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Restriction fragment length polymorphism and molecular taxonomy in *Vitis vinifera* L.

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Abstract. Forty-six accessions of grapevine (*V. vinifera* L.) were compared by restriction fragment length polymorphism (RFLP) analysis, and 111 informative or unique restriction fragments were found that revealed an important level of polymorphism. RFLP patterns were compared in two ways: by calculating electrophoretic similarity degree values further analyzed by principal component analysis and by studying the distribution of rare restriction fragments. Six taxonomic groups could be defined, which partially confirmed relationships derived from ampelographical data. Our data support the existence of ecogeographical groups.

Key words: *Vitis vinifera* – Restriction fragment length polymorphism – Molecular taxonomy – Principal component analysis

Introduction

The occurrence of *Vitis vinifera* L. in Western Europe and the Mediterranean since the Tertiary-Quaternary transition is well-documented (Levadoux 1956; Jelaska 1956; Negrul 1968b). During the last ice age, its range definitely corresponded to the areas of the western forests around the Mediterranean and probably to those of the oriental Mediterranean and Iran (Levadoux 1956). Its primitive range was not broken up completely, however, and during the Neolithicum, the geographical range became more or less identical to the present one, and some ecotypes appeared (Levadoux 1956; Negrul 1968b). By selecting wild vines for increase in fertility and improved taste, man became an important factor of diversification (Levadoux 1956; Negrul 1968a, b). The selected cultivars were vegetatively propagated, and some accumulated somatic mutations. In the case of the 'Pinot' cultivar, numerous variations have been observed: 'Pinot gris', 'Pinot blanc', 'Meunier' and 'Pinot noir' among others. Negrul (1946) called such groups sortogroups. Each member of a sortogroup is a 'cépage' (commonly translated as 'grapevine cultivar', although 'cépage' does not exactly correspond to the cultivar definition), each 'cépage' is composed of clones (Levadoux 1956; Galet 1990). The history of the 'cépages' is probably very complex, and very little is known about it (Levadoux 1954b; Bouquet 1982). *V. vinifera* is presently estimated to contain more than 5000 'cépages'.

The knowledge and study of the 'cépages' is called ampelography (from the Greek word 'ampelos': vine) (Viala and Vermorel 1901; Negrul 1946; Levadoux 1954b; Galet 1990). The ampelographical characters used so far to describe the 'cépages' may vary significantly with environmental conditions (Levadoux 1954b). The increasing use of clones with a very similar morphology and the consequent need for precise and rapid identification have stimulated the search for biochemical markers (Stavrakakis and Loukas 1983; Bénin et al. 1988; Tedesco et al. 1989). Isoenzymes have been used to identify various 'cépages', but only few markers have been found and the profiles obtained may depend on the growth conditions of the starting material (Wolfe 1976; Subden et al. 1987; Bénin et al. 1988). Restriction fragment length polymorphism (RFLP) studies yield data that are independent of the environment. Methods to detect DNA polymorphism within *Vitis* with probes derived from other organisms

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have been reported (Striem et al. 1990; Yamamoto et al. 1991). In previous papers, we have demonstrated that RFLP analysis with different grapevine probes can be used to distinguish the 16 major *Vitis* rootstock hybrids (Bourquin et al. 1991, 1992).

The degree of polymorphism within *V. vinifera* and its possible use for taxonomical analyses remained to be investigated. The study reported here presents an RFLP analysis of 46 'cépages' and discusses the results with respect to the ampelographical assumptions of their taxonomic organization.

Materials and methods

RFLP analysis

Grapevine DNA enriched in nuclear DNA was prepared as described (Gebhardt et al. 1989; Bourquin et al. 1991, 1992) with the following modifications: the leaves were not treated with ice-cold ether before grinding, and *n*-octanol and β-mercaptoethanol were omitted from the extraction buffer. Young leaves harvested in June were thoroughly ground with a Pollähne roller-press in the presence of ice-cold extraction buffer (100 ml per 30 g leaves). DNA was digested with restriction enzymes *Hin*fI or *Hae*III, and the prehybridization and hybridization were carried out in plastic bags (0.1 ml hybridization buffer per square cm) without shaking. The probes used to detect RFLP profiles were small *Pst*I fragments of a *V. vinifera* cv 'Chardonnay' library (Bourquin et al. 1991, 1992). 'Pinot teinturier' originated from the ampelographical collection of the viticultural experimental domain of Cours-les-Cosne of the INRA Institute at Dijon; the 45 other plants (Table 1) originated from the ampelographical collection of Bergheim of the INRA Institute at Colmar. Throughout the text, the actual plants studied are designated as "accessions".

Analysis of database

To construct the fragment matrix, the presence of a given fragment in a given DNA was indicated by 1 and its absence by 0. The electrophoretic similarity degree (ESD) was calculated for each couple of accessions from the fragment matrix according to the following formula:

$$\text{ESD}\,(x, y) = \Sigma nc / \Sigma(nu + nc)$$

where x and y are two accessions; nc is the number of fragments shared by x and y, and nu is the number of non-common fragments for x and y (Stavrakakis and Loukas 1983). To reduce the number of dimensions of the data, the ESD matrix was analyzed by principal component analysis (PCA) (SPAD-N software) (Jolliffe 1986; Keim 1989; Neuhausen 1992). The first three principal components were further selected to be analyzed by ascending hierarchical classification (AHC) (SPAD-N software) (results not shown). The PCA and AHC enabled the classes of accessions to be identified and their relative position to be determined. Taxonomic groups were deduced as follows: the dendrogram resulting from the AHC was cut at a level so as to liberate nine classes of accessions. Table 2 lists these different classes and, for each accession, the following parameters: its maximal ESD within its class (ESDmw); its maximal ESD outside its class (ESDmo); its average ESD derived from comparison with all accessions, and the standard deviation corresponding to the latter value. The composition of each class is discussed with respect to these values.

Another method of analysis which complements the previous one consists of grouping the accessions according to the shared restriction fragments of very low frequency. In a first approach, we arbitrarily chose to compare all accessions for fragments of frequency less than 11. Accessions which shared more than three fragments were grouped.

The synthetic bandmap was obtained by arranging the fragments in decreasing frequencies and according to the five hypothetical taxonomic groups of accessions described in the results and discussion (Powell et al. 1991).

Table 1. Plant materials used in the experiments

Abbreviation	Ampelographical designation (Galet 1990)
1. Ali	Aligoté
2. CbF	Cabernet franc
3. CbS	Cabernet-Sauvignon
4. Cha	Chardonnay
5. ChB	Chasselas
6. Che	Chenin
7. Cin	Cinsaut
8. Cla	Clairette
9. Gam	Gamay noir
10. GaF	Gamay Fréaux
11. Gew	Gewürztraminer
12. SaV	Savagnin vert
13. SaJ	Savagnin jaune
14. Gre	Grenache
15. Mer	Merlot
16. Meu	Meunier
17. PiB	Pinot blanc
18. PiG	Pinot gris
19. PiN	Pinot noir
20. MuA	Muscat noir à petits grains
21. Pou	Poulsard
22. Rie	Riesling
23. Sem	Sémillon
24. SyB	Sylvaner
25. Syr	Syrah
26. Tro	Trousseau
27. Vio	Viognier
28. Alt	Altesse
29. Car	Carignan
30. Cot	Côt
31. Enf	Enfariné
32. FoB	Folle blanche
33. Fur	Furmint
34. Gou	Gouais blanc
35. Jac	Jacquère
36. Jur	Jurançon noir
37. Mar	Marsanne
38. Mau	Mauzac
39. Mel	Melon
40. GrM	Meslier Saint-François
41. PeM	Petit Meslier
42. Mon	Mondeuse
43. PiA	Pineau d'Aunis
44. PiT	Pinot teinturier
45. Rou	Roussanne
46. Sau	Sauvignon

Table 2. Classes of accessions after hierarchical classification

A	B	C	D	E	F	G	H
	Ali	Gam	0.81	Jur	0.68	0.58	0.12
	Gam			Jur	0.71	0.57	0.12
	Mel	Ali	0.80	Mau	0.63	0.54	0.12
1	Cha	Ali	0.76	Mau, Enf	0.71	0.58	0.12
	GrM	SyB	0.72	Che	0.71	0.57	0.11
	SyB			Che	0.69	0.58	0.11
	PeM	Cha	0.72	Gew	0.68	0.54	0.13
	Gou	SyB	0.70	Jac	0.64	0.53	0.12
	Meu	PiT	0.98	Che	0.64	0.55	0.14
	PiT			Mon	0.63	0.54	0.14
	Gew	PiT	0.74	Che	0.69	0.55	0.12
2	Tro	Gew	0.70	Che	0.71	0.53	0.11
	Cot	Jur	0.67	Sem	0.61	0.52	0.11
	Jur			Gam	0.71	0.54	0.11
	Gre	Cot, Jur	0.61	SyB	0.67	0.55	0.09
	Che	Sem	0.75	GrM	0.71	0.60	0.10
	Sem			Tro	0.65	0.57	0.10
	Enf	Sau	0.71	Cha	0.71	0.57	0.10
3	Sau			PeM	0.65	0.56	0.11
	PiA	Che	0.65	Mer	0.66	0.55	0.10
	Mon	Sem	0.63	Jur, PiT	0.63	0.53	0.10
	Mau	Enf	0.63	Cha	0.71	0.55	0.11
4	CbS	Mer	0.73	CbF	0.67	0.54	0.11
	Mer			CbF	0.70	0.51	0.11
	Alt	Vio	0.73	CbS	0.63	0.52	0.11
5	Vio			Enf	0.70	0.54	0.10
	Syr	Vio	0.68	Enf	0.66	0.53	0.11
	Rie	Jac, Car	0.58	Che	0.63	0.52	0.11
6	Jac			Gou	0.64	0.53	0.09
	Car			Gre	0.64	0.49	0.10
	Mar	Car	0.49	Rou	0.60	0.48	0.10
	FoB	MuA	0.49	Gam	0.63	0.48	0.11
7	MuA			PeM	0.54	0.42	0.12
	Fur	FoB	0.48	Gre	0.64	0.49	0.11
8	Rou	Cla	0.49	Mar, Vio	0.60	0.47	0.10
	Cla			Jac	0.53	0.43	0.10
	Pou	ChB	0.58	Syr	0.64	0.46	0.11
9	ChB			CbS	0.63	0.47	0.11
	Cin	ChB, CbF	0.52	SyB	0.55	0.44	0.11
	CbF			Mer	0.70	0.44	0.12

A, Class number; B, accession; C, accession of the class corresponding to the value in column D; D, maximal ESD (ESDmw) within the class for the accession in column B; E, accession outside the class corresponding to the value in column F; F, maximal ESD (ESDmo) outside the class for the accession in column B; G, average ESD value calculated for the accession in column B with respect to all 40 accessions; H, standard deviation corresponding to the value in column G

Results

Degree of polymorphism among the 46 accessions

Fifty-six probe/enzyme combinations (PECs) were used to study the RFLP patterns of the first 27 accessions listed in Table 1. Eighteen probes were combined with *Hin*fI; 2 probes with *Hae*III; 18 probes with both enzymes. The RFLP patterns showed a considerable level of polymorphism. Five probes showed RFLPs corresponding to unique or moderately repeated sequences with both enzymes, and 15 probes showed such a pattern with only one enzyme. Six probes showed very complex patterns corresponding to highly repeated sequences. Finally, 12 probes corresponding to unique or moderately repeated sequences did not show any RFLP. Overall, 25 PECs (45%) were informative and of practical use under our experimental conditions. Twenty-one PECs of this latter group were used to analyze the last 19 accessions listed in Table 1, including 4 controls already analyzed in the first set of experiments, 'Aligoté', 'Chenin', 'Sémillon', and 'Viognier', the DNAs of which were extracted from a new batch of young leaves. A fifth control was the DNA of 'Cabernet-Sauvignon' used in the previous experiment. An example of an RFLP pattern is shown in Fig. 1. There were no significant differences between the RFLP patterns of the controls in both sets of experiments, which demonstrates the excellent reproducibility of our method.

Several accessions had identical RFLP patterns. The first group comprises 'Meunier', 'Pinot noir',

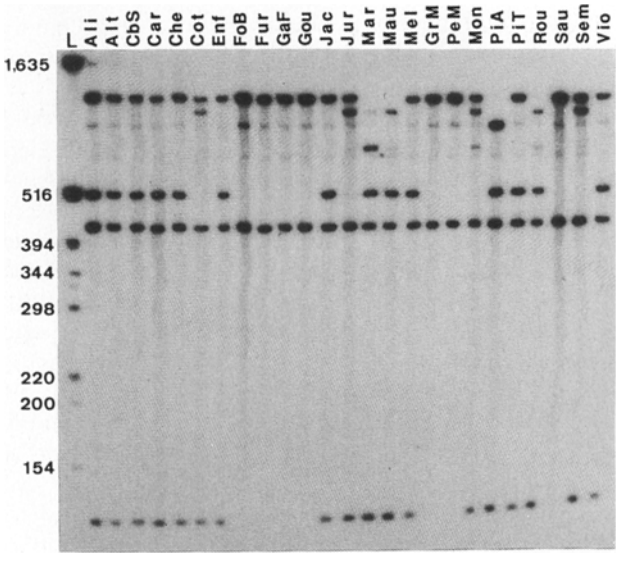

Fig. 1. Autoradiography of *Hin*fI-digested DNAs probed with the *Pst*I 'Chardonnay' DNA fragment number 307. The numbers in the *vertical column* on the *left* indicate the molecular weights of the fragments (bp) of the DNA ladder in *lane L*

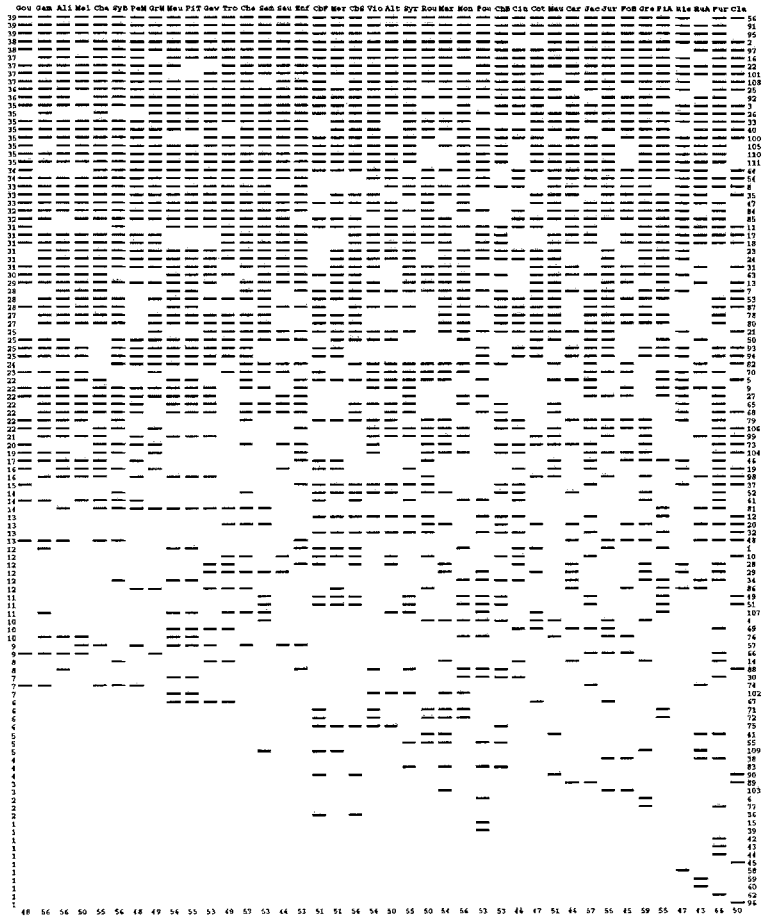

Fig. 2. Synthetic bandmap of the 111 polymorphic restriction fragments for the 40 accessions listed in Table 2. Each column corresponds to the accession indicated at the *top*; the fragments are represented by a *short horizontal line* segment. The columns are ordered according to the hypothetical taxonomic groups described in the results section. Line segments are ordered according to the decreasing frequencies of the fragments indicated in the *left margin*. The *numbers* assigned to the fragments are in the *right margin*. The number of fragments for each accession is at the *bottom of each column*

'Pinot gris', and 'Pinot blanc'; the second group 'Gewürztraminer', 'Savagnin vert', and 'Savagnin jaune'; the third group 'Gamay Fréaux' and 'Gamay noir'. Consequently, the studies described below involve only 40 accessions.

With the 21 PECs, 170 restriction fragments could be recorded among the 40 accessions. Fifty-nine fragments (34.7%) were common to all accessions; 11 fragments were unique (0.6%); and 100 fragments were informative (58.8%). The 111 informative or unique fragments yielded 179 different RFLP patterns. These results were arranged in the form of a 40 × 111 matrix of fragments, which served to establish the synthetic bandmap (Fig. 2). The average number of unique or informative fragments per accession pattern calculated from the bandmap is 52 (47% of the total fragments). The latter figure illustrates the significant level of RFLP among the 40 accessions.

PCA results and taxonomic study

The 40 × 111 matrix was further analyzed by PCA. Of the total variance 91% was explained with the 14 first principal components, and 28%, 13%, 10%, 8% and 7% were explained with the first to the fifth principal components, respectively. The plot corresponding to the first two principal components (41% of the total variance) is shown in Fig. 3.

The first three principal components served to define nine classes by AHC (Table 2). The first class is composed of 8 individuals for which the ESDmw's are higher than 0.69, with a maximal value of 0.81 for 'Aligoté' and 'Gamay noir'. These ESDmw's are higher than the corresponding ESDmo's, and are significantly higher than the average ESD values. This class is very highly correlated to the first principal component (Fig. 3). The genotype 'Gouais' is not as closely related to the other genotypes of this class.

The second class is composed of 7 individuals and is more heterogeneous. 'Meunier', 'Pinot teinturier' and 'Gewürztraminer' are very highly correlated with the first principal component. The maximal ESDmw (0.98) for the 'Meunier'-'Pinot teinturier' couple is due to a single difference between their restriction profiles. 'Gewürztraminer' is closely related to 'Pinot teinturier' and 'Meunier'; 'Trousseau' to 'Gewürztraminer' (and also to 'Chenin' which is outside the class); 'Côt' is not as closely related to 'Jurançon', which seems to be more

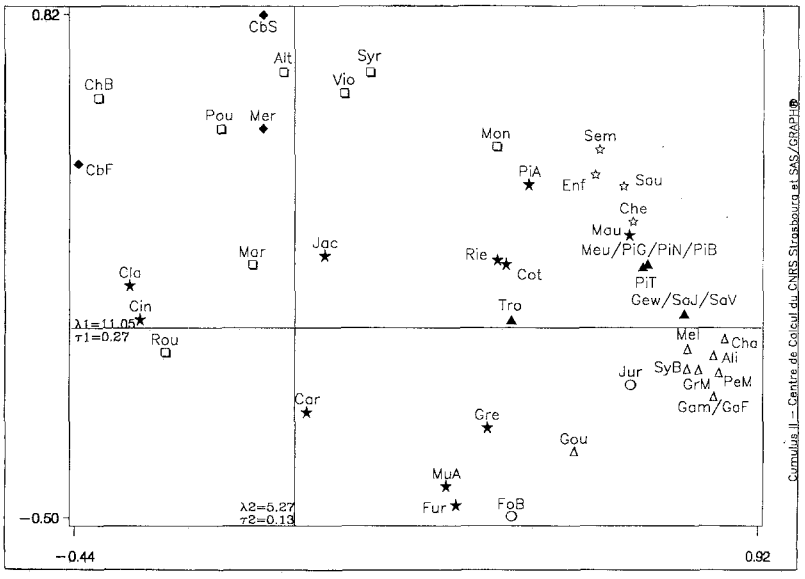

Fig. 3. Principal component analysis: the first two principal components are represented. λ_1, λ_2: eigenvalues; τ_1, τ_2: inertia rates; The position of each accession is indicated by a *symbol* identifying the hypothetical taxonomic group (see text): △ first group, ▲ second group, ☆ third group, ◆ fourth group, □ fifth group, ○ sixth group, ★ no group determined

closely related to 'Gamay noir'; the position of 'Grenache' is not clear from this study.

The third class composed of 7 individuals is also heterogeneous. 'Chenin' and 'Sémillon' are closely related. 'Chenin' has a high average ESD, and is closely related to 'Meslier Saint-François', 'Sylvaner', 'Gewürztraminer', and 'Trousseau'. 'Enfariné' is closely related to 'Sauvignon', and to 'Chardonnay' and 'Viognier', which are outside the class.

The fourth class is composed of the closely related accessions 'Cabernet-Sauvignon' and 'Merlot', which are also closely related to 'Cabernet franc' of the ninth class. 'Cabernet-Sauvignon' is highly correlated to the second principal component (Fig. 3).

The fifth class is composed of the closely related accessions, 'Altesse', 'Viognier', and 'Syrah'. This class is highly correlated to the second principal component (Fig. 3).

Classes 6 to 9 are characterized by relatively low ESDm's; all of the ESDmw's are lower than the corresponding ESDmo's.

On the basis of these results, we propose the following five hypothetical taxonomic groups: group 1 = class 1; group 2 = 'Pinot teinturier', 'Meunier', 'Gewürztraminer', 'Trousseau'; group 3 = 'Chenin', 'Sémillon', 'Enfariné', 'Sauvignon'; group 4 = class 4 and 'Cabernet franc'; group 5 = class 5.

The patterns of shared low frequency restriction fragments shown in Fig. 4 illustrate some of the correlations which did not appear clearly in the previous analysis. 'Roussanne' and 'Marsanne' share 5 low frequency fragments, and we note that ESD (Rou, Mar) = ESDm (Rou) = ESDm (Mar) = 0.60. If 'Mondeuse' is connected to 'Sémillon', 'Jurançon', and 'Pinot teinturier' according to its ESDm values (0.63), it also seems to be related to 'Viognier', 'Syrah', and 'Meunier' on the basis of the ESD values (0.62, 0.61, 0.62 respectively), and to 'Viognier', 'Marsanne', and 'Poulsard' on the basis of common low frequency fragments. The relationship between 'Poulsard' and 'Chasselas blanc' appears to be relatively weak in the ninth class; however, the 5 low frequency fragments common to both accessions suggest a stronger relationship. Furthermore, we note that ESDmo (Pou, Syr) = 0.64 and

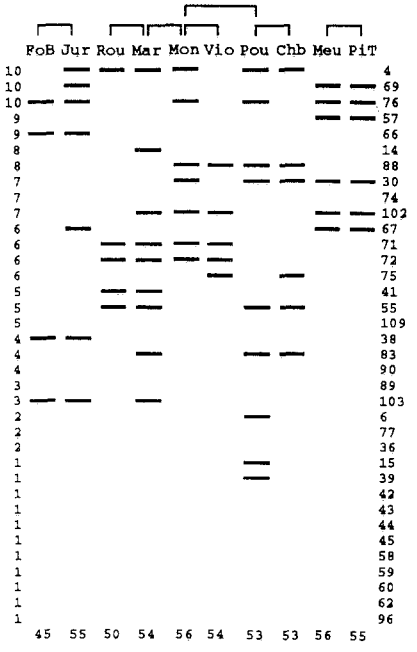

Fig. 4. Synthetic bandmap of the low frequency fragments. Accessions which share fewer than 4 fragments of a frequency less than 11 are not included. Related accessions are linked by *brackets*

that 'Poulsard' and 'Mondeuse' share 4 low frequency fragments. Consequently, the fifth hypothetical taxonomic group 'Altesse'-'Viognier'-'Syrah' may be extended to 'Marsanne'-'Roussanne', 'Mondeuse', and 'Poulsard'-'Chasselas'.

Finally, it should be noted that 'Jurançon' and 'Folle blanche' share 4 low frequency fragments and that both accessions can be connected to 'Gamay noir' (Table 2). This couple forms a sixth hypothetical taxonomic group.

Discussion

In the RFLP study presented here, genome analysis confirmed the existence of different levels of taxonomic relationships between the 'cépages' of *V. vinifera*, levels which generally agree with the conclusions drawn from certain ampelographical studies (Negrul 1938, 1946; Levadoux 1956; J.-M. Boursiquot personal communication). An important DNA polymorphism has been revealed by *Vitis vinifera* cv 'Chardonnay' probes, and it must be noted that the 46 accessions of our experiment contribute a small part to the large diversity of genotypes inferred from numerous earlier ampelographical studies.

On the basis of ampelographical criteria it has been concluded that certain 'cépages' are very closely related (sortogroups), but this has seldom been proven at the genetic level. The RFLP results obtained here for the individuals of earlier defined sortogroups confirm their very close genetic relationships. It has been observed, for example, that 'Pinot noir', 'Pinot blanc', and 'Pinot gris' can evolve from one another by somatic mutation (Galet 1990). Another example is 'Gamay Fréaux', which is a mutation of 'Gamay de Bouze'. 'Gamay Fréaux' differs from 'Gamay noir' only by the coloration of the berries, some very red young leaves, and a weakly pubescent limb; Gamay Fréaux has been observed to mutate to 'Gamay noir' (Galet 1990). 'Gamay de Bouze' is perhaps a mutation of 'Gamay noir' (Galet 1990). Finally, it should be noted that 'Gewürztraminer' is considered to be the pink aromatic form of 'Savagnin blanc'; 'Savagnin jaune' and 'Savagnin vert' are two clonal forms of 'Savagnin blanc' (Levadoux 1956; Galet 1990).

'Altesse' and 'Furmint' appear to be extremely similar when their ampelographical characters are compared (Galet 1990), but our results show an important difference in RFLP patterns for these 'cépages', with an ESD of 0.45. This proves that ampelography can be usefully complemented by RFLP analysis, the latter being moreover reproducible and environmentally independent.

The hypothetical taxonomic groups of 'cépages' revealed in this study are in good agreement with results of other studies. With the 'Pinot noir' 'cépage' as a reference plant type, Levadoux (1948) discovered that the majority of the 'cépages' of the north-east of France may form a taxonomic unit, which Negrul (1946) called the 'Pinot' sortotype. Within this group of Noiriens, Levadoux (1948) observed that 'Teinturier du Cher' is closely related to the wild grapevines of this region, a region where it has been cultivated for a long time. Consequently, Levadoux (1948) suggested that 'Teinturier du Cher' could be an ancestor of the Noiriens. The individuals belonging to hypothetical taxonomic groups 1 and 2 are all found in the group of Noiriens. The present study confirms the idea that 'Aligoté', 'Melon', 'Gamay noir', and 'Chardonnay' are particularly closely related (Negrul 1946; Levadoux 1948; J.-M. Boursiquot personal communication), whereas 'Gouais' is more distantly related to this group (Levadoux 1948; J.-M. Boursiquot personal communication).

In the third group, the relationship 'Chenin'-'Sémillon' is relatively well correlated with some ampelographical assumptions (J.-M. Boursiquot personal communication). However, this latter author considers that 'Chenin' is more related to 'Sauvignon'. Furthermore, Bisson (1986) reported an experiment on 'Sauvignon' inbreeding where 20% of the F_1 generation presented the phenotype of 'Chenin'. It should also be noted that 'Chenin' has been found in some old vineyards in the south-west of France (Galet 1962), the presumed geographical origin of 'Sémillon', and that 'Sémillon' cannot easily be related to a sortotype of this region (Levadoux 1956). The position of 'Enfariné' within this group is not confirmed by ampelography (J.-M. Boursiquot personal communication).

As far as the fourth group is concerned, it has already been mentioned that 'Cabernet franc' and 'Cabernet-Sauvignon' have both been cultivated in the south-west of France for a long time; 'Merlot' has perhaps appeared more recently in this region and is of an unknown origin (Viala and Vermorel 1901). These three 'cépages' are related on the basis of some ampelographers (Levadoux 1956; J.-M. Boursiquot personal communication) and our studies.

The 'cépages' of the fifth group have been cultivated for a long time in neighbouring geographical regions. 'Altesse' and 'Mondeuse' are cultivated in Savoy, 'Viognier' in the northern part of the Rhone valley, 'Chasselas blanc' in Savoy and in Switzerland, 'Marsanne', and 'Roussanne' in the Rhone valley, and 'Poulsard' in the Jura. With respect to ampelography (J.-M. Boursiquot personal communication), 'Marsanne', 'Roussanne', 'Viognier', 'Syrah', 'Altesse', and 'Mondeuse' would form one group, and 'Poulsard' and 'Chasselas blanc' another one.

Some ampelographical observations and our studies suggest the following relations: 'Meslier Saint-

François'-'Chenin'; 'Gouais'-'Jacquère'; 'Riesling'-'Jacquère'; 'Jurançon'-'Folle blanche'; 'Côt'-'Jurançon'; 'Grenache'-'Carignan'; 'Pineau d'Aunis'-'Chenin' (Levadoux 1948, 1956; Bisson 1986; J.-M. Boursiquot personal communication).

Finally, on the basis of ampelography and historical considerations, a majority of the 'cépages' that we have studied have been proposed to be part of an occidental ecogeographical race named *proles occidentalis* (Negrul 1946, 1968b; Jelaska 1956; Levadoux 1956). Such is the case for the 'cépages' of hypothetical taxonomic groups 1 and 2. For the same reasons, 'Muscat noir à petits grains' and 'Furmint' are supposed to be related to 'cépages' of the *proles orientalis* and *proles pontica*, respectively (Negrul 1946, 1968b; Jelaska 1956; Levadoux 1956). The bandmap, PCA, and ESD data for the above-mentioned 'cépages' and groups of 'cépages' plead for the existence of large taxonomic units within *V. vinifera*. However, they do not yet suffice to put the *proles* concept on a firm genetic basis.

In general, our RFLP data show that various 'cépages' of the same presumed geographical origin are related. Some autochthonous wild grapevines may be the ancestors of these regional families (Cecuk 1955; Levadoux 1954a, 1956). To prove this hypothesis, RFLP analysis has to be further refined by using more probes and restriction enzymes. However, there are probably numerous and complex connections at the different taxonomic levels. For these reasons, it will probably be necessary to analyze many more 'cépages' and wild grapevines to draw up a precise taxonomy of the *V. vinifera* population. In spite of the large effort this will require, such studies will have a number of interesting applications. A knowledge of the origin of the 'cépages' and their degree of relationship will be useful to breeders. It may be possible to approach questions concerning the mechanisms which allowed the transitions from the autochthonous plants to the more evolved 'cépages' as seen in some oriental forms (Levadoux 1954a, 1956). Ampelography now disposes of new powerful means by which to identify plants deriving from distinct ancestral cultivars; the RFLP technique can also be used to develop better controls in the commercial trade of 'cépages' and rootstocks (Bourquin 1991, 1992). Finally, this work prepares the way for genomic mapping and the detection of traits of agronomical interest within the *V. vinifera* species.

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