

## Evidence of Gene Flow between Wild and Cultivated Grapevine (*Vitis vinifera* L.) in France

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### Abstract

The wild grapevine, *Vitis vinifera* subsp. *silvestris*, is an endangered species. In 1999, we initiated an inventory and a characterization of wild grapevine in France. Since this subspecies is dioecious and since, in many cases, wild grapevine have been identified near vineyards, we have been concerned about gene flow between cultivated and wild grapevines. In order to identify such a gene flow, we sampled individuals issued from open pollination in natural environment of the same mother. We identify pollinators by parentage analysis using the 20 SSR markers. Most of the pollen originated from male wild individuals, but evidence of gene flow, even if at a low level, was revealed.

### INTRODUCTION

The wild grapevine, *Vitis vinifera* subsp. *silvestris*, is the ancestral form of *V. vinifera* L. This subspecies represents the only endemic taxon of *Vitaceae* in Europe. The repartition of this subspecies extends from Portugal to Turkmenistan and from Rhine riversides to northern forests of Tunisia (Arnold et al., 1998). In this area, it is rare to find wild grapevine in natural environments because it is an endangered subspecies. Wild grapevine (*V. vinifera* subsp. *silvestris*) is dioecious, while domesticated grapevine (*V. vinifera* subsp. *sativa*) is hermaphrodite (excluding rare exceptions of female cultivars). In the core of a research program, we have initiated an inventory of wild grapevine in France. About 300 individuals have been inventoried (Lacombe et al., 2003a, b) and many of those have been identified in close proximity to vineyards. This proximity may increase risk of gene flow between compartments, especially because *V. vinifera silvestris* is dioecious. In order to analyze the relationships between wild and cultivated grapevine the pollen flux for wild individuals has to be investigated. Direct estimation of contemporary gene flow is possible by comparing genotypes of potential parents with genotypes of seeds collected from maternal individuals or of naturally established seedlings (Kameyama et al., 2001; Di Vecchi Staraz et al., 2006a). Such exclusion analysis has shown great progress with the emergence of microsatellite markers, which are characterized by high polymorphism and codominant alleles. Largely utilized in grapevine genetic studies, they have proven very usefulness in parentage analysis (Bowers and Meredith, 1997; Bowers et al., 1999; Dettweiler et al., 2000; Sefc et al., 1998; Vouillamoz et al., 2003; Di Vecchi Staraz et al., 2006b).

In the present work, we investigated pollen mediated gene flow by the parentage analysis of individuals derived from seeds harvested on one female wild grapevine located in the south of France, using 20 SSR markers.

## MATERIALS AND METHODS

### Plant Material and Microsatellite Markers

We collected seeds from the female wild grapevine ('PSL 12') belonging to the population (26 wild individuals) located in the south of France (near the town of Montpellier), described first by This et al. (2001). The nearest vineyards are at 500 m. The 48 seedlings obtained from seeds were sampled and analyzed using 20 microsatellite loci (nSSR) well scattered on the genome: VVMD5, VVMD7, VVMD21, VVMD24, VVMD25, VVMD27, VVMD28, VVMD32 (Bowers et al., 1996, 1999); VVIn16, VVIv67, VVIv37, VVIq52, VVIp60, VVIh54, VVIb01, VVIn73, VVIp31 (Merdinoglu et al., 2005); VVS2 (Thomas and Scott, 1993) and VMC1b11, VMC4f3 (Vitis Microsatellite Consortium).

### Parentage Analysis

In order to find the potential fathers of the seedlings analyzed, we compared their genotypes to the large database realized by Laucou et al. (2006), consisting of 2704 *Vitis vinifera* L. unique genotypes from the Domaine de Vassal INRA (Institut National de la Recherche Agronomique, France) grape repository, and 30 genotypes corresponding to the individuals from the site studied. Parentage analysis and handling of the data set were performed as described by Di Vecchi Staraz et al. (2006b), using FaMoz software (Gerber et al., 2003). Paternities were assigned calculating a logarithm of the likelihood ratio, Log of odds ratio (LOD-score), and on the basis of the LOD-scores of parent pairs.

## RESULTS AND DISCUSSION

We identified the probable father of 39 of the studied descendant seedlings (Table 1). Twenty-nine are issued from the pollination of a single nearest wild male (60% of the samples). This illustrates the effectiveness of the pollination of the nearest male. Seven more seedlings are from wild grapevine located in the neighbourhoods of the mother plant, for a total of 36 (75%) fathers belonging to wild compartment of *Vitis vinifera* L. The identification of two descendants from the wild female 'PSL 13' required further investigations about its flowers and viability of pollen.

Nevertheless, we identified two fathers belonging to the cultivated compartment. They are two well-known cultivars: 'Cabernet-sauvignon' and 'Cinsaut'. They are cultivated not far (less than 2 km) from the area where we collected the samples (Fig. 1). Thus, we observed gene flow between wild and cultivated grapevine, but at a low level (4%). We did not identify the fathers of ten descendant seedlings, because we have to further investigate the area, searching other wild grapevines that could be the unidentified fathers.

## CONCLUSIONS

We showed the usefulness of parentage analysis by microsatellite markers in inferring gene flow in grapevine (*Vitis vinifera* L.). Although in a limited area located in the south of France, we demonstrated the existence of gene flow between wild and cultivated grapevine. It is now necessary to further investigate, expanding the area involved and increasing the number of individuals studied.

This finding will have impacts on our understanding of grapevine evolution. Furthermore, this will have an impact on the in situ conservation of wild grapevine (also ex situ, if wild accessions are collected in the vicinity of vineyards).

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## Tables

Table 1. The results of the parentage analysis of the 48 seedlings issued from pollen-mediated fertilization.

Father (sex)	Descendant
PSL 9 (male)	2
PSL 11 (male)	29
PSL 13 (female)	2
PSL 21 (male)	1
PSL 25 (male)	1
PSL 26 (male)	1
Cabernet-sauvignon (hermaphrodite)	1
Cinsaut (hermaphrodite)	1
Non-identified	10
Total	48

## Figures

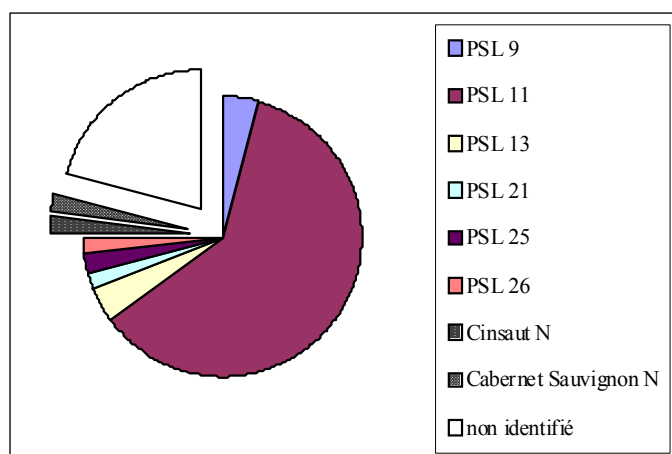


Fig. 1. The pie-chart illustrating the fathers of the 48 seedlings assigned by the parentage analysis.