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A banned variety was the mother of several major wine grapes

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A number of widely grown varieties of *Vitis vinifera* ssp. *sativa*, the grape used for wine production, are known to have resulted from crosses between Pinot noir and Gouais blanc, although it is not known which was the maternal parent in these crosses. We have analysed microsatellites and a single nucleotide polymorphism (SNP) in chloroplast DNA from these two varieties and twelve progeny strains, including Chardonnay, Gamay noir and Aligoté. The results demonstrate that Gouais blanc was the maternal parent for nine of these strains, including Chardonnay, Gamay noir and Aligoté. This is a striking conclusion, as Gouais is generally considered a highly inferior variety, and its cultivation was banned for many years in parts of Europe.

Keywords: *Vitis vinifera*; Chardonnay; maternal inheritance; chloroplast DNA; microsatellite; SNP

1. INTRODUCTION

Wine is made from cultivars of the Eurasian grape (*Vitis vinifera* ssp. *sativa*), which is believed to have been domesticated from wild populations of the vine *V. vinifera* ssp. *sylvestris* on at least two separate occasions (Arroyo-Garcia *et al.* 2006). Although vines of existing grape varieties are propagated vegetatively, new varieties have typically resulted from spontaneous crosses between cultivars. The parents of such crosses were probably unknown and certainly unrecorded at the time of the crossing. Using a set of nuclear microsatellite loci, Bowers *et al.* (1999) showed that repeated crossing of just two parental cultivars, Pinot noir and Gouais blanc, which were both widely grown in north-eastern France during the Middle Ages, produced an exceptionally large number of new varieties, including several that are now of enormous international importance. Bowers *et al.* (1999) point out that in spite of this, Gouais blanc is considered to be a poor variety, and attempts were made in historical times to ban its use. However, it is not known which of the parental varieties was the male (pollen donor) and which the female (egg donor), and whether it was different in different crosses. This information would be significant, not only for its historical interest but also because in many plants, including *Vitis*, the female parent is the source of chloroplast DNA (Corriveau & Coleman 1988). In other economically significant plants important characteristics can be determined by the chloroplast genome, including tolerance to chilling (Chung *et al.* 2007) or to the fungal toxin tentoxin (Avni *et al.* 1992). The plant mitochondrial genome is usually inherited from the same parent as the chloroplast genome, and can also determine important traits, such as cytoplasmic male sterility (Schnable & Wise 1998). It has been reported that anthocyanin content may be influenced by the maternal parent in *Vitis* (Liang *et al.* 2009), although it is not clear if this depends on the organelle genomes.

We set out to determine which way round the Pinot × Gouais crosses occurred for 12 progeny varieties, Aligoté, Aubin vert, Auxerrois, Bachet, Chardonnay, Franc noir, Gamay noir, Knipperlé, Melon, Romorantin, Roublot and Sacy, using chloroplast DNA markers from these and the parental varieties Pinot noir and Gouais blanc.

2. MATERIAL AND METHODS

The varieties Aubin vert E-1, Bachet E-1, Franc noir E-1, Knipperlé E-1, Sacy 783 and Romorantin 466 were obtained from the Espiguette Estate of the Institut Français de la Vigne et du Vin (IFV, formerly ENTAV). Aligoté 01, Auxerrois 01, Chardonnay 102, Gamay noir 06, Pinot noir 74 and Pinot noir 102 were obtained from the Foundation Plant Services vineyard at UC Davis. Gouais blanc (Plant ID 39794 and 39798) was obtained from the Foundation Plant Services greenhouse.

Leaves were crushed onto Whatman FTA cards. Discs punched from the cards were washed with three aliquots of 800 µl of the elution buffer provided by the manufacturer, followed by two washes with 800 µl of 10 mM Tris-HCl pH 8.0, 0.1 mM EDTA. Triton X100 was added to the washes to 1% v/v, followed by overnight incubation at room temperature. A 400 µl sample of each of these washes was taken and 800 µl of 96 per cent ethanol and 120 µl of 4 M sodium acetate were added. Samples were then incubated at −80°C for 20 min followed by centrifugation at 13 000 rpm in an Eppendorf microcentrifuge. The supernatant was discarded and the pellets were washed with 500 µl of 70 per cent ethanol and then dissolved in 100 µl water. Whichever DNA preparation gave best yields in PCR from the five washes of a given card was used for subsequent analysis.

Primers incorporating M13 tails were synthesized for analysis of the chloroplast microsatellite loci cpSSR3, cpSSR5 and cpSSR10 (Arroyo-Garcia *et al.* 2002) as follows:

cpSSR3F CACGACGTTGTAAAACGACTCAAGCCAATCG TTTTGAATGCC;
cpSSR3R ACTTTGGTTTCATTCGGCTC;
cpSSR5F CACGACGTTGTAAAACGACTCTCTCTTCCAAA TTGATGTTCCA;
cpSSR5R TTAATGGCTTGGATCGTGTATC;
cpSSR10F CACGACGTTGTAAAACGACTCTCGCCGCCG TAGTAAGTAGT;
cpSSR10R CGTTCGCCCAAACTAAACAT.

Primers for a SNP, identified within the region covered by the primers for the microsatellite locus cpSSR4, and designated cp4527, were synthesized as follows:

Forward AGCAACCCGAATAAGATAAA.
Reverse AGATGATTGAATAGACCCGC.

The polymorphic locus corresponded to position 4527 in the GenBank accession DQ424856.1. PCR was carried out using Taq polymerase from Bioline according to the manufacturer's instructions with 1 µl of DNA template solution prepared as described above. A FAM label was incorporated onto cpSSR PCR products for genotyping as described by Boutin-Ganache *et al.* (2001). For the cpSSR loci the cycling parameters were 3 min at 94°C, 33 cycles of (30 s at 94°C, 45 s at Tm, 1 min at 72°C), 10 cycles of (30 s at 94°C, 45 s at 53°C, 1 min at 72°C), 10 min at 72°C. Tm was set at 1°C below the predicted annealing temperature for the primers used. For cp4527 the cycling parameters were 2 min at 94°C,

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Table 1. Sequence of the newly identified single nucleotide polymorphism locus cp4527 (bold) and microsatellite allele types at four cpSSR loci for the parental and twelve progeny varieties under study. Identical results for cp4527 and cpSSR10 were obtained for two separate vines of Gouais blanc and two of Pinot noir. Data for cpSSR9, 14 and 23 are from Arroyo-Garcia *et al.* (2006). S, short allele; L, long allele.

variety	cp4527 SNP	SSR9	SSR10	SSR14	SSR23
Gouais blanc	TAAAAATT**G**ATAGACCAA	S	L	L	L
Pinot noir	TAAAAATT**T**ATAGACCAA	L	S	S	S
Aligoté	TAAAAATT**G**ATAGACCAA	S	L	L	L
Auxerrois	TAAAAATT**G**ATAGACCAA	S	L	L	L
Bachet	TAAAAATT**G**ATAGACCAA	S	L	L	L
Chardonnay	TAAAAATT**G**ATAGACCAA	S	L	L	L
Franc noir	TAAAAATT**G**ATAGACCAA	S	L	L	L
Gamay noir	TAAAAATT**G**ATAGACCAA	S	L	L	L
Melon	TAAAAATT**G**ATAGACCAA	S	L	L	L
Romorantin	TAAAAATT**G**ATAGACCAA	S	L	L	L
Sacy	TAAAAATT**G**ATAGACCAA	S	L	L	L
Aubin vert	TAAAAATT**T**ATAGACCAA	L	S	S	S
Knipperlé	TAAAAATT**T**ATAGACCAA	L	S	S	S
Roublot	TAAAAATT**T**ATAGACCAA	L	S	S	S

35 cycles of (1 min at 94°C, 1 min at 51°C, 2 min at 72°C), 7 min at 72°C. Products were analysed in 2.5 per cent agarose gels, stained with ethidium bromide and visualized using UV-light. PCR products were purified when necessary using an Illustra GFX PCR Purification kit (GE Healthcare, Amersham, UK) according to the manufacturer's instructions. Genotyping was carried out on an ABI3730 genotyping machine at the National Institute of Agricultural Botany, Huntingdon Road, Cambridge, UK and DNA sequencing was carried out by Geneservice, Cowley Road, Cambridge, UK. PCR and sequencing of the cp4527 locus were performed in duplicate. No evidence of polymorphism within individual cultivars at any of the chloroplast loci was found, either within our data or when comparing our data with those of Arroyo-Garcia *et al.* (2006). This is consistent with the fact that cultivars are invariably propagated asexually and only very low levels of somaclonal variation are seen within cultivars, even with nuclear microsatellites (Bowers *et al.* 1999; Riaz *et al.* 2002).

3. RESULTS AND DISCUSSION

Using DNA isolated from leaf samples, we amplified a range of chloroplast DNA microsatellites previously used to analyse earlier events in *Vitis* history and domestication (Arroyo-Garcia *et al.* 2006). No variation was found for cpSSR3 or cpSSR5, but variation was found for cpSSR10. Two alleles were found, differing by two nucleotides. These results were in agreement with those of Arroyo-Garcia *et al.* (2006), who found three additional chloroplast microsatellites polymorphic for the varieties under study (cpSSR9, 14 and 23), but did not use them to infer the direction of the crosses under investigation. In addition, variation in the annealing efficiency of PCR primers for another microsatellite (cpSSR4) allowed us to identify a novel single nucleotide polymorphism (SNP), designated cp4527, that also varied between the parents. The microsatellite and SNP chlorotypes of all the varieties used are shown in table 1. The results from both kinds of marker are completely consistent. Gouais blanc and one group of varieties contained the long alleles of cpSSR10, cpSSR14 and cpSSR23, a short allele of cpSSR9 and a G nucleotide in the SNP. Pinot noir and the remainder had shorter alleles of cpSSR10, cpSSR14 and cpSSR23, a long allele of cpSSR9 and a T nucleotide in the SNP.

Given that chloroplast DNA is maternally inherited in *Vitis* (Corriveau & Coleman 1988), we can say that Gouais blanc was the maternal parent for Aligoté, Auxerrois, Bachet, Chardonnay, Franc noir, Gamay noir, Melon, Romorantin and Sacy. Pinot noir was the maternal parent for Aubin vert, Knipperlé and Roublot. It is particularly ironic that the despised grape Gouais blanc was not just a parent for several of the world's best known and most important varieties, such as Chardonnay and Aligoté, it was the maternal parent, providing additional DNA and potentially determining important characteristics of the offspring.

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