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VINE AND WINE CULTURAL HERITAGE

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Abstract: Genetic and genomic information available in grapevine in combination with the analyses of archaeological remains can help to shed light on questions regarding its domestication process, such as the number and location of domestication events, the temporal sequence of the domestication process or the identification of some of the genes that drove their cultivar diversification. The available genetic evidence suggests the existence of limited grapevine domestication from its wild ancestor *Vitis vinifera* ssp. *sylvestris*, with almost no reduction in genetic variation due to the extensive use of vegetative multiplication. Genetic evidence also suggests that domestication would have taken place in the Eastern end of the distribution of the wild ancestor, although both nuclear and chloroplast markers point out the existence of introgression or secondary domestication events from Western wild populations into Western cultivars. The relevance of vegetative multiplication is also pointed out by the large accumulation of somatic mutations selected as drivers of phenotypic diversification. However, spontaneous hybridizations among cultivars are in the basis of the origin of current cultivars and cultivar families associated to different geographic regions.

Resumen: La información genética y genómica disponible en vid (*Vitis vinifera* subsp. *vinifera*) junto con el análisis de restos arqueológicos pueden ayudar a conocer aspectos relacionados con su proceso de domesticación, tales como la localización y el número de sucesos de domesticación, la secuencia temporal del proceso de domesticación o incluso la identificación de alguno de los genes que han participado en la diversificación fenotípica de las variedades. La evidencia genética disponible sugiere la existencia de una domesticación de la vid muy limitada a partir de su ancestro silvestre *Vitis vinifera* subespecie. *sylvestris*, en la que casi no ha habido reducción de variación genética debido al amplio uso de la multiplicación vegetativa. La evidencia genética también sugiere que la domesticación tuvo lugar principalmente en el extremo oriental del área de distribución del ancestro silvestre, aunque tanto los marcadores nucleares como cloroplásticos ponen de manifiesto la existencia de introgresión o de sucesos secundarios de domesticación entre las poblaciones silvestres y las variedades occidentales. La importancia que ha tenido la multiplicación vegetativa en la historia del cultivo se vuelve a poner de manifiesto en el amplio número de mutantes somáticos seleccionados como generadores de diversificación fenotípica. Sin embargo, las hibridaciones espontáneas entre variedades están en la base del origen de las variedades y familias varietales actuales, que se asocian con distintas regiones geográficas.
De acuerdo con el limitado síndrome de domesticación que se observa en la vid, la reducción en diversidad genética derivada del proceso de domesticación también ha sido muy reducida. Aún así, se pueden identificar algunos caracteres morfológicos como el hermafroditismo de las flores, el gran tamaño de racimos y bayas y el gran contenido en azúcares de las bayas, que podrían haberse fijado en todas las variedades cultivadas. Para otros caracteres, como la forma de la baya, su color o su aroma, se observa un importante aumento de su diversidad en las formas cultivadas con respecto a las silvestres. El avance de la genética molecular de la vid está permitiendo identificar algunos de los genes responsables de estos caracteres. Recientemente se ha identificado un gen que participa en la regulación del tamaño del racimo y del tiempo de apertura de las flores, así como los genes responsables de gran parte de la variación que se observa en la vid para el color de la baya o para el aroma Moscatel.
Finalmente, aunque la secuencia genómica de una especie conserva mucha información sobre su pasado evolutivo, sólo el estudio de las secuencias de ADN pertenecientes a los genotipos antiguos nos abre una ventana al pasado. Por ello es especialmente importante la puesta a punto de métodos de extracción y análisis del ADN antiguo que todavía puede encontrarse en algunos restos arqueológicos.

Key words: molecular evolution, domestication, grapevine, ancient DNA, genome.
Palabras clave: evolución molecular, domesticación, uva, ADN antiguo, genoma

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The history written in the grapevine genome

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INTRODUCTION

Archeology is the discipline that traditionally deals with the reconstruction of human history. However, molecular genetics and, recently, the information provided by the human genome sequence are making increasing contributions to this discipline (Pääbo, 2003). In addition, genetic and genomic information available in domesticated animal and plant species are also helping to understand domestication processes in many different areas, such as the identification of the wild progenitors, the number and location of the domestication events as well as the temporal sequence of domestication (Zeder *et alii*, 2006a; Burke *et alii* 2007; Purugganan & Fuller 2009). Domestication processes can be considered as a type of human and target species co-evolution. When the target of domestication coincides with the archeological remains, as it is the case of grain crops, archeology and molecular genetics working together can provide a very clear picture on the above mentioned questions (Zeder *et alii* 2006a; Purugganan & Fuller, 2009). Based on the grain remains, archeology can follow phenotypic changes in time and space and trace the micro-evolutionary dynamics that accompany species diversification. On the other hand, genetic information on the target species provides a molecular framework in this evolutionary process, linking selective mechanisms inferred from archaeological studies to the genes that drove the diversification of the domesticated species (Burke *et alii*, 2007).

When considering grapevine, the recent publication of its reference genome sequence (Jaillon *et alii*, 2007; Velasco *et alii*, 2007) offers new possibilities for its application to help answering historical and biological questions on the domestication process and the use of grape and wine by human cultures. The biological and evolutionary features of grapevine will condition these applications as well as the fact that the main archeological remains (seeds) are not the targets of selection during the domestication process or that the major grape product, the wine, does not generally leave biological remains.

In this review, we will first consider the particular biological and evolutionary aspects of grapevine as well as the available information on its genome nucleotide sequence variation. Second, we will describe several examples of the information that grapevine molecular genetics is providing on the origin and evolution of grapevine cultivation. Finally, we will discuss future possibilities and comment on the requirements for those new applications.

BIOLOGY AND EVOLUTIONARY HISTORY OF GRAPEVINE

Grapevine (*Vitis vinifera L.*) is the only species of the genus *Vitis* native of Eurasia. Two forms of this species can still be identified, a wild form which populations are located in river bank forests and that have been considered by taxonomists as *Vitis vinifera* subespecies *sylvestris*, and the cultivated form grouping cultivated varieties under the botanical denomination of subspecies *sativa* or *vinifera*. There is some controversy on whether maintaining this taxonomic distinction as subspecies given the fact that all the observed phenotypic differentiation results from domestication and not from a natural selection driven process (This *et alii*, 2006). However, domestication itself can be considered as conceptually similar to the evolutionary diversification driven by other multispecies interactions (Zeder *et alii*, 2006a).

Wild grapevines are woody vines climbing to the forest canopy where they flower and fruit. These wild forms are dioecious, populations being composed of males and female plants. Pollination is anemophilous and fruits in small clusters are round, small and black and become sweet when ripen to attract birds that perform their seed dissemination (Hegi, 1925). Dioecy prevents plants from selfing and consequently grapevine plants are highly heterozygous. Generation time of these wild forms could be around ten years from seed to seed and plants also reproduce vegetatively to extend themselves along the forests. By contrast, cultivated forms are mostly hermaphrodites, are heavily pruned, and grown as bushes in vineyards. Cultivars are highly heterozygous and their vegetative propagation has contributed to maintain their high heterozygosity (Arroyo-García *et alii*, 2006).

Domestication processes have two major effects on plant and animal species. First the selection of specific domestication traits resulting in distinctive morphologies and physiologies that distinguish domesticated plants from their wild ancestors. These traits can differ between crop plants depending on the way they are used and together define what is known as the domestication syndrome (Zeder *et alii*, 2006b). Second, different selective processes after domestication leads to crop diversification and to local adaptation to preferred uses, qualities, environments, etc. (Purugganan & Fuller, 2009). The primary domestication traits in grape can be considered the flower hermaphroditism, the size of berries and clusters and the higher sugar content (Levadoux, 1956; Olmo, 1995). In addition cultivar diversification as a response to different selective pressures is clearly observed for berry color (Fournier-Level *et alii*, 2010), Muscat flavor (Emanuelli *et alii* 2010) or table grape seedlessness (Cabezas *et alii*, 2006). Finally, other traits appear to have been modified with domestication such as seed size and leaf size and morphology although their biological significance is not known (This *et alii*, 2006).

THE CURRENT MOSAIC OF *VITIS VINIFERA* L. IN THE MEDITERRANEAN BASIN

Several studies have analyzed the genetic diversity of wild and cultivated forms of grapevine in the Mediterranean basin, frequently considered as the wild and cultivated compartments of the species (Laucou *et alii*, 2011). The situation observed nowadays is the result of the interaction along time of wild and cultivated forms of *Vitis vinifera* and human populations. This analysis indicates that wild and cultivated forms have never been closed compartments and have influenced each other tremendously.

Only a few centuries ago wild grapevines were distributed in a broad range of habitats along the Mediterranean basin while nowadays they are only found in very limited locations, in populations generally constituted by a very few individuals (Arnold *et alii*, 1998; Arroyo-García *et alii*, 2006; Grassi *et alii*, 2006; Di Vecchi-Staraz *et alii*, 2009). This drastic reduction is the result of two major factors, the development of human populations and communications systems causing habitat fragmentation, river management and tree removal and the expansion of the cultivated forms. In fact the distribution of wild grapevine has been dramatically reduced over the last 150 years related to the spread of *Vitis* pathogens and pests from North America (Arrigo & Arnold, 2007). The current remnant populations cannot be considered as pure populations of the subspecies *sylvestris*. In fact, wild grapevine populations in France have been shown to be a mixture of wild forms, naturalized cultivated forms and rootstocks escaped from vineyards as well as hybrids derived from spontaneous hybridizations among those species and forms (Lacombe *et alii*, 2003). A similar situation has also been described in Italy (Di Vecchi-Staraz, 2007) and is also been observed in Spain (de Andrés *et alii* in preparation). The existence of gene flow from cultivated to wild plants has been demonstrated in French populations (Di Vecchi-Staraz *et alii*, 2009) what can have significant effects on the evolution of those populations. On the other hand, gene flow does not seem to be frequent between naturalized rootstocks and wild grapevines due to different ecological behaviors (Arrigo & Arnold, 2007).

Regarding the cultivated forms, this term refers to the varieties that are currently cultivated in the world plus those much more numerous that are stored in germplasm stock centers. In total they are estimated to be between 6000 and 10000 different genotypes (This *et alii*, 2006; Laucou *et alii*, 2011). These cultivated forms are the result of multiple interactions since the establishment of viticulture with the initial domestication events in the late Neolithic, dated around 8000-7000 BP in the Near-East (Goor, 1966; McGovern *et alii*, 1996). Factors influencing the actual composition of the cultivated gene pool are: i) the easy vegetative propagation of grapevine facilitating its diffusion in an East to West pattern following human migrations (McGovern, 2003) as well as the later exchange of cultivars and the mixture of genotypes and generations (Myles *et alii*, 2011); ii) the possible existence of secondary domestication events along the Mediterranean basin (Grassi *et alii*, 2003; Arroyo-García *et alii*, 2006); iii) the spontaneous hybridization with wild plants (Myles *et alii*, 2011) and among different cultivated genotypes (Cipriani *et alii*, 2010, Myles *et alii*, 2011, Laucou *et alii*, 2011), iv) the diversifying selection directed by different uses of grapes for wine making in the West and table consumption on the East (Fournier-Level *et alii*, 2010); v) the accumulation of somatic mutations that has generated a wide range of phenotypic variation within specific genotypes, likely the oldest or most extended in ancient times (This *et alii*, 2006). All these factors have led to a very complex pattern of admixture for the cultivated genotypes (Myles *et alii*, 2011).

THE GRAPEVINE GENOMES

To investigate the evolutionary history of domesticated plants, geneticists use neutral nuclear loci and organelle genomes. Plant cells contain

two organelles, the chloroplast and the mitochondrion bearing their own genomes. The rates of evolution of the nuclear, chloroplast and mitochondrial genomes are not equal, what affects their relative usefulness for evolutionary studies in the short periods required for domestication (Zeder *et alii*, 2006b). Plant mitochondrial genomes have a slower mutation rate than chloroplast genomes and evolve slowly (Wolfe *et alii*, 1987). In addition, the size of the plant mitochondrial genome is variable among different species and frequently displays multipartite organizations resulting from frequent intramolecular recombination events (Levings & Brown, 1989). These features of plant mitochondrial genomes limit their use in domestication studies.

Regarding the nuclear and chloroplast genomes, the nuclear plant genomes seem to evolve at a rate approximately four times faster than the chloroplast genome (Wolfe *et alii*, 1987) what generally makes nuclear DNA markers to be the molecular tools of choice for plant domestication studies (Zeder *et alii*, 2006b). Still chloroplast genomes that are uniparentally transmitted in most species (usually maternal in angiosperms and paternal in gymnosperms) are very useful to elucidate the relative contribution of seed and pollen flow to the genetic structure of populations (Provan *et alii*, 2001), to test hypotheses of crop-wild gene flow or to establish the maternal origin of specific genotypes.

Chloroplast, nuclear and mitochondrial genomes of *Vitis vinifera* have recently been sequenced for reference genotypes (Jansen *et alii*, 2006; Jaillón *et alii*, 2007; Velasco *et alii*, 2007; Goremykin *et alii*, 2009) providing a basic tool for studies in sequence diversity and domestication in grapevine. The grapevine mitochondrial genome is the largest organelle genome so far sequenced in plants (773279 nt) mainly due to incorporation of DNA from the chloroplast genome and its genetic diversity in the species has not been analyzed. In the next sections we will summarize the available information on genetic diversity of the nuclear and chloroplast genomes of grapevine.

NUCLEAR GENOME AND SEQUENCE DIVERSITY

The completion of the nucleotide sequence of the Pinot Noir derived inbred line PN40024 (Jaillón *et alii*, 2007) as well as the heterozygous cultivar Pinot Noir (Velasco *et alii*, 2007) estimated the grapevine genome in a length of *circa* 470 Mbp with a the number of annotated genes close to 30,000.

Nucleotide sequence variation among grapevine genomes has not been deeply analyzed so far. Expected variation among nucleotide sequences can be classified in two basic types: 1) nucleotide substitutions that cause the so called single nucleotide polymorphisms or SNP and 2) insertions or deletions known as INDEL. This group includes all sorts of insertion-deletions, from small single nucleotide deletions or duplications, till larger events. INDELs also include repetitions of simple nucleotide sequences commonly known as microsatellites that are very frequently used as molecular markers to trace ancestry and measure genetic diversity in grapevine given their higher evolutionary rate.

Microsatellites have been used as reliable codominant multiallelic molecular markers since the nineties in grapevine (Thomas *et alii*, 1994). They are very frequent in the genome of any species and grapevine genome sequence has identified a total of 239,634 microsatellite elements in PN40024

(Jaillon *et alii*, 2007), including 26,962 perfect microsatellite markers containing tri-, tetra-, and penta-nucleotide repeats (Cipriani *et alii*, 2008). A recent large scale comparative analysis of grapevine genetic diversity using microsatellites points out that average genetic diversity is 0.769 for *Vitis vinifera* ssp. *vinifera* and 0.707 for the ssp. *sylvestris* (Laucou *et alii*, 2011). These genetic diversity values represent an expectation of the ratio of heterozygous loci in the genome that is very high. The figures change when heterozygosity is measured. The average observed heterozygosity (Ho) remains high for *Vitis vinifera* ssp. *vinifera* (0.76) but falls down for the ssp. *sylvestris* (0.62) (Laucou *et alii*, 2011) in agreement with the observed loss of biodiversity in their natural populations.

Considering single nucleotide polymorphisms, the complete genome sequence of the heterozygous cultivar Pinot Noir detected a total of 1,751,176 SNPs among its two parental genomes (Velasco *et alii*, 2007). In addition, re-sequencing experiments in grape cultivars have provided values of polymorphism frequency ranging from 1 SNP per 129 bases (Salmaso *et alii* 2004), 1 SNP every 64 bp (Lijavetzky *et alii*, 2007) up to 1 SNP per 49 bases (Le Cunff *et alii*, 2008). Genetic diversity values for SNP are generally lower than microsatellite ones due to their bi-allelic nature and in grapevine ranged from 0 to 0.66 with a mean value of 0.30 (Lijavetzky *et alii*, 2007). Having lower diversity values than SSR, SNP are less informative markers than SSR. In fact, the average PIC (polymorphism information content) value for SNPs is 0.25 as compared to 0.70 for microsatellites (Lijavetzky *et alii*, 2007). This potential drawback of SNP is clearly overcome by the use of larger sets of markers. In this sense massive re-sequencing approaches of grapevine genotypes have so far permitted the identification of over 70000 high-quality SNP and the construction of a validated 9000 SNP genotyping array (Myles *et alii*, 2010). Genotyping the collection of grapevine accessions stored at the USDA with this array has recently provided a first global view of the genetic structure and domestication history of this crop (Myles *et alii*, 2011).

CHLOROTYPE GENETIC DIVERSITY IN GRAPEVINE

The chloroplast genome of grapevine is 160,928 bp in length and its gene content and gene order are identical to many other unrearranged angio - sperm chloroplast genomes (Jansen *et alii*, 2006). Chloroplast genetic diversity in grapevine has been mainly analyzed at microsatellite loci. Out of 34 different loci tested, only five were found to be polymorphic due to differences in the number of mononucleotide repeats in poly T/A stretches (Arroyo-García *et alii*, 2006; This *et alii*, 2011): cpSSR3 (equivalent to NTCP-8), cpSSR5 (equivalent to NTCP-12 and ccSSR5), cpSSR10 (equivalent to ccSSR14), ccSSR9 and ccSSR23. The genotypic analysis of these loci in over 1200 *V. vinifera* genotypes detected 2-3 alleles per locus combined in eight chlorotypes. Only four of them (A, B, C and D) had global frequencies greater than 5%. One problem associated to these chloroplast microsatellites is their high homoplasy due to the recurrent generation of alleles of the same length, what creates alleles that being identical by state are not identical by descent. These high levels of homoplasy can confound estimates of population differentiation (Goldstein & Pollock, 1997).

As an alternative to chloroplast microsatellites we have search the chloroplast genome for the presence of SNPs. We re-sequenced 34 intergenic

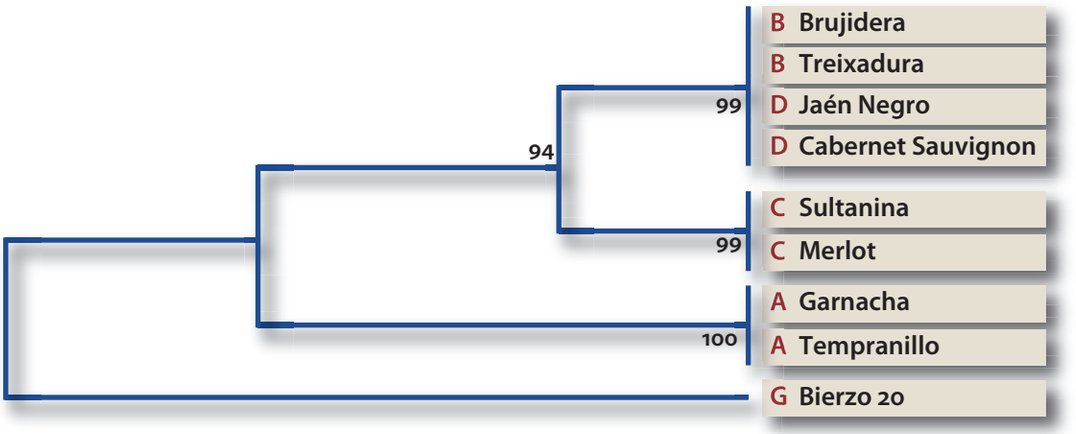

FIG. 1
Genetic relationships among the four chlorotypes identified in nine different grapevine varieties. Genetic relationships have been inferred based on the presence of chloroplast SNPs. Phylogenetic tree was built using the UPGMA method. Analyses were conducted in MEGA5

regions in the chloroplast genome in cultivars belonging to the five chlorotypes detected within the Iberian Peninsula. Out of them, 28 sequences showed polymorphisms of either SNP (23) or INDEL (5) type. These polymorphisms clearly separated chlorotypes A and G that are very different from the rest (B, C and D) but were unable to distinguish B from D [Fig. 1]. These SNP results support previous studies on the differentiation of Western and Eastern most frequent chlorotypes that were based on chloroplast microsatellites (Arroyo-García *et alii*, 2006) and exemplify the homoplasy problem presented by chloroplast microsatellite loci.

GENETIC DIVERSITY AND GRAPEVINE DOMESTICATION

Genetic diversity has been studied for a long time in grapevine with different sets of molecular markers, mainly directed to neutral evolving genome sequences, what has provided partial views of the genetic relationships among cultivated and wild germplasm as well as on the expansion and evolution of domesticates (see This *et alii*, 2006 for a very recent review). More recently, with the completion of the reference genome sequence, the possibilities of resequencing genomes and the development of SNP and SNP sets permitting the rapid genotyping of hundreds or thousands genotypes at thousands of loci, the genetic view of the grapevine domestication process is evolving into a more general integrative theory. In this section, we will review different topics regarding the origins of domestication, the multiplicity of domestication events or the relationships among cultivated genotypes during the history of the crop that gave rise to today cultivars.

GENETIC RELATIONSHIPS BETWEEN WILD AND CULTIVATED FORMS OF GRAPEVINE

The identification of the wild progenitor of cultivated grapevine is out of question given the presence of the *sylvestris* subsp. still growing in most of the area of distribution of the crop and the reduced morphological divergence observed between wild and cultivated forms (This *et alii*, 2006). Archaeological and historical evidences also suggest that primo-domestication events occurred in the Near-East (McGovern, 2003). However, confirmatory genetic proofs are still scarce. Part of the problem when studying the genetic relationships between the existent wild populations of *sylvestris* and *vinifera* cultivars is the identification

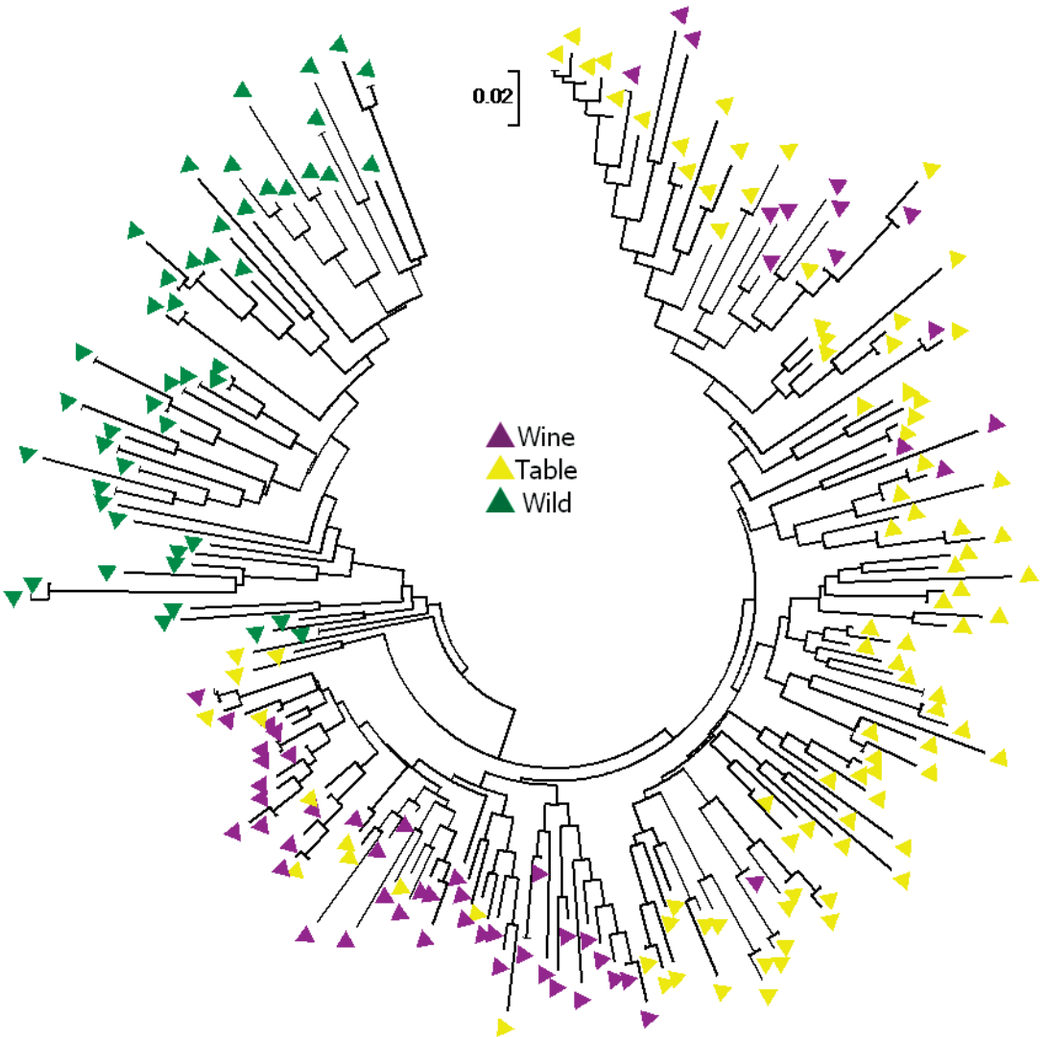

FIG. 2
Genetic relationships among cultivated and wild grapevine taxa. Genetic distances were computed using the number of differences method and are in the units of the number of base differences per sequence. Phylogenetic tree was built using the Neighbor-Joining method. The analysis involved 217 SNPs. Analyses were conducted in MEGA5

and collection of true *sylvestris* samples in the wild given the existence of feral *vinifera* that can be misidentified as *sylvestris* and the ease with which wild and cultivated grapevines can hybridize and have likely hybridized along thousands of years of common history.

Most studies using nuclear genome molecular markers have compared Western populations of *sylvestris* with different sets of Western *vinifera* cultivars (from Western and Central Europe or Northern African locations). Analyses using microsatellites markers show that wild and cultivated genotypes commonly cluster in different genetic groups when using microsatellites markers (Grassi *et alii,* 2003; Snoussi *et alii,* 2004; Dzhambazova *et alii* 2009; Zinelabidine *et alii* 2010). Similar results were obtained when using SNP markers which separate both table and wine grape *vinifera* cultivars from Western *sylvestris* genotypes **[Fig. 2]**.

Only a very recent study combines the analyses of *vinifera* cultivars with *sylvestris* samples from both the Western and Eastern ends of the wild

species distribution. The results of this study suggest a much closer genetic similarity between Eastern *vinifera* cultivars and Eastern *sylvestris* than between Western *vinifera* cultivars and Western *sylvestris* (Myles *et alii*, 2011). These results represent the first genetic evidence supporting the archaeological evidence on the initial Eastern domestication of grapevine followed by its dissemination in an East to West direction. In addition, these studies also point out the possible existence of introgression of genetic materials from Western *sylvestris* into Western *vinifera* cultivars what would explain the slightly higher genetic similarity observed between Western *vinifera* and Western *sylvestris* genotypes. Still, these results should be interpreted with caution given the small number of samples analyzed and the mentioned uncertainties on their true wild origin.

The morphological similarities observed between *vinifera* and *sylvestris* are also correlated with a high similarity at the genome sequence level and so far no single molecular marker has been found that could be claimed as being characteristic of either *sylvestris* or *vinifera* subspecies. Only through the statistically analyses of data from several loci it is possible to establish the probabilities of origin of a given specimen to specific subspecies.

CHLOROTYPES AND DOMESTICATION ORIGINS

Chloroplasts are maternally inherited in grapevine as in many other angiosperm species (Strefeler *et alii*, 1992; Arroyo García *et alii*, 2002). This mode of transmission in a woody species with sexual and vegetative reproduction means that chloroplast molecular markers can be transmitted to progeny plants either by seeds or cuttings from a given plant but never through pollen. The low number of effective chlorotypes (4) identified in grapevine limits many of the applications of chlorotype markers in the study of the domestication history of this crop. Fortunately, the different chlorotype frequencies observed in wild populations of *Vitis vinifera* ssp. *sylvestris* along the range of the species distribution as well as in cultivated plants from different regions and uses can be informative on the maternal origin of cultivars (Arroyo-García *et alii*, 2006).

Interestingly, chlorotype A, that is a very divergent chlorotype from chlorotypes B, C and D on the basis of chloroplast microsatellites and SNPs, has been mainly found in Western and Central European wild populations but is absent in Near East and Asian populations. In addition, chlorotypes C and D are highly frequent in Near East and Asian populations but are absent in Western European populations (Arroyo-García *et alii*, 2006). The distribution of chlorotypes in grapevine cultivars follows similar patterns, with chlorotype A being highly abundant in Western Europe and, particularly, in wine cultivars from the Iberian Peninsula and chlorotype C being characteristic of Eastern cultivars and highly abundant among table grape cultivars (Arroyo-García *et alii*, 2006). This pattern of distribution has also been confirmed for the same chlorotypes in cultivated and wild samples from Portugal (Cunha *et alii*, 2010) and from Northern Africa, where table grape cultivars frequently bear chlorotype C whereas wild populations bear chlorotype A (Snoussi *et alii*, 2004; Laiadi *et alii*, 2009; Zinelabidine *et alii*, 2010). Parallel analyses of chlorotype distribution have also been performed in additional cultivated and wild samples along the Mediterranean basin although only based on two polymorphic chloroplast microsatellites. Although chlorotype names

and alleles are not coincident due to different analytical methods, the results follow a similar trend. Only six chlorotypes were detected in samples collected along the Mediterranean basin, with chlorotype VI (equivalent to Chlorotype A) being highly frequent in the Iberian Peninsula (Imazio *et alii*, 2006). Furthermore, as described for chlorotype A, chlorotype VI is neither detected in cultivated nor in wild samples from Iran where chlorotypes I and III (equivalent to D and C) are the most abundant (Baneh *et alii*, 2007).

Taken together these results suggest the participation of both Western and Eastern native germplasm in the origin of current grapevine cultivars together with the existence of an important flow of cultivars following a predominant East to West direction (Arroyo-García *et alii*, 2006). At the level of nuclear markers, the analysis of genotypes for the previously mentioned 9K SNP arrays in a small sample of cultivated and wild samples from Western Europe also support the introgression of genetic material from Western *Vitis Vinifera* ssp *sylvestris* in Western *Vitis vinifera* ssp. *sativa* cultivars (Myles *et alii*, 2011) in agreement with the chlorotype results. Since, as mentioned before, chloroplast are maternally inherited, the only possibility to get chloroplast A transmitted to cultivated materials is by the cultivation of either cuttings or seeds coming from Western wild populations. Thus, these events could correspond to secondary domestication events taking place in the Western side of the Mediterranean basin under the influence of the wine culture spread over the area from the East. Combined archaeological and genetic research on different plant species domestication, suggest that multiple domestication events are more common in plants than originally thought (Zeder *et alii*, 2006b). This is the case of olive trees that seem to have been domesticated in Eastern and Western sides of the Mediterranean basin (Besnard *et alii*, 2001; Terral *et alii*, 2004) similarly to the situation proposed for grapevine. Therefore, in spite of the low genetic diversity detected in grapevine chlorotypes, they provide interesting and complementary information regarding the existence of multiple domestication events and can also provide information on the geographic origin of specific cultivars given the low number of generations separating wild and cultivated genotypes.

GENETIC IDENTIFICATION AND GENETIC RELATIONSHIPS AMONG CULTIVARS

The availability of multiallelic codominant molecular markers such as microsatellites in the early nineties allowed the rapid genetic identification of grapevine cultivars (Thomas *et alii*, 1994). Many polymorphic microsatellite markers are now available for grapevine as well as genotype databases for the most commonly used markers (see This *et alii*, 2011, for a recent review). The increase in the number of loci analyzed in large number of cultivars allowed studying the genetic relationships among cultivated genotypes. A primordial conclusion derived from those analyses was the identification that some cultivars were in fact derived by spontaneous hybridizations from other still existent cultivars. The first demonstrated case was Cabernet Sauvignon, shown to be a spontaneous hybrid from the cross between Cabernet Franc and Sauvignon Blanc (Bowers & Meredith 1997). This initial work has been followed by many others pointing out that this not the exception but the rule (Bowers *et alii*, 1999). In fact, the possible reason why cultivars from a given region seem to be

more closely related among them than to cultivars from other regions (Sefc *et alii*, 2000) is because they have close family relationships. One extreme situation is the parentage of over 300 French cultivars that seem all derived from spontaneous hybridizations between Pinot Noir and Gouais or Gueche Blanc, two cultivars that were already relevant during the Middle Age (This *et alii*, 2006). Similar patterns of hybridization are also described in Italy (Cipriani *et alii*, 2010), or Switzerland (Vouillamoz *et alii*, 2003) and are also found in the Iberian Peninsula as being responsible for many of the current cultivars in Spain and Portugal (Ibañez *et alii*, 2011). A recent study using SNPs on the whole USDA grapevine collection describes a situation in which almost 75% of the non-redundant genotypes are related to at least one other cultivar by a first degree relationship. These first degree relationships are rare between wine and table grape cultivars and among cultivars from geographically distant regions (Myles *et alii*, 2011). Given the combination of sexual hybridization with vegetative propagation of grapevine genotypes for hundreds or even thousands of years there has been a potential for crosses across generations making impossible the reconstruction of accurate genealogies. The conditions in which vineyards are managed today make it practically impossible that these spontaneous hybridizations will continue generating new cultivars. However, mixed vineyards with reduced management have been the melting pot for the origin of new cultivars for centuries.

A historical event that can serve as a pilot experiment to follow the timing of viticulture expansion and the origin of new cultivars is the history of American viticulture. The first *Vitis vinifera* subsp. *vinifera* cultivars were carried to America by Spaniards in the beginning of the 16th century. From that time, a few successful genotypes corresponding to the Spanish Listán Prieto, Moscatel de Málaga or Muscat of Alexandria and Mollar Cano have spread from North America to South America under many different synonyms. In addition, in the five hundred years elapsed since the first introduction, they have produced a first generation of criollan cultivars, mostly derived from spontaneous hybridizations among them. Well known cultivars like Criolla Sanjuanina, Torontel, Torrontés Riojano o Huasquina Pisquera, all derive from these spontaneous crosses (Milla-Tapia *et alii*, 2007).

GENES INVOLVED IN THE DOMESTICATION SYNDROME AND PHENOTYPIC DIVERSIFICATION

Consistent with the limited domestication syndrome observed in grapevine, the reduction in genetic diversity attributable to domestication on a genome-wide scale appears to be weak (Myles *et alii*, 2011). Still there are a few relevant morphological changes that have almost become fixed in domesticated grapevine genotypes and that include hermaphrodite flowers, large clusters and large berries and higher sugar content (Olmo, 1995) (see **Fig. 3** for cluster evolution). In other domesticated species fixation of morphological traits are related with signatures of selection at the genome level or regions in which nucleotide diversity falls down drastically (Wang *et alii*, 1999, 2001). A few regions with this signature have so far been identified on chromosomes 18 (Houel *et alii*, 2010) and 17 (Myles *et alii*, 2011) of *Vitis vinifera* ssp. *vinifera*. However the related morphological features are unknown.

Alternatively, genes controlling traits affected by the domestication syndrome would likely show

FIG. 3
Evolution of cluster size related with the domestication process of grapevine.
A. Cluster of female wild plant.
B. Cluster of a putative hermaphrodite wild plant.
C. Cluster of domesticated cultivar

domestication signatures at the level of nucleotide diversity. None of these genes have clearly been identified in grapevine till now. Recently, we have identified the grapevine homolog of the Arabidopsis *TERMINAL FLOWER* (*TFL1*) named as *VvTFL1A*, as responsible in part for a large increase in cluster size and a delay in flowering anthesis shown by a somatic variant of cultivar Carignan/Cariñena (Fernández *et alii*, 2010). The biological function of this gene has been related with inflorescence structure and size in other species (Prusinkiewicz *et alii*, 2007) and it could have been a target of selection for cluster size during domestication. A nucleotide genetic diversity study is currently been performed for *VvTFL1A* sequence in a core collection of grapevine genotypes selected to maximize phenotypic variation with the objective of understanding its role in domestication.

Other grapevine domestication traits would not be constitutive of the domestication syndrome because they are not fixed in cultivated genotypes. However, they have been targets of selection for crop diversification driven by different human groups developing varieties with desirable visual or gustatory features. This is the case of the berry colour or the Muscat aroma that are quite polymorphic among cultivated genotypes in contrast to what is found in wild populations (Fournier-Level *et alii*, 2010; Emanuelli *et alii*, 2010). Berry colour variation is due to nucleotide variation in a complex locus on chromosome 2 constituted by four linked genes encoding MYB transcription factors which regulate anthocyanins biosynthesis in the berry skin (Walker *et alii*, 2007). Among them, mutations at two genes *VvMybA1* and *VvMybA2* are related with variation in berry colour and white berries are only produced by plants in which both genes carry null mutations in homozygosis (Walker *et alii*, 2007). A recent analysis of the existent alleles at these two genes in a core collection of grapevine genotypes maximizing phenotypic variation (Barnaud *et alii*, 2006) identified a very large number of gene haplotypes following a model in which a first mutation in *VvMybA2* giving rise to light coloured berries is followed by the appearance of a mutation (insertion of a *Gret1* Retrotransposon, Kobayashi *et alii*, 2004) in *VvMybA1* leading to the appearance on white berries (Fournier-Level *et alii*, 2010). This haplotype containing both mutations was rapidly selected and currently it is present not only in all the white berry cultivars but carried by nearly all the coloured table grapes and many of the wine grapes cultivars (Lijavetzky *et alii*, 2006).

Similarly, the genetic analyses of variation for Muscat flavour identified a major QTL on chromosome 5 of grapevine (Doligez *et alii* 2006; Duchene *et alii*, 2009) and a gene encoding a deoxy-D-xylulose synthase involved in a primary regulatory step of monoterpene biosynthesis as a putative candidate gene (Duchene *et alii*, 2009). A recent study of sequence variation at this gene in a selected core collection of cultivated genotypes identified a specific SNP highly represented in the Muscat flavoured varieties suggesting that it could had been the molecular target of selection for this trait. This SNP causes a non-synonymous amino acid substitution that could affect the regulatory role of the encoded protein.

These two examples demonstrate the possibilities offered by the combination of genetic analyses and genomic information in the identification of the specific polymorphisms responsible for the phenotypic variation that has been the target of human selection during domestication.

GRAPEVINE ANCIENT DNA

The information present in the genome of actual domesticated and wild cultivars, although useful to trace initial domestication and dispersal events as we have seen, can be confounded by thousands of years of hybridization, introgression and selection across interleaved generations. For this reason, the possibility to extract and analyze ancient DNA (aDNA) from archeological remains offers a more direct approach to study the genetic basis of those past events (Zeder *et alii*, 2006b). Currently, the development of specific extraction procedures, control approaches to verify the ancient DNA identity and quality, as well as the engineering of modified enzymes activities with improved capabilities using aDNA as template (Green *et alii*, 2009) allow the extraction and amplification of ancient DNA with enough efficiency. Ancient DNA has been more widely used in the study of animals than plants domestication processes. The reason is that animal DNA is generally better preserved in bone remains and also the high usefulness of animal high-copy mtDNA in domestication studies. The extraction of aDNA from plant archaeological remains is more challenging, because of preservation issues and also because usually it is the low-copy nuclear DNA the most useful in the study of plant domestication. Until now only two publications report the isolation of DNA from archeological grapevine seed samples from either waterlogged and charred environments from the iron age (5th century BC), the Greek (5th century BC) and the Roman (2nd-4th century AD) times (Manen *et alii*, 2003) as well as from the early medieval (7th-8th century) and late medieval (14th-15thcentury) times (Cappellini *et alii*, 2010). The main goal of both works was the genetic identification of the cultivars grown at the age of the archeological sites or at least the identification of their region of origin. So far the results do not allow drawing strong conclusions on the identity of the cultivars in any case. DNA can only be recovered in some samples and few microsatellites can be amplified. In addition, there are problems with the sizing of the amplified fragments, the fact that seeds are segregants of the original cultivar genotypes and the lack of comprehensive grapevine genotype databases. Altogether they make it difficult to reach conclusions on the origins of these materials.

In any case, these preliminary experiments point out the possibility to use these remains and perhaps other plant remains, such as stems or berry skins, to recover DNA. With good markers and databases, it should be possible to get information on the genetic identity of these remains in terms of genotype characterization, geographical origin and genetic relationships to currently known cultivars. As methods become standardized, this information will permit to perform statistical analyses providing information not only on genetic identities but on viticulture practices.

CONCLUDING REMARKS

Combination of archeological markers and genetic information can help shed light on multiple aspects of the common history of humans and grapevines. In the last four years, the completion of the grapevine reference sequence and the development of next generation sequencing technologies have multiplied the possibilities to obtain information on genetic diversity at the genome level that can be very useful in the study of gra-

pevine domestication. As more and more grapevine genomes are sequenced or characterized with large SNP platforms the available information will rapidly improve our knowledge on the history of this crop. The tremendous sequence capacity generated by recent technological developments will have to be matched by paralleled improvement in the characterization, conservation, and public availability of genetic resources, the generation of comprehensive and public databases and a renovated effort in the phenotypic characterization of genetic resources. These tools will set a new frame to analyze and understand the archeological grapevine remains providing new information on the evolution of viticulture and wine culture. The complexity and multidisciplinary context of these tasks will require to strength the links among researchers, disciplines, and research institutes sharing common interests.

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