wine and table grape production in Austria and in small areas of Germany, Switzerland, Italy and the Czech Republic. The siblings Neuburger and Frühroter Veltliner share 70% of their alleles at 32 SSR loci.

Another cultivar with the name 'Veltliner', Grüner Veltliner, is not related to Roter and Frühroter Veltliner. Thus, the common term 'Veltliner group' can only be applied to the varieties Roter Veltliner and its progeny Frühroter Veltliner, Neuburger and Rotgipfler. The so-far assumed origin of Jubiläumsrebe, a white variety for the production of dessert wine, from a cross between the red wine cultivars Blauer Portugieser and Blaufränkisch (Ambrosi et al. 1994), caused some astonishment, but was hardly questioned. However, our results demonstrate that Jubiläumsrebe is actually an offspring of Portugieser and Frühroter Veltliner. Jubiläumsrebe was bred in Klosterneuburg in the 1920s. This vineyard site was destroyed during the 2nd world war and misinterpretation of the remaining plants may have occurred. Breeding reports of the 1920s denote crosses between Blauer Portugieser and Blaufränkisch as well as a cross between Grauer Portugieser and Frühroter Veltliner. The different Portugieser types (Blauer, Grauer and Grüner Portugieser) cannot be distinguished by microsatellite analysis, but considering the information in the breeding reports it is very likely that Jubiläumsrebe is descended from a cross with Grauer Portugieser and therefore is an offspring of two white wine varieties. Crosses between Jubiläumsrebe and Rheinriesling yield progeny with white as well as with gray and red berries, which also supports its origin from Grauer Portugieser (gray berries) and Frühroter Veltliner (red berries).

The parentages presented in this paper are supported by all 32 investigated loci (for genotypes see http://www.boku.ac.at/zag/forsch/grapeSSR.htm) and by high likelihood-ratio values. Likelihood-ratio values compare the probability of the parenthood of the suggested cultivars (derived from the genotypes of the parents) with the probabilities that: (1) two random cultivars are the parents, (2) one of the suggested cultivars and a random cultivar are the parents, and (3) one of the suggested cultivars and a close relative to the second suggested parent are the parents (Table 2). The relative allelic frequencies required for this calculation are shown in Table 3. At least two individuals per cultivar were analysed independently to ascertain cultivar

Table 2 Likelihood ratios of the probability of the suggested parentages of Silvaner, Rotgipfler, Frühroter Veltliner and Jubiläumsrebe versus other possibilities. Probability values were calculated from relative allele frequencies derived from our cultivar collection and from the 95% upper confidence limits (see Table 3). The calculations are based on the data of 52 cultivars and 32 microsatellite loci (see Materials and methods). The order of the parents in the table does not indicate the actual direction of the cross

Cultivar	Suggested parents	Cumulative likelihood ratios of the suggested parentage (1) × (2) versus				
		$X \times Y^{a,b}$	$(1) \times X^{a,c}$	$(1) \times \text{rel } (2)^{a,d}$	$(2) \times X^{a,c}$	$(2) \times \text{rel } (1)$
Silvaner	(1) Österreichisch Weiß	7.2×10^{16}	1.3×10^{8}	2.0×10^{3}	1.9×10^{14}	3.3×10^{4}
	(2) Traminer	(1.6×10^{9})	(6.9×10^{4})	(1.4×10^{2})	(6.7×10^{9})	(2.6×10^{3})
Rotgipfler	(1) Traminer	9.0×10^{20}	1.7×10^{16}	3.5×10^{4}	8.2×10^{9}	5.8×10^{3}
	(2) Roter Veltliner	(4.2×10^{12})	(1.9×10^{11})	(2.9×10^{3})	(3.0×10^{6})	(3.9×10^{2})
Frühroter Veltliner	(1) Silvaner	9.1×10^{18}	2.7×10^{13}	2.0×10^{4}	5.9×10^{9}	2.3×10^{3}
	(2) Roter Veltliner	(9.1×10^{10})	(1.3×10^{9})	(1.5×10^{3})	(1.2×10^{6})	(1.5×10^{2})
Jubiläumsrebe	(1) Portugieser	2.9×10^{18}	2.6×10^{10}	8.0×10^{3}	2.3×10^{12}	2.7×10^{4}
	(2) Frühroter Veltliner	(7.1×10^{10})	(4.9×10^{6})	(5.3×10^{2})	(2.8×10^{8})	(1.9×10^{3})

[a] Values in parentheses are the cumulative likelihood ratios calculated with the 95% upper confidence limits for the allele frequencies.
[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.

Table 3 Relative allelic frequencies and the 95% upper confidence limit of the frequencies derived from the genotypes of 52 *V. vinifera* individuals

Locus	Allele size (bp)	Allele frequencies:		Locus	Allele size (bp)	Allele frequencies:	
		Observed	Upper 95% confidence limit			Observed	Upper 95% confidence limit
VVS 1	179	0.08	0.12	VVMD 31	203	0.15	0.21
	180	0.25	0.33		209	0.15	0.21
	189	0.45	0.54		223	0.03	0.06
VVS 2	132	0.23	0.30	VVMD 32	251	0.10	0.16
	150	0.27	0.35		263	0.07	0.11
	152	0.02	0.05		271	0.41	0.49
VVS 3	212	0.35	0.43	VVMD 36	252	0.28	0.36
	218	0.62	0.70		262	0.41	0.49
VVS 4	167	0.53	0.61		274	0.14	0.20
	174	0.20	0.27	ssrVrZAG 7	155	0.76	0.84
VVS 29	168	0.55	0.63		157	0.14	0.20
	176	0.35	0.43	ssrVrZAG 15	165	0.74	0.81
VVMD 5	224	0.20	0.27		173	0.03	0.06
	230	0.15	0.21	ssrVrZAG 21	200	0.28	0.36
	238	0.14	0.20		206	0.43	0.52
	244	0.02	0.05	ssrVrZAG 25	225	0.56	0.64
VVMD 6	189	0.12	0.18		236	0.19	0.26
	199	0.33	0.41		238	0.08	0.13
	206	0.16	0.22		245	0.09	0.15
	207	0.21	0.28	ssrVrZAG 29	112	0.65	0.73
VVMD 7	236	0.31	0.39		114	0.07	0.11
	240	0.18	0.25		116	0.26	0.34
	244	0.18	0.25	ssrVrZAG 30	149	0.72	0.79
	250	0.06	0.10	ssrVrZAG 47	159	0.15	0.21
	254	0.11	0.17		161	0.04	0.07
VVMD 8	138	0.24	0.31		167	0.29	0.37
	140	0.43	0.52		172	0.16	0.22
	144	0.20	0.27	ssrVrZAG 62	187	0.23	0.30
VVMD 17	220	0.32	0.40		191	0.08	0.12
	221	0.24	0.31		195	0.11	0.17
	222	0.26	0.34		203	0.20	0.27
VVMD 21	242	0.08	0.12	ssrVrZAG 64	139	0.24	0.31
	248	0.70	0.78		143	0.13	0.19
	257	0.07	0.11		163	0.17	0.23
VVMD 24	205	0.05	0.09	ssrVrZAG 67	126	0.21	0.28
	207	0.37	0.45		132	0.08	0.13
	211	0.27	0.35		149	0.09	0.15
	215	0.14	0.20		159	0.06	0.10
VVMD 25	242	0.22	0.29	ssrVrZAG 79	248	0.05	0.09
	250	0.40	0.48		250	0.25	0.32
VVMD 26	249	0.39	0.47		258	0.17	0.23
	251	0.43	0.52	ssrVrZAG 83	188	0.36	0.44
	263	0.05	0.09		190	0.18	0.25
VVMD 27	180	0.18	0.25		194	0.24	0.31
	182	0.02	0.05	ssrVrZAG 112	229	0.20	0.27
	188	0.29	0.37		234	0.22	0.29
	193	0.16	0.22		238	0.04	0.07
VVMD 28	228	0.15	0.21		240	0.24	0.31
	236	0.14	0.20				
	268	0.12	0.18				

identity. The probability that two different, non-related, cultivars display the same genotype at all investigated loci is 3.4×10^{-24}.

As demonstrated by the examples given here and elsewhere (Regner et al. 1996; Bowers and Meredith 1997; Sefc et al. 1997), microsatellite analysis is a powerful tool for the clarification of the origins of grapevine cultivars. However, its success in reconstructing crosses which occurred long ago is dependent on the availability of the ancient cultivars. While many old varieties are still grown today, others have been abandoned and have become extinct. Only a few and probably the last individuals of Österreichisch Weiß, the parent of Silvaner, are still kept in the cultivar

collection of the HBLA Klosterneuburg. This example demonstrates the importance to maintain cultivars, which are currently of minor economic impact, in collections, since they may provide links for pedigree analysis.

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