

## ‘Sangiovese’ and ‘Garganega’ are two key varieties of the Italian grapevine assortment evolution

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### Summary

Two synonymous cases have been found using a set of 11 SSR markers: ‘Garganega’ and ‘Grecanico dorato’; ‘Catarratto bianco comune’, ‘Catarratto bianco lucido’ and ‘Catarratto bianco extra lucido’. Molecular data at 36 SSR loci showed that ‘Sangiovese’ and ‘Garganega’ represent two key varieties in the Italian ampelographic assortment evolution, as they both have a first degree relationship with numerous wine varieties. ‘Sangiovese’ showed this link with ten varieties: ‘Foglia tonda’, ‘Frappato’, ‘Gaglioppo’, ‘Mantonicone’, ‘Morellino del Casentino’, ‘Morellino del Valdarno’, ‘Nerello mascalese’, ‘Susumaniello’, ‘Tuccanese di Turi’ and ‘Vernaccia nera del Valdarno’. Seven varieties resulted closely related to ‘Garganega’: ‘Trebiano toscano’ *alias* ‘Ugni blanc’, ‘Albana’, ‘Empibotte’, ‘Malvasia bianca di Candia a sapore semplice’, ‘Marzemina bianca’, ‘Catarratto’ and ‘Greco del Pollino’. However, being ‘Sangiovese’ parents disputed and those of ‘Garganega’ still unknown, it was not possible to determine the univocal direction of the various crosses. Identification of the “missing” parents would allow these genealogical trees to be drawn up with greater precision.

**Key words:** Catarratto, Trebbiano toscano, Grecanico dorato, pedigree, SSR, synonyms.

### Introduction

Researches on grapevine varieties pedigree determination have been increased in the last years by means of microsatellite markers (SSR) (SEFC *et al.* 2001), contributing to clarify the evolution of the current ampelographic assortment. One of the most interesting examples is the discovery of the origin of dozens of French varieties from a single pair of parents, ‘Pinot’ and ‘Gouais’ (BOWERS *et al.* 1999 a, BOURSICQUOT *et al.* 2004). This explains why they have less allelic variability than groups of varieties in other countries with strong viticultural traditions and, using specific tests, they can be precisely assigned to their corresponding geographical region of origin (SEFC *et al.* 2000).

‘Sangiovese’ and ‘Garganega’ are ancient and renowned wine varieties, the former being widely cultivated throughout Italy, the latter in Veneto region, mainly in the

provinces of Verona and Vicenza. ‘Sangiovese’ is better known than ‘Garganega’ and was mentioned for the first time by SODERINI (1590) as ‘Sangiogheto’; actually it is the most commonly cultivated black grape variety in Italy and is the basis for the production of famous wines, such as Chianti and Brunello di Montalcino. ‘Garganega’ is probably less known but perhaps even older than ‘Sangiovese’, as it was cited under this name as one of the grapes in the province of Padova by Pier de’ Crescenzi in the 13th century (CALÒ and COSTACURTA 2004). It is famous for the excellent Soave and Gambellara wines and its origins are still unknown.

As a consequence, the hypothesis is plausible that they could have generated progenies over the centuries or could be related to other cultivars growing in the same area. Many possible close kinships emerged from the comparison of ‘Sangiovese’ and ‘Garganega’ molecular profiles with those of hundreds of genotypes collected in the database of Centro di Ricerca per la Viticoltura. These indications were further investigated by increasing the molecular analyses up to 36 SSR loci. Many varieties showed to be involved in a parent-offspring link, some of them having economic or historical importance, such as ‘Trebiano toscano’ *alias* ‘Ugni blanc’, ‘Catarratto’, ‘Albana’, ‘Frappato’, ‘Gaglioppo’, ‘Nerello mascalese’ and ‘Marzemina bianca’. These results trace out the role played by ‘Sangiovese’ and ‘Garganega’ in the appearance of many and well known Italian varieties.

### Material and Methods

**Plant material:** More accessions belonging to 22 varieties were used for genotyping (Tab. 1). They came from the Centro di Ricerca per la Viticoltura collections of Conegliano (Treviso), Arezzo and Turi (Bari), and also from private vineyards in Tuscany and Sicily.

**Nuclear SSR loci analysis:** Genotyping was performed with 11 SSR loci (basic set) routinely employed at the Centro di Ricerca per la Viticoltura of Conegliano for cultivar identification (VVS2, THOMAS and SCOTT 1993; VVMD5, VVMD7, VVMD27 and VVMD28, BOWERS *et al.* 1996 and 1999 b; VrZAG62 and VrZAG79, SEFC *et al.* 1999; ISV2, ISV3, ISV4 and VMCNG4b9, CRESPIAN 2003). Since two groups of synonymous varieties were found (‘Grecanico dorato’ and ‘Garganega’; ‘Catarratto

Table 1

List of 22 analysed varieties

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|                                             |
|---------------------------------------------|
| Sangiovese                                  |
| Ciliegiolo                                  |
| Foglia tonda                                |
| Morellino del Casentino                     |
| Morellino del Valdarno                      |
| Tuccanese di Turi                           |
| Gaglioppo di Cirò                           |
| Vernaccia nera del Valdarno                 |
| Nerello mascalese                           |
| Mantonicone                                 |
| Susumaniello                                |
| Garganega                                   |
| Grecanico dorato                            |
| Marzemina bianca                            |
| Catarratto bianco comune                    |
| Catarratto bianco lucido                    |
| Catarratto bianco extra lucido              |
| Trebbiano toscano                           |
| Malvasia bianca di Candia a sapore semplice |
| Empibotte                                   |
| Albana                                      |
| Greco del Pollino                           |

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bianco comune', 'Catarratto bianco lucido' and 'Catarratto bianco extra lucido'), only one sample for each cultivar was used and the analysis was continued for 19 varieties with 25 nSSR loci, making totally 36 nSSR loci: VVS1 and VVS29 (THOMAS and SCOTT 1993); VVMD17, VVMD21, VVMD24, VVMD25, VVMD26, VVMD31, VVMD32 and VVMD36 (BOWERS *et al.* 1996 and 1999 b); VrZAG21, VrZAG64 and VrZAG83, SEFC *et al.* 1999; VMC1e12, VMC4g6, VMC2h9, VMC3d7, VMC2g2, VMC6e10, VMC4h6, VMC4c6, VMC2h4 and VMC5g6.1 (Vitis Microsatellite Consortium); scu05, SCOTT *et al.* 2000; UCH11, LEFORT *et al.* 2002.

Multiplex PCR of two or three SSR loci were suitably arranged based on expected allele lengths. The PCR reaction mixture (25 µl final volume) contained 20 ng total DNA, 10 µl Eppendorf HotMasterMix (2.5 x) and 5 pmoles of each primer. The PCR was performed in an AB 9700 thermal cycler with the following steps: 1 min 30 s at 94 °C; 35 cycles at 94 °C for 30 s, 55 °C for 30 s, 65 °C for 30 s; 65 °C for 7 min and a final step of at least 10 min at 8 °C to stop the reaction. Five µl of the PCR product were tested on 2 % agarose gel; on the basis of signal intensity, 0.75-1.5 µl of amplified DNA were used for electrophoresis onto a sequencing gel (5 % polyacrylamide, 1 x TBE, 7 M urea). Amplification products of cultivars with alleles of known molecular size were used as references for allele sizing. Allele bands were revealed by silver staining and visually scored at least twice, as reported in CRESPIAN and MILANI (2001).

**Statistical analyses:** The molecular database used for data elaboration encompassed the SSR profiles of 668 wine and table cultivars. Two different approaches were applied: the Kingroup v2 software (KONOVALOV *et al.* 2004) and the haplotype probability (HP).

Kingroup v2 program was used to calculate the likelihoods of the hypothesized pedigree relationships and their significance *versus* alternative relations. The kinship relatedness estimator was applied; parent-offspring relation as primary hypothesis and unrelated as null hypothesis.

The haplotype probability, *i.e.* the expected frequency of an *i* allele at a particular locus was calculated, given the Hardy and Weinberg law assumptions and basing on the third Mendel's law, with the following formula:

$$HP = 2p_i - p_i^2$$

where  $p_i$  is the frequency of allele *i*, computed with Cervus 3.0.3 software ([www.fieldgenetics.com](http://www.fieldgenetics.com)). The loci non in HW equilibrium, as calculated by Cervus, were discarded (VVMD7, VrZAG62 and ISV3). The SSR loci localized on the same linkage group (ADAM-BLONDON *et al.* 2004, MERDINOGLU *et al.* 2005) were considered as a single locus (15 LGs were used) and the allele univocally shared with 'Sangiovese' or 'Garganega' having the lowest frequency was chosen for HP computation; when the common allele was not univocally identifiable, that with greater frequency was selected, consequently the corresponding estimate may be biased in excess. The calculations were done with Excel computation sheet. The total haplotype probability, therefore the probability to find a particular allele combination, obtainable from the supposed parentage relationship, was computed as the product of the HPs at all LGs considered.

## Results and Discussion

**Synonyms:** Genotyping results with the basic set of SSR markers revealed two synonymies (probability of identity:  $7.3 \times 10^{-15}$ ). Firstly, 4 accessions of 'Garganega' and 4 of 'Grecanico dorato' showed the same molecular profile, confirming what previously pointed out by VANTINI *et al.* (2003) on just 2 samples. 'Garganega' has been known in Veneto since at least 1200 and has great morphological variability (COSMO and POLSINELLI 1960, CALÒ and COSTACURTA 2004). The first citation relating to 'Grecanico dorato', growing in Sicily, dates back to the end of 17th century (PASTENA 1969) and different phenotypes were described also in this case.

In order to detect possible synonymies, suggested by the Sicilian name of this cultivar reminding to a hypothetical Greek origin, its molecular profile was compared with those of the *Vitis* microsatellite databases of Centro di Ricerca per la Viticoltura of Conegliano (Italy), University of California, Davis (USA) and University of Crete, Iraklion (Greece) <http://www.biology.uoc.gr/gvd/> as well as with genotypes from various references in literature. 'Garganega'/'Grecanico dorato' showed to be different from all previously described varieties.

The second case of synonymy regarded 'Catarratto', the most widely white wine variety actually growing in Sicily. Three phenotypes were selected in the course of time: 'Catarratto bianco comune', 'Catarratto bianco lucido' and 'Catarratto bianco extra lucido'; the first two are registered in the Italian National Catalogue as distinct varieties. Nevertheless, all the accessions analyzed with the basic set of SSR markers showed identical profile. 'Catarratto bianco comune', 'Catarratto bianco lucido' and 'Catarratto bianco extra lucido' were

ratto' is an ancient variety with historical presence on the island and not cultivated elsewhere, to our knowledge. The type with glossy clusters was first reported by the Ampelographic Commission of Palermo (1883), who distinguished it from 'Catarratto bianco comune', characterized by very pruinose berries. 'Catarratto extra lucido', showing clusters completely without bloom, was selected by PASTENA (1971) among 'Catarratto lucido' vines and our data confirmed what this ampelographer stated *i.e.* the 'Catarratto's are variants obtained by massal selection exploiting the intravarietal variability, therefore the three typologies are somatic mutants derived from vegetative propagation from the same original seedling (Fig. 1).



Fig. 1: From left to right: clusters of 'Catarratto bianco comune', 'Catarratto bianco lucido' and 'Catarratto bianco extra lucido'.

**Pedigree:** 'Sangiovese' displayed first degree relationships with the following black skinned wine varieties (in brackets the percentage of shared alleles): 'Foglia tonda' (0.611), 'Frappato' (0.625), 'Gaglioppo' (0.653), 'Mantonicone' (0.667), 'Morellino del Casentino' (0.722), 'Morellino del Valdarno' (0.625), 'Nerello mascalese' (0.639), 'Susumaniello' (0.625), 'Tuccanese di Turi' (0.625) and 'Vernaccia nera del Valdarno' (0.570), sharing with them at

least one allele at each of the 36 SSR loci analysed (Tab. 2). 'Foglia tonda' and 'Morellino del Casentino' were also linked by a first degree relationship, but for only one allele at VMC 5g6.1 locus. The 'Sangiovese' parents 'Ciliegiolo' and 'Calabrese di Montenuovo' detected by VOULLAMOZ *et al.* (2007) were disputed by DI VECCHI STARAZ *et al.* (2007), since their molecular data indicated a different pedigree for 'Ciliegiolo' as an offspring of 'Sangiovese' and no reliable parent pair could be identified for 'Sangiovese' among the very large number of individuals they tested. The varieties closely related to 'Sangiovese' included cultivars growing in different areas: some were minor Tuscan cultivars, *i.e.* 'Foglia tonda', 'Morellino del Casentino', 'Morellino del Valdarno' and 'Vernaccia nera del Valdarno', this last different from 'Vernaccia' of Marche region (central Italy). Among these, only 'Foglia tonda' is listed in the Italian Catalogue of Vine Varieties and has a certain notoriety, whereas the others have been identified recovering local germplasm and are currently undergoing characterisation (CRESPIAN *et al.*, 2004). Others were typical varieties of Sicily and Calabria, forming part of the viticultural tradition of southern Italy, *i.e.* 'Frappato', 'Gaglioppo', 'Nerello mascalese' and 'Mantonicone'. Lastly there were two varieties from Apulia: 'Susumaniello', registered in the Italian Catalogue, and the unacknowledged 'Tuccanese di Turi' (Bari) (Fig. 2). 'Sangiovese' cannot be generated by any pair of these cultivars, not even taking into account 'Ciliegiolo', nor by selfing of one of them, because it shows alleles absent in each one SSR profile considered.

Similarly to 'Sangiovese', 'Garganega' also exhibited close relationships with many varieties, having at least one allele in common at each of the 36 SSR loci used (Tab. 3); most of them are well-known and fully described. They were 'Trebiano toscano' *alias* 'Ugni blanc' (0.653), 'Albana' (0.667), 'Empibotte' (0.667) and 'Malvasia bianca di Candia a sapore semplice' (0.639), all spread in central Italy; 'Marzemina bianca' (0.639), an ancient cultivar of

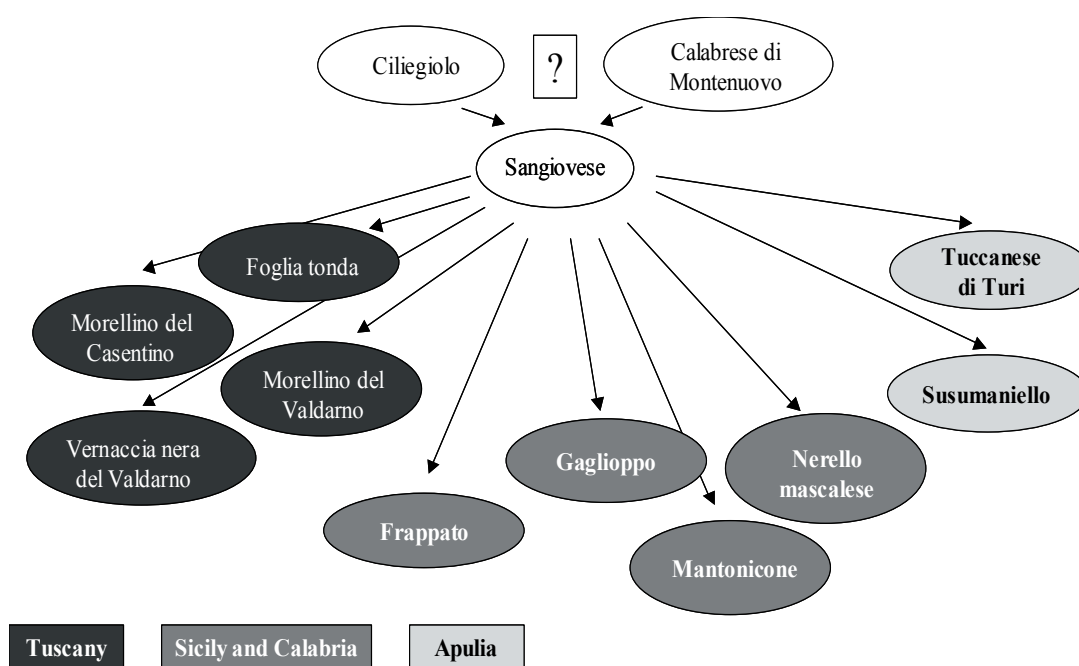


Fig. 2: 'Sangiovese' and first degree related varieties.

Table 2

'Sangiovese' and its progeny molecular data at 36 SSR loci. Allele lengths are in bp

| SSR loci   | LG | Sangiovese | Foglia tonda | Frappato | Gaglioppo | Mantonicone | Morellino del Casentino | Morellino del Valdarno | Nerello mascalese | Susumanuello | Tuccanese di Turi | Vernaccia nera del Valdarno |
|------------|----|------------|--------------|----------|-----------|-------------|-------------------------|------------------------|-------------------|--------------|-------------------|-----------------------------|
| VVS1       | 20 | 181        | 181          | 181      | 181       | 181         | 181                     | 181                    | 181               | 181          | 181               | 181                         |
|            |    | 181        | 190          | 181      | 181       | 181         | 190                     | 181                    | 190               | 181          | 181               | 190                         |
| VVS2       | 11 | 133        | 133          | 133      | 133       | 133         | 133                     | 133                    | 133               | 133          | 133               | 133                         |
|            |    | 133        | 143          | 143      | 151       | 151         | 133                     | 143                    | 143               | 143          | 143               | 143                         |
| VVS29      | 1  | 171        | 171          | 171      | 171       | 171         | 171                     | 171                    | 171               | 171          | 171               | 171                         |
|            |    | 171        | 171          | 171      | 171       | 171         | 171                     | 171                    | 171               | 171          | 171               | 171                         |
| VVMD5      | 16 | 226        | 228          | 226      | 226       | 226         | 228                     | 226                    | 226               | 226          | 228               | 226                         |
|            |    | 236        | 236          | 226      | 226       | 240         | 236                     | 236                    | 236               | 232          | 236               | 240                         |
| VVMD7      | 7  | 239        | 239          | 239      | 239       | 249         | 239                     | 239                    | 239               | 239          | 247               | 239                         |
|            |    | 263        | 263          | 239      | 263       | 263         | 263                     | 247                    | 249               | 249          | 263               | 239                         |
| VVMD17     | 18 | 212        | 212          | 221      | 220       | 212         | 212                     | 212                    | 212               | 212          | 221               | 221                         |
|            |    | 221        | 222          | 222      | 221       | 220         | 221                     | 224                    | 221               | 221          | 224               | 222                         |
| VVMD21     | 6  | 243        | 249          | 243      | 243       | 243         | 249                     | 249                    | 243               | 243          | 243               | 243                         |
|            |    | 249        | 256          | 249      | 249       | 249         | 256                     | 249                    | 249               | 249          | 266               | 243                         |
| VVMD24     | 14 | 210        | 208          | 210      | 210       | 210         | 210                     | 210                    | 210               | 216          | 214               | 210                         |
|            |    | 216        | 216          | 210      | 210       | 216         | 216                     | 216                    | 216               | 219          | 216               | 216                         |
| VVMD25     | 11 | 245        | 245          | 245      | 245       | 245         | 245                     | 245                    | 245               | 245          | 245               | 245                         |
|            |    | 245        | 259          | 259      | 245       | 259         | 245                     | 259                    | 245               | 259          | 259               | 259                         |
| VVMD26     | 1  | 249        | 249          | 249      | 249       | 249         | 249                     | 249                    | 249               | 249          | 249               | 249                         |
|            |    | 249        | 249          | 249      | 251       | 249         | 249                     | 251                    | 251               | 251          | 249               | 249                         |
| VVMD27     | 5  | 179        | 185          | 179      | 179       | 179         | 181                     | 185                    | 179               | 179          | 179               | 179                         |
|            |    | 185        | 185          | 185      | 185       | 185         | 185                     | 189                    | 179               | 189          | 185               | 189                         |
| VVMD28     | 3  | 237        | 239          | 237      | 231       | 231         | 247                     | 247                    | 231               | 237          | 237               | 237                         |
|            |    | 247        | 247          | 261      | 237       | 247         | 261                     | 247                    | 247               | 239          | 261               | 239                         |
| VVMD31     | 7  | 212        | 204          | 212      | 210       | 212         | 212                     | 212                    | 212               | 210          | 212               | 204                         |
|            |    | 212        | 212          | 216      | 212       | 216         | 212                     | 212                    | 216               | 212          | 216               | 212                         |
| VVMD32     | 4  | 253        | 257          | 251      | 253       | 253         | 257                     | 241                    | 253               | 257          | 253               | 257                         |
|            |    | 257        | 273          | 253      | 257       | 257         | 273                     | 257                    | 257               | 263          | 253               | 273                         |
| VVMD36     | 3  | 264        | 258          | 264      | 264       | 264         | 264                     | 264                    | 264               | 250          | 264               | 258                         |
|            |    | 264        | 264          | 276      | 296       | 296         | 270                     | 264                    | 296               | 264          | 270               | 264                         |
| VrZAG 21   | 4  | 202        | 204          | 190      | 200       | 190         | 204                     | 204                    | 190               | 190          | 202               | 204                         |
|            |    | 204        | 206          | 202      | 204       | 204         | 206                     | 206                    | 204               | 204          | 214               | 206                         |
| VrZAG 62   | 7  | 193        | 193          | 193      | 193       | 193         | 187                     | 195                    | 195               | 195          | 193               | 193                         |
|            |    | 195        | 193          | 195      | 201       | 201         | 193                     | 203                    | 201               | 199          | 193               | 195                         |
| VrZAG 64   | 10 | 137        | 139          | 137      | 139       | 137         | 139                     | 137                    | 137               | 139          | 137               | 137                         |
|            |    | 139        | 159          | 139      | 141       | 137         | 159                     | 137                    | 141               | 159          | 139               | 139                         |
| VrZAG 79   | 5  | 242        | 238          | 242      | 242       | 242         | 242                     | 242                    | 250               | 250          | 242               | 244                         |
|            |    | 258        | 242          | 248      | 250       | 250         | 242                     | 244                    | 258               | 258          | 258               | 258                         |
| VrZAG 83   | 8  | 190        | 190          | 190      | 190       | 190         | 190                     | 190                    | 190               | 188          | 194               | 188                         |
|            |    | 194        | 200          | 194      | 194       | 190         | 200                     | 200                    | 190               | 190          | 194               | 190                         |
| ISV2       | 14 | 143        | 143          | 157      | 165       | 141         | 143                     | 143                    | 141               | 143          | 143               | 143                         |
|            |    | 165        | 151          | 165      | 169       | 143         | 169                     | 165                    | 143               | 143          | 143               | 151                         |
| ISV3       | 2  | 139        | 139          | 139      | 133       | 133         | 133                     | 133                    | 133               | 133          | 133               | 139                         |
|            |    | 139        | 145          | 139      | 139       | 139         | 139                     | 139                    | 139               | 139          | 139               | 145                         |
| ISV4       | 11 | 177        | 169          | 177      | 177       | 177         | 177                     | 169                    | 169               | 177          | 191               | 169                         |
|            |    | 197        | 177          | 183      | 191       | 191         | 197                     | 197                    | 177               | 183          | 197               | 177                         |
| VMC 1e12   | 14 | 254        | 250          | 250      | 250       | 254         | 250                     | 246                    | 250               | 254          | 260               | 250                         |
|            |    | 260        | 260          | 254      | 260       | 254         | 254                     | 254                    | 254               | 260          | 260               | 254                         |
| VMC NG 4b9 | 6  | 158        | 158          | 158      | 150       | 150         | 158                     | 168                    | 150               | 168          | 166               | 158                         |
|            |    | 168        | 168          | 164      | 168       | 158         | 168                     | 176                    | 168               | 172          | 168               | 162                         |
| VMC 4g6    | 6  | 127        | 127          | 129      | 127       | 127         | 127                     | 127                    | 127               | 127          | 127               | 125                         |
|            |    | 143        | 143          | 143      | 129       | 129         | 143                     | 133                    | 129               | 129          | 137               | 143                         |
| VMC 2h9    | 6  | 117        | 117          | 117      | 117       | 117         | 117                     | 117                    | 117               | 117          | 117               | 117                         |
|            |    | 117        | 139          | 123      | 117       | 117         | 117                     | 123                    | 123               | 117          | 123               | 139                         |
| VMC 3d7    | 10 | 163        | 163          | 163      | 161       | 163         | 163                     | 161                    | 161               | 163          | 163               | 161                         |
|            |    | 163        | 169          | 177      | 163       | 163         | 169                     | 163                    | 163               | 177          | 163               | 163                         |
| VMC 2g2    | 6  | 119        | 119          | 119      | 119       | 119         | 119                     | 119                    | 119               | 119          | 119               | 119                         |
|            |    | 119        | 141          | 125      | 119       | 119         | 119                     | 125                    | 125               | 119          | 125               | 141                         |
| VMC 6e10   | 5  | 95         | 95           | 95       | 113       | 95          | 95                      | 97                     | 95                | 119          | 93                | 97                          |
|            |    | 119        | 119          | 117      | 119       | 111         | 119                     | 119                    | 113               | 121          | 95                | 119                         |
| VMC 4h6    | 9  | 158        | 158          | 152      | 158       | 158         | 158                     | 158                    | 158               | 158          | 152               | 158                         |
|            |    | 158        | 162          | 158      | 158       | 158         | 162                     | 158                    | 158               | 164          | 158               | 162                         |
| VMC 4c6    | 5  | 157        | 163          | 157      | 163       | 157         | 157                     | 163                    | 157               | 157          | 157               | 163                         |
|            |    | 163        | 166          | 163      | 163       | 163         | 163                     | 163                    | 163               | 163          | 163               | 166                         |
| VMC 2h4    | 12 | 214        | 214          | 198      | 198       | 198         | 208                     | 200                    | 198               | 214          | 214               | 214                         |
|            |    | 214        | 234          | 214      | 214       | 214         | 214                     | 214                    | 214               | 214          | 214               | 234                         |
| scu 05     | 12 | 156        | 156          | 156      | 160       | 156         | 160                     | 156                    | 156               | 160          | 156               | 160                         |
|            |    | 160        | 160          | 171      | 165       | 165         | 169                     | 160                    | 156               | 163          | 156               | 160                         |
| UCH11      |    | 236        | 242          | 246      | 236       | 242         | 236                     | 236                    | 236               | 236          | 242               | 236                         |
|            |    | 262        | 262          | 262      | 236       | 262         | 262                     | 244                    | 262               | 263          | 262               | 242                         |
| VMC 5g6.1  |    | 125        | 125          | 121      | 139       | 139         | 139                     | 142                    | 139               | 125          | 142               | 133                         |
|            |    | 142        | 141          | 142      | 142       | 142         | 142                     | 151                    | 142               | 151          | 151               | 142                         |

Table 3

Molecular data of 'Garganega' and first degree related varieties at 36 SSR loci. Allele lengths are in bp

| SSR loci   | LG | Garganega<br><i>alias</i><br>Grecanico<br>dorato | Albana | Catarratto | Empibotte | Greco bianco<br>del Pollino | Malvasia bianca<br>di Candia a<br>sapore semplice | Marzemina<br>bianca | Trebbiano<br>toscano <i>alias</i><br>Ugni blanc |
|------------|----|--------------------------------------------------|--------|------------|-----------|-----------------------------|---------------------------------------------------|---------------------|-------------------------------------------------|
| VVS1       | 20 | 181                                              | 181    | 181        | 181       | 181                         | 181                                               | 181                 | 181                                             |
|            |    | 181                                              | 181    | 181        | 181       | 182                         | 181                                               | 190                 | 190                                             |
| VVS2       | 11 | 133                                              | 133    | 143        | 133       | 143                         | 133                                               | 133                 | 133                                             |
|            |    | 143                                              | 143    | 151        | 143       | 145                         | 143                                               | 133                 | 143                                             |
| VVS29      | 1  | 171                                              | 171    | 171        | 171       | 171                         | 171                                               | 171                 | 171                                             |
|            |    | 171                                              | 171    | 171        | 171       | 171                         | 171                                               | 171                 | 171                                             |
| VVMD5      | 16 | 226                                              | 226    | 226        | 226       | 232                         | 226                                               | 226                 | 226                                             |
|            |    | 232                                              | 232    | 226        | 232       | 232                         | 238                                               | 238                 | 232                                             |
| VVMD7      | 7  | 249                                              | 247    | 239        | 249       | 239                         | 249                                               | 239                 | 249                                             |
|            |    | 253                                              | 249    | 249        | 253       | 249                         | 263                                               | 253                 | 253                                             |
| VVMD17     | 18 | 222                                              | 222    | 221        | 221       | 222                         | 220                                               | 212                 | 212                                             |
|            |    | 222                                              | 222    | 222        | 222       | 222                         | 222                                               | 222                 | 222                                             |
| VVMD21     | 6  | 249                                              | 249    | 243        | 249       | 249                         | 249                                               | 249                 | 243                                             |
|            |    | 249                                              | 256    | 249        | 249       | 249                         | 256                                               | 249                 | 249                                             |
| VVMD24     | 14 | 210                                              | 210    | 210        | 210       | 210                         | 210                                               | 210                 | 210                                             |
|            |    | 210                                              | 210    | 210        | 216       | 210                         | 210                                               | 210                 | 210                                             |
| VVMD25     | 11 | 245                                              | 245    | 259        | 243       | 245                         | 245                                               | 245                 | 245                                             |
|            |    | 259                                              | 259    | 259        | 259       | 259                         | 259                                               | 245                 | 259                                             |
| VVMD26     | 1  | 251                                              | 255    | 249        | 249       | 251                         | 249                                               | 249                 | 251                                             |
|            |    | 263                                              | 263    | 251        | 263       | 251                         | 251                                               | 263                 | 263                                             |
| VVMD27     | 5  | 179                                              | 179    | 179        | 183       | 179                         | 185                                               | 185                 | 179                                             |
|            |    | 194                                              | 189    | 179        | 194       | 185                         | 194                                               | 194                 | 183                                             |
| VVMD28     | 3  | 239                                              | 237    | 231        | 239       | 249                         | 249                                               | 239                 | 247                                             |
|            |    | 251                                              | 239    | 239        | 251       | 251                         | 251                                               | 239                 | 251                                             |
| VVMD31     | 7  | 210                                              | 204    | 210        | 210       | 213                         | 210                                               | 213                 | 210                                             |
|            |    | 213                                              | 210    | 210        | 213       | 213                         | 210                                               | 216                 | 212                                             |
| VVMD32     | 4  | 251                                              | 251    | 251        | 251       | 251                         | 259                                               | 251                 | 251                                             |
|            |    | 259                                              | 273    | 253        | 273       | 253                         | 259                                               | 253                 | 273                                             |
| VVMD36     | 3  | 254                                              | 248    | 254        | 266       | 264                         | 264                                               | 244                 | 264                                             |
|            |    | 266                                              | 254    | 296        | 296       | 266                         | 266                                               | 254                 | 266                                             |
| VrZAG 21   | 4  | 190                                              | 202    | 190        | 202       | 202                         | 190                                               | 190                 | 202                                             |
|            |    | 202                                              | 206    | 200        | 206       | 214                         | 190                                               | 202                 | 206                                             |
| VrZAG 62   | 7  | 199                                              | 193    | 199        | 199       | 187                         | 199                                               | 193                 | 193                                             |
|            |    | 199                                              | 199    | 201        | 203       | 199                         | 201                                               | 199                 | 199                                             |
| VrZAG 64   | 10 | 137                                              | 137    | 139        | 137       | 137                         | 137                                               | 139                 | 137                                             |
|            |    | 139                                              | 139    | 141        | 139       | 143                         | 139                                               | 143                 | 163                                             |
| VrZAG 79   | 5  | 250                                              | 250    | 250        | 246       | 250                         | 238                                               | 248                 | 244                                             |
|            |    | 250                                              | 250    | 250        | 250       | 250                         | 250                                               | 250                 | 250                                             |
| VrZAG 83   | 8  | 190                                              | 188    | 194        | 188       | 190                         | 190                                               | 194                 | 188                                             |
|            |    | 194                                              | 194    | 194        | 194       | 194                         | 194                                               | 194                 | 194                                             |
| ISV2       | 14 | 141                                              | 165    | 165        | 141       | 141                         | 141                                               | 141                 | 141                                             |
|            |    | 165                                              | 169    | 169        | 157       | 169                         | 169                                               | 165                 | 161                                             |
| ISV3       | 2  | 133                                              | 133    | 139        | 133       | 139                         | 133                                               | 133                 | 133                                             |
|            |    | 139                                              | 139    | 145        | 139       | 145                         | 139                                               | 139                 | 139                                             |
| ISV4       | 11 | 177                                              | 169    | 177        | 169       | 169                         | 177                                               | 169                 | 177                                             |
|            |    | 187                                              | 187    | 191        | 187       | 177                         | 187                                               | 177                 | 187                                             |
| VMC 1e12   | 14 | 240                                              | 240    | 240        | 240       | 240                         | 240                                               | 240                 | 240                                             |
|            |    | 240                                              | 260    | 254        | 240       | 260                         | 260                                               | 256                 | 246                                             |
| VMC NG 4b9 | 6  | 176                                              | 158    | 150        | 158       | 158                         | 150                                               | 166                 | 162                                             |
|            |    | 178                                              | 176    | 176        | 176       | 176                         | 176                                               | 176                 | 176                                             |
| VMC 4g6    | 6  | 129                                              | 133    | 129        | 127       | 133                         | 125                                               | 129                 | 125                                             |
|            |    | 133                                              | 143    | 133        | 133       | 143                         | 129                                               | 133                 | 133                                             |
| VMC 2h9    | 6  | 123                                              | 117    | 117        | 117       | 117                         | 117                                               | 117                 | 117                                             |
|            |    | 125                                              | 125    | 123        | 125       | 123                         | 125                                               | 123                 | 123                                             |
| VMC 3d7    | 10 | 163                                              | 163    | 161        | 163       | 163                         | 159                                               | 163                 | 161                                             |
|            |    | 175                                              | 175    | 175        | 163       | 163                         | 175                                               | 175                 | 175                                             |
| VMC 2g2    | 6  | 125                                              | 119    | 119        | 119       | 119                         | 119                                               | 119                 | 119                                             |
|            |    | 127                                              | 127    | 125        | 127       | 125                         | 127                                               | 125                 | 125                                             |
| VMC 6e10   | 5  | 93                                               | 93     | 93         | 113       | 93                          | 93                                                | 97                  | 117                                             |
|            |    | 117                                              | 121    | 113        | 117       | 93                          | 113                                               | 117                 | 117                                             |
| VMC 4h6    | 9  | 152                                              | 152    | 152        | 158       | 158                         | 152                                               | 152                 | 158                                             |
|            |    | 158                                              | 182    | 162        | 158       | 162                         | 162                                               | 158                 | 158                                             |
| VMC 4c6    | 5  | 163                                              | 163    | 163        | 163       | 163                         | 163                                               | 163                 | 157                                             |
|            |    | 163                                              | 166    | 163        | 163       | 163                         | 166                                               | 163                 | 163                                             |
| VMC 2h4    | 12 | 206                                              | 206    | 206        | 214       | 214                         | 206                                               | 214                 | 206                                             |
|            |    | 214                                              | 214    | 232        | 214       | 214                         | 214                                               | 214                 | 214                                             |
| scu 05     | 12 | 165                                              | 160    | 165        | 156       | 169                         | 165                                               | 160                 | 165                                             |
|            |    | 169                                              | 169    | 165        | 169       | 184                         | 165                                               | 169                 | 165                                             |
| UCH11      |    | 242                                              | 242    | 236        | 236       | 242                         | 242                                               | 244                 | 246                                             |
|            |    | 246                                              | 260    | 242        | 246       | 242                         | 244                                               | 246                 | 246                                             |
| VMC 5g6.1  |    | 139                                              | 129    | 139        | 151       | 142                         | 141                                               | 141                 | 139                                             |
|            |    | 151                                              | 139    | 151        | 151       | 151                         | 151                                               | 151                 | 151                                             |



North East Italy, which originated 'Raboso veronese' (CRESPAN *et al.* 2006); the before described 'Catarratto' (0.611) and finally 'Greco del Pollino' (0.611), a minor variety present in southern Italy, in particular in Calabria, Basilicata and Apulia, under different synonymous names (COSTACURTA *et al.* 2004) (Fig. 3). Unlike 'Sangiovese' related varieties, those found to be close to 'Garganega' are spread from North to South Italy and cover a broader area, even if they are less numerous. 'Trebiano toscano' is greatly spread also in France (GALET 2000).

No couple of these cultivars could originate 'Garganega' and even selfing of one of them must be excluded, since no combination can provide the alleles matched in its SSR profile. Therefore, being the parents of 'Garganega' still undiscovered, it was difficult to make any hypotheses on its exact relationship with these varieties; the identification of the "missing" parents will allow to clarify the correct pedigree direction.

The presence of 'Malvasia bianca di Candia a sapore semplice' in this kinship is interesting, since Malvasias form a complex group of highly heterogeneous varieties (COSTACURTA *et al.* 2005, LACOMBE *et al.* 2007), the majority of which are growing in Italy and whose geographical origin is often unknown.

**Statistical analyses:** The results obtained elaborating SSR data with KinGroup vs2 program are reported in Tab. 4. The probability ratios between the hypothesis that the listed varieties could be really linked to 'Sangiovese' or 'Garganega' by a first degree relationship, in respect to the probability that they were not, gave positive values, highly significant in respect to the whole population mean, which had a strongly negative value,  $-3.39 \times 10^{-38}$ .

The haplotype probabilities of each one 'Sangiovese' and 'Garganega' first degree related cultivar are shown in Tab. 5. The computed values, in spite of the great restric-

Table 4

Kingroup likelihood ratios of parent-offspring relationships vs. unrelated, computed with 668 only cvs and over 35 SSR loci

|                             |                             |           |
|-----------------------------|-----------------------------|-----------|
| Sangiovese                  | Morellino del Casentino     | 25.57     |
|                             | Tuccanese di Turi           | 24.66     |
|                             | Mantonicone                 | 22.77     |
|                             | Morellino del Valdarno      | 22.71     |
|                             | Nerello mascalese           | 21.68     |
|                             | Gaglioppo                   | 20.74     |
|                             | Susumaniello                | 19.80     |
|                             | Foglia tonda                | 19.22     |
|                             | Frappato                    | 16.69     |
|                             | Vernaccia nera del Valdarno | 16.01     |
| Garganega                   | Empibotte                   | 29.43     |
|                             | Malvasia di Candia a.s.s.   | 26.82     |
|                             | Trebiano toscano            | 25.28     |
|                             | Albana                      | 24.53     |
|                             | Marzemina bianca            | 24.17     |
|                             | Greco bianco del Pollino    | 21.74     |
|                             | Catarratto                  | 17.55     |
| Whole population mean ratio |                             | -3.39E+38 |

tions imposed in the calculation, go from  $3.24 \times 10^{-6}$  to  $8.98 \times 10^{-9}$  and indicate clearly that these particular combinations can not be due to instance, but are well explainable with parent-offspring relationships. In summary, both computational approaches contributed to corroborate the hypotheses issued from simple molecular data scrolling.

## Conclusions

Our findings indicate that 'Sangiovese' and 'Garganega' were crucial in the evolution of the Italian ampelo-

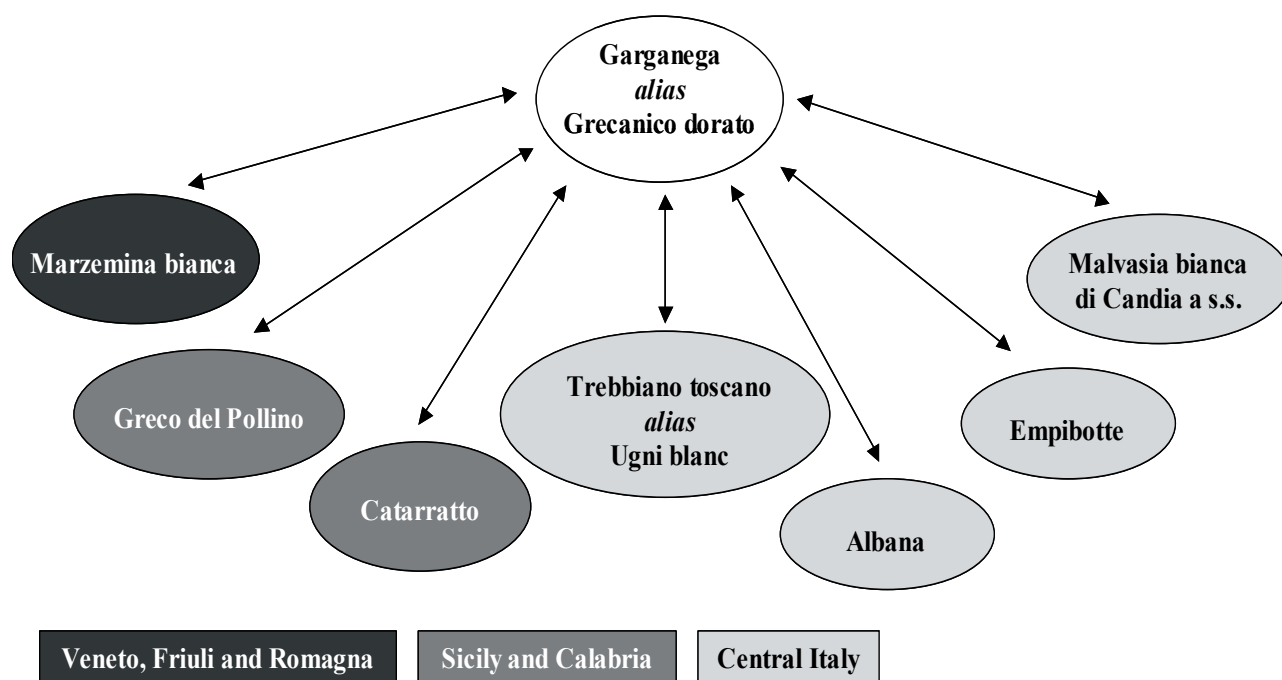


Fig. 3: 'Garganega' and first degree related varieties.

Table 5

Haplotype probability (HP) of Sangiovese's presumed progeny and first degree related varieties linked to Garganega, computed on 15 linkage groups

|            |                             |                         |
|------------|-----------------------------|-------------------------|
| Sangiovese | Foglia tonda                | 2.25 x 10 <sup>-7</sup> |
|            | Frappato                    | 3.24 x 10 <sup>-6</sup> |
|            | Gaglioppo                   | 6.31 x 10 <sup>-7</sup> |
|            | Mantonicone                 | 3.12 x 10 <sup>-7</sup> |
|            | Morellino del Casentino     | 4.29 x 10 <sup>-7</sup> |
|            | Morellino del Valdarno      | 4.71 x 10 <sup>-8</sup> |
|            | Nerello mascalese           | 1.58 x 10 <sup>-7</sup> |
|            | Susumaniello                | 8.03 x 10 <sup>-8</sup> |
|            | Tuccanese di Turi           | 7.73 x 10 <sup>-8</sup> |
|            | Vernaccia nera del Valdarno | 1.15 x 10 <sup>-6</sup> |
| Garganega  | Albana                      | 1.89 x 10 <sup>-8</sup> |
|            | Catarratto                  | 4.02 x 10 <sup>-8</sup> |
|            | Empibotte                   | 6.13 x 10 <sup>-9</sup> |
|            | Greco bianco del Pollino    | 8.98 x 10 <sup>-9</sup> |
|            | Malvasia bianca di Candia   | 3.79 x 10 <sup>-9</sup> |
|            | a sapore semplice           |                         |
|            | Marzemina bianca            | 7.37 x 10 <sup>-9</sup> |
|            | Trebbiano toscano           | 2.42 x 10 <sup>-8</sup> |

graphic assortment and demonstrate that 'Sangiovese' has clear and dated relationships with Southern Italian varieties. Our data confirm and corroborate, on one side, some of the indications given by DI VECCHI STARAZ *et al.* 2007 about the role played by these two cultivars, also using a mostly different set of SSR markers; on the other side, our work adds new members to the respective families of 'Sangiovese' and 'Garganega'. The detection of missing parents in the two family trees will contribute to clarify the respective temporal appearance of close related varieties. It is interesting to notice an additional synonym of 'Garganega'/'Greco dorato' reported by GALET (2000), who stated: "Selon Carpentieri ce plant serait identique au greco d'Arcetri (*i.e.* south of Florence), ou decanico, synonymes non recontrés en Sicile": this would fill the strange gap on the apparent absence of this variety in central Italy.

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