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## The parentage of 'Sangiovese', the most important Italian wine grape

J. F. VOUILLAMOZ<sup>1</sup>, A. MONACO<sup>2</sup>, L. COSTANTINI<sup>1</sup>, M. STEFANINI<sup>1</sup>, A. SCIENZA<sup>3</sup> and M. S. GRANDO<sup>1</sup><sup>1</sup> IASMA Research Centre, Genetics and Molecular Biology Department, San Michele all'Adige (TN), Italy<sup>2</sup> Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università Federico II, Portici (NA), Italy<sup>3</sup> Dipartimento di Produzione Vegetale, Sezione Coltivazioni Arboree, Università degli Studi di Milano, Milano, Italy

### Summary

A previous microsatellite study pointed out a possible parent-offspring relationship between 'Sangiovese', the most widespread red grape cultivar in Italy, and 'Ciliegiolo', an ancient Tuscan variety. Testing 'Sangiovese' as a parent of 'Ciliegiolo', we searched for the putative other parent in our extensive, private and standardized database, but we did not find any candidate. Testing 'Ciliegiolo' as a parent of 'Sangiovese', we found four candidate cultivars. After the analysis of 50 microsatellites, only one stood the paternity test and we established with a strong statistical support that 'Sangiovese' is a progeny of 'Ciliegiolo' and 'Calabrese di Montenuovo', an obscure grapevine from Campania, Italy. This cultivar does not have a registered name and is supposed to have been introduced from Calabria. Among 180 additional local grape cultivars from Calabria, Campania or Tuscany, we did not find any matching variety. As a consequence, we propose to adopt the name 'Calabrese di Montenuovo' for this grape cultivar. In addition, we found relatives of 'Sangiovese' and 'Calabrese di Montenuovo' in Calabria, thus strongly suggesting a Calabrian origin for 'Calabrese di Montenuovo' and indicating that 'Sangiovese' has ancestors and/or progenies in Tuscany and in Southern Italy.

**Key words:** Microsatellite, *Vitis vinifera*, kinship, fingerprinting, pedigree.

### Introduction

'Sangiovese' is the most widespread grape cultivar in Italy (about 85,000 ha), producing the famous Chianti and Brunello di Montalcino wines in Tuscany. With 22 microsatellites, which are co-dominantly inherited molecular markers commonly used in pedigree reconstruction (SEFC *et al.* 2001), CRESPIAN *et al.* (2002) found a likely parent-offspring relationship between 'Sangiovese' and 'Ciliegiolo' (meaning "small cherry"), an ancient Tuscan variety often blended with 'Sangiovese' in Chianti wines. However, in the absence of the second parent, it was impossible to determine which of 'Sangiovese' or 'Ciliegiolo' could be the parent and which could be the progeny. We investigated the two possibilities by searching our database containing microsatellite genotypes of almost 2,000 grape cultivars from all over the world (including over 500 from Italy) and with

50 microsatellites we were able to isolate a single candidate. In this paper, we provide strong likelihood evidence that 'Sangiovese' is the progeny of 'Ciliegiolo' and an obscure variety fortuitously sampled in Montenuovo (Campania). This variety does not have a registered name and almost certainly originates from Calabria. We genotyped 180 additional Southern Italian accessions in search of its true-to-type identity. We also searched for putative relatives of this mysterious accession from Montenuovo, as well as putative relatives of 'Sangiovese' and 'Ciliegiolo'.

### Material and Methods

**Plant material:** In addition to our private and standardized database containing almost 2,000 distinct grape cultivars from all over the world (VOUILLAMOZ *et al.* 2006), including 511 distinct Italian cultivars, a total of 180 grape accessions ( $n = 146$  from Calabria,  $n = 25$  from Campania,  $n = 6$  from Tuscany,  $n = 2$  from Basilicata and  $n = 1$  from Apulia) were sampled and genotyped for this study. All Calabrian accessions come from the private collection at Librandi winery. As the collection is in the process of characterization, all samples were analysed blind (numbered). Other accessions were sampled in vineyards, and most of them had local and unregistered names. All genomic DNAs were extracted from small dried leaves with Qiagen DNeasy Plant Mini Kit.

**Microsatellite analysis:** In order to discard identical genotypes, the putative parents of 'Sangiovese' or 'Ciliegiolo' detected in our database as well as the 180 accessions selected for this study were initially analysed at 10 microsatellite markers (VVMD5, VVMD7, VVMD24, VVMD27, VVMD28, VVMD31, VVMD32, VVS2, VrZAG62, VrZAG79). 'Sangiovese' and 'Ciliegiolo' as well as varieties that stood the test of being their putative parents were then genotyped at 40 additional microsatellites (listed in Tab. 1). PCR amplifications and allele sizing were performed as in VOUILLAMOZ *et al.* (2006).

**Likelihood ratios:** The program Identity version 1.0 (WAGNER and SEFC 1999) was used to calculate the total probability of identity (PI) and the cumulative likelihood ratios (LRs) for the proposed parentage. Likelihood ratios were calculated as in VOUILLAMOZ *et al.* (2003). For comparison, we calculated the allele frequencies based on 93 cultivars (the 89 cultivars listed in VOUILLAMOZ and GRANDO 2006 and the four cultivars in Tab. 1) genotyped

Table 1

Genotypes at 50 microsatellite markers. The proposed parentage 'Sangiovese' = 'Ciliegiolo' x 'Calabrese di Montenuovo' is consistent at 49 out of 50 markers. The only discrepancy is at VMC5H2 (bold allele). The Calabrian cv. 'Negrello-39' (unregistered name) shares at least one allele at each locus with 'Sangiovese', thus strongly supporting its being a progeny of 'Sangiovese'

SSR	Ciliegiolo	Sangiovese	Calabrese di Montenuovo	Negrello-39
VVMD5	236-226	236-226	232-226	236-226
VVMD6	214-212	212-194	212-194	212-212
VVMD7	263-247	263-239	239-239	249-239
VVMD8	143-135	147-143	147-141	143-143
VVMD17	222-212	221-212	221-212	221-212
VVMD21	266-243	249-243	258-249	253-243
VVMD24	219-216	216-210	216-210	216-210
VVMD25	245-245	245-245	259-245	245-245
VVMD26	251-249	249-249	251-249	251-249
VVMD27	181-179	185-179	189-185	179-179
VVMD28	247-237	247-237	237-231	247-231
VVMD31	216-212	212-212	212-210	216-212
VVMD32	253-253	257-253	257-257	257-253
VVMD34	240-240	240-240	240-240	248-240
VVMD36	264-244	264-264	276-264	294-264
VVS2	133-133	133-133	135-133	143-133
VVS4	175-168	168-168	168-168	176-168
VVS29	171-171	171-171	171-171	171-171
VMC1B11	184-166	166-166	188-166	170-166
VMC1C10	142-142	142-142	142-142	156-142
VMC1E8	230-208	222-208	222-208	222-208
VMC2A5	171-157	157-157	157-157	177-157
VMC2B3	188-180	188-180	180-180	188-180
VMC2B11	176-172	182-176	182-180	176-168
VMC2E7	160-154	160-158	158-158	160-160
VMC2F10	93-93	93-89	109-89	93-89
VMC2H4	218-202	218-218	218-216	218-218
VMC3D12	205-199	205-205	222-205	205-199
VMC4C6	163-163	163-157	157-157	163-157
VMC5A1	171-169	171-169	171-161	171-169
VMC5C5	120-116	116-116	124-116	116-116
VMC5G8	317-309	309-309	309-301	309-301
VMC5H2	194-194	<b>209</b> -194	194-194	209-194
VMC5H5	188-176	194-176	194-184	194-188
VMC6E1	141-139	165-141	165-141	141-139
VMC6E10	115-113	115-91	91-91	109-91
VMC6G1	178-178	198-178	198-170	178-170
VMC8D1	219-209	219-209	219-209	219-209
VMC8F10	233-197	233-197	197-197	233-233
VMC8G6	161-155	155-155	161-155	155-135
VMC8G9	171-171	195-171	195-171	199-171

at 49 microsatellite markers (one locus showing a discrepancy was discarded) and the allele frequencies based on 400 cultivars (the default limit of the program Identity) genotyped at the first 32 microsatellites in Tab. 1.

### Results and Discussion

**D a t a b a s e s e a r c h :** Based on 18 common microsatellite markers, our 'Sangiovese' and 'Ciliegiolo' accessions (IASMA collection) perfectly matched those in CRESpan *et al.* (2002). Given that CRESpan *et al.* (2002)

evidenced a possible parent-offspring relationship between 'Sangiovese' and 'Ciliegiolo', we first assessed 'Sangiovese' as a parent of 'Ciliegiolo', and we did not find any candidate cultivar in our database. With 'Sangiovese' as a progeny of 'Ciliegiolo', we found four putative candidate cultivars, all available in our IASMA grape collection. When analysed at a total of 32 microsatellite markers as in other parentage analyses (SEFC *et al.* 2001), the first three candidates were excluded for not sharing at least one allele with 'Sangiovese' at 5 or 6 loci (data not shown). Only the accession named 'Calabrese di Montenuovo' genotyped by COSTANTINI *et al.* (2005) stood the allele-sharing test, thus strongly

suggesting a parent-offspring relationship with 'Sangiovese'. This accession is an obscure plant retrieved by one of us (Antonella Monaco) in an ancient vineyard around a winery in Campania. It was locally named 'Calabrese di Montenuovo', simply because the vineyard is situated on the Montenuovo hill near Naples and the winery founders came from Calabria. This accession a) does not have an officially registered name, b) is different from the variety 'Calabrese' also called 'Nero d'Avola' in Sicily, c) is one of the numerous Italian varieties named 'Calabrese' followed by an epithet (even 'Sangiovese' is sometimes called 'Calabrese' in Tuscany) and d) does not match any genotype in our database. We only found a dozen of plants of 'Calabrese di Montenuovo' around the winery and we carefully took cuttings in order to propagate this key cultivar in the IASMA grape collection.

**Investigating the Southern Italian germplasm:** We genotyped 180 grape accessions from Southern Italy at 10 microsatellites. After discarding redundant genotypes and accessions already existing in our database, we obtained 73 new and unique genotypes (data not shown). Yet, none matched 'Calabrese di Montenuovo'. However, 'Sangiovese' and 'Calabrese di Montenuovo' shared at least one allele at each locus with nine and four distinct genotypes from Calabria, respectively. All were subsequently analysed at a total of 32 microsatellites (first 32 in Tab. 1, data not shown). 'Sangiovese' still shared at least one allele at each locus with six distinct genotypes having unregistered and various names made of 'Nerello' or 'Negrello' followed by an epithet according to their origins or characteristics (SCULLI 2004). 'Calabrese di Montenuovo' still shared at least one allele at each locus with 'Castiglione' (genotyped at the University of California, Davis and in COSTANTINI *et al.* 2005), a widespread variety in Calabria. Though at least 57 markers would be recommended to demonstrate a parent-offspring relationship in the absence of one parent (VOUILLAMOZ and GRANDO 2006), sharing one allele at each 32 microsatellites most likely indicates a very close relationship, such as parent-offspring

or full-siblings. Therefore, our results strongly suggest that 'Sangiovese' and 'Calabrese di Montenuovo' both have relatives in Calabria. At this point, the parentage 'Sangiovese' = 'Calabrese di Montenuovo' x 'Ciliegiolo' was challenged by the parentage 'Sangiovese' = 'Calabrese di Montenuovo' x 'Negrello-39' (one of the 11 accessions in Librandi collection having the same genotype under different names based on Negrello) that only showed one discrepancy locus (VVMD7).

**Parentage discovered:** 'Sangiovese' = 'Ciliegiolo' x 'Calabrese di Montenuovo': After the analysis of 50 microsatellites, the putative parentage 'Sangiovese' = 'Calabrese di Montenuovo' x 'Negrello-39' was not supported anymore, as it was excluded at 8 loci (VVMD7, VMC5H5, VMC6E10, VMC16F3, VrZAG21, VrZAG64, VrZAG67, VrZAG83 in Tab. 1). However, 'Negrello-39' still shared at least one allele at each locus with 'Sangiovese', thus strongly supporting its being a progeny of 'Sangiovese'. The putative parentage 'Sangiovese' = 'Ciliegiolo' x 'Calabrese di Montenuovo' was still supported at 50 microsatellites, being excluded at only one marker (VMC5H2, bold allele in Tab. 1). Indeed, a discrepancy level of 2 % is quite common in parentage analysis (JONES and ARDREN 2003), and this discrepancy is most likely due to a somatic mutation in 'Sangiovese', as in VOUILLAMOZ *et al.* (2003). Discarding the discrepancy locus, likelihood ratios (LRs) analysis strongly supported that 'Sangiovese' is a progeny of 'Ciliegiolo' and 'Calabrese di Montenuovo' (Tab. 2). LR of the proposed parentage versus any other two parents were extremely high:  $6.64 \times 10^{18}$  (400 cvs at 32 microsatellites) and  $2.81 \times 10^{37}$  (93 cvs at 49 microsatellites). LR of the proposed parentage versus a cross between one of the parents and a relative of the other parent were obviously much lower but still over 3,000 (400 cvs at 32 microsatellites) and  $3'000'000$  (93 cvs at 49 microsatellites). Despite the discrepancy at one locus, LR undisputedly support the proposal that 'Sangiovese' is the progeny of a cross between 'Ciliegiolo' and 'Calabrese di Montenuovo'.

Table 2

Likelihood-ratio (LR) values for the proposed parentage 'Sangiovese' = 'Ciliegiolo' x 'Calabrese di Montenuovo' versus other possibilities. Relative allele frequencies were calculated from 400 cultivars at 32 microsatellite markers and from 93 cultivars at 49 microsatellites (one discrepancy locus was discarded). Values in parentheses are the cumulative likelihood ratios calculated with the 95 % upper confidence limits for the allele frequencies

Proposed parents <sup>a</sup> of 'Sangiovese': (1) 'Ciliegiolo', (2) 'Calabrese di Montenuovo'					
Cumulative likelihood ratios of the proposed parentage (1) x (2) versus:					
Allele frequencies	X x Y <sup>b</sup>	(1) x X <sup>c</sup>	(1) x (2) relative <sup>d</sup>	(2) x X <sup>c</sup>	(2) x (1) relative <sup>d</sup>
400 cvs at 32 micros.	$6.64 \times 10^{18}$ ( $4.97 \times 10^{14}$ )	$3.32 \times 10^{10}$ ( $4.53 \times 10^8$ )	$4.37 \times 10^3$ ( $1.45 \times 10^3$ )	$3.07 \times 10^{11}$ ( $1.59 \times 10^9$ )	$3.01 \times 10^3$ ( $9.75 \times 10^2$ )
93 cvs at 50 micros.	$2.81 \times 10^{37}$ ( $1.62 \times 10^{26}$ )	$2.99 \times 10^{20}$ ( $9.28 \times 10^{14}$ )	$3.36 \times 10^6$ ( $1.30 \times 10^5$ )	$1.68 \times 10^{23}$ ( $3.20 \times 10^{17}$ )	$1.95 \times 10^7$ ( $8.89 \times 10^5$ )

<sup>a</sup> The order of the parents does not indicate the actual direction of the cross.

<sup>b</sup> X and Y are random unrelated cultivars.

<sup>c</sup> The identity of one of the suggested parents is assumed and the other parent is unknown.

<sup>d</sup> The identity of one of the suggested parents is assumed and the other parent is a close relative to the other suggested parent.

### Conclusion

We have shown with 50 microsatellite markers that 'Sangiovese' is the progeny of 'Ciliegiolo' and 'Calabrese di Montenuovo'. As it did not match any other accession, we propose here to adopt the name 'Calabrese di Montenuovo' (reference plants are kept alive at IASMA grape collection). In Calabria, we found six putative relatives of 'Sangiovese', of which the unregistered 'Negrello-39' most likely is an offspring of 'Sangiovese', and one putative relative of 'Calabrese di Montenuovo' named 'Castiglione'. This strongly suggests that 'Sangiovese' has been cultivated in Southern Italy for a long time and that 'Calabrese di Montenuovo' most likely comes from Calabria.

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