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Multivariate analysis of *Vitis* subgenus *Vitis* seed morphology

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Summary

We studied 142 grapevine seed samples belonging to 5 *Vitis* species, 92 cultivars of *Vitis vinifera*, 12 feral/wild populations and 4 hybrid rootstock cultivars. Eleven different characters from the seed and one allometric index have been used. Seeds show a wide range of variation in body dimensions, and in other parameters. Two largely differentiated clusters were obtained. Coincidences with previous seed classifications are discussed. Wild extra-European species have smaller seeds. The index breadth/length (STUMMER's index) doesn't allow to separate wild grapevines and cultivars. It defines, however, the "wild syndrome" values above 0.8 corresponding to wild extra-European *Vitis* species, occasionally used as rootstocks. Ferals/wild individuals tend to display smaller berries and plumper pips than their cultivated relatives therefore "looking wild". The multivariate analysis place together ferals/wild and related cultivars in their respective clusters and does not discriminate a cluster of wild European grapevine.

Key words: Morphometrics, ampelography, archaeobotany, grapevine origins.

Introduction

Grape seeds are highly polymorphic. As such, they have been used as a basis for taxonomy within the genus *Vitis*, specifically for European grapevine, *Vitis vinifera* L. The grapevine berries have (0) 1-4 (5) seeds. The seed is rounded or ovoid more or less gradually tapering into a beak, ending into the micropile, a swollen dorsal face with a deep furrow and enlarged in the center, the chalaza, a ventral part with two fossettes separated by the raphe, a prominent ridge (TERPÓ 1976, REYNIER 1995).

PLINIUS described "Alexandrian" grapes as having soft seeds, but the consistence of seeds have not often been considered as a relevant character afterwards. KROCHMAL and NAWABI (1961) described Afghan cultivars with very soft seeds such as 'Kata-i-Shumh Sur', with soft seeds ('Aquili-i-Siah', 'Fakhri Safid', 'Husaini-i-Dabah', 'Khalili'), and others with hard and brittle seeds. EBADI *et al.* (1996) described soft and very soft types in terms of "Floater" (late abortive hollow seeds with testa) opposed to "Sinkers" (normal hard fertile seeds).

CLEMENTE (1807, 1814, 1879) occasionally used seed length, color and number per berry (0-5) as descriptive

characters. Although 'Teta de Vaca Blanca' had some seedless berries, while others had 1-2 seeds, he did not actually describe any seedless cultivar. KOLENATI (1846) described the seedless 'Apyrena persica' from the Caucasus. His classification however is based on leaf and berry characters not on seed parameters.

ENGELMANN (1875) first used seed parameters for classifying American *Vitis* species. PLANCHON (1887) defined *Euvitis* as bearing pear-shaped seeds, versus *Muscadinia* = *Lenticellosis* with seeds oblong to ovoid, with oval-oblong chalaza surrounded by radial ridges and furrows. This difference is accepted in the Floras and monographs in the US (GRAY and FERNALD 1989, MUNSON 1909, REHDER 1990). VIALA and PÉCHOUTRE (1910) show that seed dimensions are extremely variable within cultivated species (*V. vinifera*, *V. labrusca*, *V. aestivalis*) and less within wild species.

PLANCHON (1887), VIALA and PÉCHOUTRE (1910) and NEGRUL (1960) mention the taxonomic relevance of the position of the chalaza.

POTEBNJA (1911) uses seed weight, length, diameter, size, outline, shape and position of the chalaza, type of raphe and fossettes, shape and size of the beak to classify grapevine cultivars. With these data, he defined the "European" cultivar group.

STUMMER (1911) studied the ferals/wild grapevines of the Austrian Danube and numerous cultivars from the Klosterneuberg Centre of Oenological Research. He attempted to distinguish *V. vinifera* and *V. sylvestris* Gmel. using the ratio length - diameter (Fig. 1).

The distinction between wild and cultivated grapevines is based in the interplay of taxonomic characters, physiological features and ecological data. Grapevine individuals living far from vineyards and gardens, in natural habitats, are often called "wild". However, as LEVADOUX (1956) underlined, these could generally and properly be named "lambrusques". This category includes remnants of vineyards abandoned, sub-spontaneous plants originating from seeds of cultivated plants and spontaneous populations. Even the latter are extremely complex, since spontaneous populations could derive from several generations of sub-spontaneous plants, from wild populations, which never have been in cultivation or to be the result of hybridization among different types. If we add to this picture the presence of American and French rootstocks and hybrid direct producers, which freely set seeds and hybridize with all of the above categories the result is that grapevine populations in natural habitats of Europe are extremely complex and often ancestral wild grapevine is rare or missing (LAGUNA 2003 a, b and 2004). Thus we use through this paper "ferals/wild" or "lambrusques" to refer

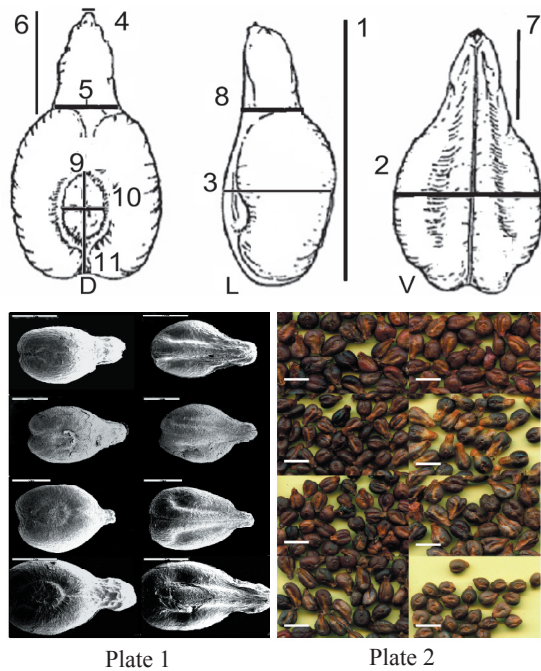


Fig. 1: Seed of *Vitis vinifera* L. Dorsal side (D), side view (L) and ventral side (V). Parameters studied: total length of seed (1), maximum breadth (= total diameter *sensu* TERPÓ) (2), thickness of seed (3), breadth of the beak at the hilum (4), breadth of the beak at the seed base (5), beak length in dorsal view (6), beak length in ventral view (7), thickness of the beak at the seed base (8), total length of chalaza scutellum (9), maximum breadth (diameter) of the chalaza scutellum (10), distance from the chalaza apex to the apex of the seed (11). The index maximum breadth (diameter) / total length is also calculated for each seed. **Plate 1:** From top to bottom: Dorsal, left, and ventral, right SEM images of seeds, of *Vitis vinifera* L. cvs 'Korinthiaki' (Group 8), 'Pinot Noir' (Group 5), 'Malvasia' (Group 7) and 'Semillon' (Group 1). Scale bars: 2 mm. **Plate 2:** From left to right and top to bottom: Digital desktop scanning images of seeds, of *Vitis vinifera* L. cvs 'Albillo' (Group 1), 'Graciano' (Group 2), 'Albariño' (Group 3), 'Airén' (Group 4), 'Chardonnay Blanc' (Group 5), 'Parellada' (Group 6), 'Silvaner Grün' (Group 7) and *Vitis riparia* Michx. (Group 8). Scale bars: 4 mm.

to all grapevine plants seemingly living in a wild status. ANDRASOVSKY (1915, 1917, 1925) used seed shape as principal character for *Vitis* systematics, and classified *V. vinifera* L. cultivar groups according to their seed morphology. NEGRUL (1946 a, b and 1960) underlined the systematic value of seed dimensions, fossette and chalaza. However, recent versions of his classification in eco-geographical groups excluded seed parameters from the list of useful characters (TROSHIN *et al.* 1990, TROSHIN 1999).

The systematics of LEVADOUX (1948) is based on leaf characters; the fruit, as the focus of human selection during millennia, may have led to separate phylogenetically close cultivars, therefore producing a false taxonomy.

FACSAR (1972 a) has shown that, in oval berries, the position and number of seeds determines the seed shape. In large-berried varieties the growth of berry along the longitudinal axis is not accompanied with an equivalent longitudinal growth of seeds, because they reach their full size earlier. He recognized 6 different types of berry structure on the basis of the position of the seeds.

FACSAR (1970, 1972 b) recognized rounded, pentagonal, quadrangular and triangular seeds (Tab. 1), and proposed morpho-genetic threads (phylogenetic?) connecting 41 seed types to their basic ancestral forms within each group (rounded, triangular, pentagonal, etc.). FACSAR (1975) examined archaeological pips from the Buda Castle, belonging to Hungarian cultivars and recognized 13 seed types in three groups: pillar-like, cone-shaped and rounded-stemmed, which are related to the former.

GALET (1970) used seed length, breadth, beak shape, chalaza, fossettes, and occasionally color in describing Afghan cultivars, however seeds are not analyzed in his ampelography (GALET 1970, 1988, 1990).

The Organisation International de la Vigne et du Vin (OIV 1983) included in the code of descriptors absence/presence (seedless/rudimentary/well developed), seed length, weight and transversal ridges (absent/present). Transversal ridges are common through section *Muscadinia*. Seed length, breadth and weight are used in the standardized berry de-

Table 1

Seed types and type groups *sensu* FACSAR (1970, 1972, 1975) and TERPÓ (1977). L: seed length; W: seed breadth; T: seed thickness; BL: beak length (dorsal); S-R: position within the square-stemmed vs. round-stemmed gradient (scored 0 to 1)

A: Parameters

Type Group	L	W	W/L	T	BL	S-R
Pillar-like beaked	5.5-6.7	3.3-4.1	0.6-0.7	2.7-3.5	1.3-1.9	0.1-0.5
Triangular	6.1-6.5	3.7-3.8	0.5-0.6	2.9-3.0	1.6-1.8	0.5-0.6
Rounded	4.7-7.1	3.7-4.6	0.6-0.8	2.4-3.5	0.9-1.8	0.5-0.9
Pentagonal	4.9-7.3	3.4-4.5	0.5-0.7	2.6-3.2	1.2-1.8	0.5-0.8

B: Examples

Type Group	Cultivars
Pillar-like beaked (Quadrangular, Quadrangularis, Quadratic, Prismatic, Obovate, Elliptical)	Cornichon blanc, Gohér, Fügér, Mézes, Lisztes, Fehérszanka, Szagos bajnár, Fügeszölő
Triangular (Cone-shaped beaked, Turbinatus, Cordatus)	Balafánt, Dinka
Rounded (Rounded-stemmed, Rotundatus)	Furmint, Vékonyhájú, Berkenyekevelű, Erdei, Dodrelabi, Bánáti, Mavrud, Chasselas, Cinsaut, Muskotaly
Pentagonal (Quinquangularis)	Aramon, Folle Noir, Pinot, Cabernet, Gamay, Dinka, Szemerdiai, Kéknyelű

scriptions (GENRES 2003). TERPÓ (1976, 1977) described the seeds from 94 (mainly Hungarian) wine cultivars as small, globular, short beaked, with chalaza scutellum centered in the seed.

Grapevine seeds are often found in archaeological contexts. Archaeobotanists have to deal with seed characters for identifying the materials in terms of wild or cultivated (JONES and LEGGE 1987, RIVERA and WALKER 1989). This is important for determining the origin and spread of grapevine domestication and cultivation. In fact the assumption that seed morphology is sufficient for tracing this process is often found in archaeobotanical literature. STUMMER (1911) and LEVADOUX (1956) attributed the smaller globose seeds (practically lacking beaks) to wild grapevines (*Vitis sylvestris* Gmel.). Cultivated *Vitis vinifera* L. accordingly show large and elongated, ovoid or pear-shaped seeds with a rather long beak (WEBB 1968, ZOHARY and HOPF 1994, BUXÓ 1997, DELWEN 2003). Nevertheless, many factors determine the form of the seeds. The number of seeds in each berry, the size of the berry and its ripening have a decisive influence in this respect. MORDECHAI KISLEV (Bar-Ilan University, Israel; cited by DELWEN 2003) vigorously questioned the use of STUMMER's index in archaeobotanical reports. He pointed out that although it is already well known to be unreliable it is still used, while other detailed studies, such as those by FACSAR (1970, 1975) and TERPÓ (1976, 1977) have been largely ignored. DELWEN (2003) proposed a major wide-ranging study of modern and ancient grape pip morphology, including taphonomic issues such as charring distortion, a study started by SMITH and JONES (1990).

The purpose of this work is to study the seeds of *Vitis vinifera* cultivars, compared with ferals/wild *V. vinifera*, hybrid rootstocks and other *Vitis* species, using a series of quantitative morphological parameters - useful for both taxonomic and archaeological research - by means of a multivariate analysis.

Material and Methods

We studied 142 grapevine seed samples belonging to 5 *Vitis* species, 92 cultivars of *Vitis vinifera*, 12 feral/wild populations (lambrusques in LEVADOUX (1956) terms) and 4 rootstocks. The seeds were collected in two repositories in Spain: CIDA of La Rioja at Mendavia (Navarra) and Casa de las Vides/CAPA at Agullent (Valencia). Seeds of wild species came from botanical gardens, or were collected in wild populations cited by OCETE (1999) and MARTÍNEZ DE TODA and SANCHA (pers. comm.). The samples are listed in Tab. 2. Names and origin are standardized according to ALLEWEDT and DETTWEILER-MÜNCH (1992). Voucher specimens and seed samples are kept in the UMH (Herbarium of the Universidad Miguel Hernández).

We used a Mitutoyo Digital calliper (resolution 0.1 mm). For each sample, 7-20 seeds (average 14) were measured depending on availability. Five seeds were selected at random from each sample for building the data matrix. Eleven different characters and one allometric index have been considered corresponding to the significant parts of the seed: beak, body of the seed and chalaza scutellum (Fig. 1).

Table 2

List of samples analysed with identification: Cluster (I: 1, II: 2) and Group (1-8). Outline type (P: pentagonal, Q: quadrangular, R: rounded, T: triangular). Type of Fossettes (TERPÓ 1977) (P, parallel, V, v-shaped = furcate, C, convergent towards the apex = turning inwards, D, divergent towards the apex = turning outwards = hook shaped). Use (W: Wine, T: Table, O: Others). Eco-geographical group (TROSHIN 1999) (AME: American species, ANT: Orientalis Antasiatica, ASI: Asiatic species, CAS: Orientalis Caspica, OCC and OCG: Occidentalis, PON: Pontica, SYL: Wild European). Country of Origin. Codes and Repository. Repositories in Spain: 1, Casa de las Vides, Agullent, Valencia, 2, CIDA of La Rioja, Mendavia, Navarra, 4, Bodegas Viña Ijalba, Rioja. From the fields in Spain: 3, Abengibre, Albacete, 5, Aspe and Font Calent Alicante, 6, Peñafiel, Valladolid, 7, Pozo Amargo, Cuenca, 8, Robledo, Cacabelos, León, 9, Jumilla, Murcia. From wild populations in Spain: 10, Valle del Borosa (Jaén), 11, Between Anguiano and Bovadilla, Rioja, 12, Roncal Valley, Navarra, 13, Santa Cruz de Campezo, Alava, Botanic Gardens: 14, Vácrtót, Hungary, 15, Montreal, Canada, 16, Siena, Italy. Names in italics, marked with asterisk. * = denote labelling errors in the origin

Standardized name	Cluster	Out.	Fos.	Use	Eco	Origin	Rep.
Airén	I4	P	P	W	OCC	ESP	7
Airén	I4	P	P	W	OCC	ESP	1
Albarín Negro	I3	P	P	W	OCC	ESP	2
Albariño	I3	P	P	W	OCC	ESP	1
Albillo	I6	P	P	W	OCG	ESP	3
Albillo*	I1	P	V	W	OCG	ESP	6
Albillo	I1	P	P	W	OCG	ESP	1
Aledo	I6	T	P	T	ANT	ESP	5
Alicante Bouschet	I3	P	V	W	PON	FRA	1
Alphonse Lavallée	I1	P	V	W	OCC	FRA	4
Alphonse Lavallée	I1	P	V	W	PON	FRA	3
Aragonés	II5	Q	V	W	ANT	ESP	6
Barbera	II7	T	V	W	PON	ITA	2
Beba	I1	P	V	T	PON	ESP	1
Beba	I1	P	V	T	PON	ESP	9
Bobal	I3	T	V	W	OCC	ESP	3
Bobal	I3	T	V	W	OCC	ESP	7
Bobal	I3	T	V	W	OCC	ESP	1
Bobal	I3	R	D	W	ANT	ESP	5
Bobal Negro	I3	T	V	W	OCC	ESP	3
Bonicaire	I1	Q	V	W	OCC	ESP	2
Brancellao	II5	R	D	W	OCC	PRT	2
Cabernet Franc	II8	P	P	W	OCC	FRA	8
Cabernet Sauvignon	I1	P	P	W	PON	FRA	1
Cainho	I6	P	D	W	PON	PRT	2
Calop Blanco	I1	T	V	W	PON	ESP	2
Calop Blanco	I1	P	V	W	PON	ESP	3
Canega	I4	T	V	W	OCG	ESP	2
Chardonnay Blanc	II5	R	P	W	PON	FRA	1
Carignan Noir	II5	P	V	W	PON	ESP	1
Cayetana Blanca	I2	P	D	W	PON	ESP	2
Cayetana Blanca	I2	R	P	W	PON	ESP	1
Cinsaut	I1	T	P	W	PON	FRA	2
Chelva	I6	P	V	W	OCG	ESP	1
Chenin Blanc	I1	T	P	W	PON	FRA	1
Feral / Wild (lambrusque)	I6	P	P	O	OCC	ESP	4
Feral / Wild (lambrusque)	I1	P	V	O	SYL	ESP	3
Feral / Wild (lambrusque)	I1	P	P	O	SYL	ESP	4
Feral / Wild (lambrusque)	I2	P	V	O	SYL	ESP	10
Feral / Wild (lambrusque)	II7	P	V	O	SYL	ESP	10
Feral / Wild (lambrusque)	I2	R	V	O	SYL	ESP	10
Feral / Wild (lambrusque)	II7	P	V	O	SYL	ESP	10
Feral / Wild (lambrusque)	II5	R	P	O	SYL	ESP	12
Feral / Wild (lambrusque)	II5	R	P	O	SYL	ESP	12
Feral / Wild (lambrusque)	II5	P	V	O	SYL	ESP	10
Feral / Wild (lambrusque)	II5	P	V	O	SYL	ESP	10
Feral / Wild (lambrusque)	II5	R	V	O	SYL	ESP	13
Flame Tokay	I6	P	V	T	POG	HUN	2
Folle Blanche	I3	T	V	W	OCC	FRA	2
Forcallat Tinta	I1	T	V	W	PON	ESP	2
Frasco	I3	P	V	W	POG	ESP	3
Frasco	I3	P	V	W	POG	ESP	3
Gamay Noir	I1	P	V	W	OCG	FRA	2
Garnacha Blanca	II5	T	V	W	ANT	ESP	1
Garnacha Peluda	I1	P	V	W	OCC	ESP	1
Garnacha Roja	I1	R	P	W	ANT	ESP	4
Garnacha S. Vicente	I3	T	V	W	ANT	ESP	4
Garnacha Tinta	I1	T	V	W	PON	ESP	2
Garnacha Tinta	I1	P	V	W	PON	ESP	1
Godello	I3	R	D	W	PON	ESP	1

Tab. 2 continued

Standardized name	Cluster	Out.	Fos.	Use	Eco	Origin	Rep.
Graciano	I2	R	P	W	OCC	ESP	1
Gran Negro	I3	T	P	W	ANT	ESP	2
Imperial Napoleón	I2	R	P	T	ANT	ESP	2
Italia	I2	P	P	W	PON	ITA	9
Jaén Blanco	I1	T	V	W	OCC	ESP	4
Jerez	I15	P	V	W	PON	ESP	1
Kadin Barmak	I14	Q	P	T	CAG	AM	2
Königin der Weingärten	I16	Q	V	W	ANT	HUN	2
Korinthiaki	I18	P	V	R	OCC	GRC	2
Listán Negro	I13	P	V	W	PON	ESP	2
Loureiro Blanco	I15	R	V	W	OCC	ESP	1
Malvar	I12	R	V	W	OCC	ESP	1
Malvasia	I17	P	P	W	PON	ESP	8
Malvasia Bianca	I15	T	D	W	OCG	ITA	1
Malvasia Bianca*	I17	P	V	W	PON	ITA	6
Merlot*	I17	R	V	W	OCC	FRA	7
Merlot	I15	R	V	W	OCC	FRA	1
Merlot	I15	R	V	W	OCC	FRA	9
Merseguera	I15	R	V	W	OCC	ESP	1
Miguel de Arco	I11	T	V	W	ANT	ESP	2
Monastrell	I12	R	P	W	OCC	ESP	1
Monastrell*	I13	R	P	W	OCC	ESP	5
Monastrell	I12	P	P	W	POG	FRA	2
Morato	I17	P	V	W	CAG	ESP	4
Moravia Agria	I16	P	D	W	OCC	ESP	3
Moravia Dulce*	I13	P	V	W	CAG	ESP	3
Moravia Dulce	I11	P	V	W	CAG	ESP	3
Moscato Giallo	I11	P	P	W	PON	ITA	6
Moscato Giallo	I16	T	V	W	PON	ITA	8
Moscato Giallo	I12	T	V	W	OCG	ITA	2
Moscato Giallo	I16	P	P	W	OCG	ITA	5
Moscato Giallo	I16	P	C	W	OCG	ITA	5
Muscat of Alexandria	I17	T	P	W	OCG	ITA	2
Navarra	I12	P	P	W	PON	ESP	4
Negramoll	I12	P	P	W	PON	ESP	2
Palomino Fino	I15	P	V	W	PON	ESP	2
Pardillo	I15	P	V	W	OCG	ESP	3
Pardillo	I15	R	V	W	OCG	ESP	2
Parellada	I16	R	D	W	OCC	ESP	1
Parraleta	I17	R	P	W	PON	ESP	2
Pedro Ximénez	I16	P	D	W	CAS	ESP	1
Perle von Csaba	I15	R	P	T	OCG	ITA	2
Pinot Noir	I15	P	P	W	OCC	FRA	1
Pintaillo A	I11	P	V	W	OCC	ESP	3
Pintaillo B	I17	P	P	W	OCC	ESP	3
Planta Nova	I18	R	V	W	ANT	ESP	1
Riesling Weiss	I11	R	P	W	PON	FRA	1
Ruby Cabernet	I15	R	V	W	PON	FRA	1
Sangiovese	I15	P	D	W	OCG	ITA	2
Sauvignon Blanc	I11	P	P	W	PON	FRA	7
Sauvignon Blanc	I11	Q	D	W	PON	FRA	1
Semillon	I11	P	P	W	OCC	FRA	1
Servant (sub Pedro Juan)	I15	P	D	W	PON	ESP	3
Servant	I15	P	P	W	ANT	FRA	1
Silvaner Grün	I17	Q	P	W	OCG	FRA	1
Tempranillo	I11	P	P	W	PON	ESP	8
Tempranillo (Cencibel)	I11	Q	P	W	POG	ESP	3
Cencibel*	I13	Q	V	W	PON	ESP	3
Tempranillo	I17	P	D	W	PON	ESP	7
Tempranillo*	I15	P	V	W	PON	ESP	1
Tinto Roriz	I17	R	D	W	OCC	PRT	2
Tempranillo Blanca	I17	P	P	W	PON	ESP	6
Tinta Grossa	I13	R	D	W	ANT	PRT	1
Torrentés	I16	P	V	W	OCC	ESP	1
Touriga Nacional	I15	P	P	W	PON	PRT	1
Ugni Blanc	I13	T	V	W	PON	ITA	1
Uzbekistan	I16	P	D	W	OCG	SU	1
V. berlandieri Hybrid	I18	R	P	O	AME	USA	3
V. berlandieri Hybrid	I18	R	P	O	AME	USA	3
V. berlandieri Hybrid	I18	R	V	O	AME	USA	11
V. berlandieri Hybrid	I18	R	V	O	AME	USA	11
V. bryoniifolia	I18	R	V	O	ASI	Asia	14
V. riparia	I18	R	V	O	AME	USA	15
V. sylvestris*	I17	R	V	O	SYL	ITA	16
V. thunbergii	I18	R	P	O	ASI	JAP	14
Valenci Negro	I13	T	V	W	OCC	ESP	6
Verdejo Blanco	I12	P	D	W	PON	ESP	1
Verdil	I15	R	V	W	OCC	ESP	1
Vermentino	I13	P	P	W	PON	ITA	1
Viura*	I15	R	V	W	OCC	ESP	7
Viura	I17	R	V	W	OCC	ESP	1
Viura	I17	R	P	W	OCC	ESP	5

It was impossible to reduce the morphology of ventral fosses as cited by TERPÓ (1977) (parallel, furcate, convergent towards the apex, divergent towards the apex) to quantitative measurable parameters. However the observed types are nonetheless displayed in Tab. 2.

The package R was used for the analysis of data (IHAKA and GENTLEMANT 1997) with subroutines based on the procedures of calculation of the program SPAD (LEBART and MORINEAU 1984, 1985), according to routines implemented by PALAZÓN and CALVO (1999).

The crude data matrix consists of 710 rows (5 seeds by 142 samples) and 17 columns, 4 for references and 13 for the parameters. References are the code of sample, code of species or cultivar, origin, and number of seeds measured from each sample. The data matrix was previously filtered by means of an analysis of principal components (MURTAGH 1985, MURTAGH and HECK 1987). The resulting matrix was processed by hierarchical agglomerative clustering of the objects described by the first 4 main components that account for 90 % of the inertia, using the Ward's method (minimum-variance) (WARD 1963, LEBART and MORINEAU 1984). In order to gain stability, the analyses were repeated considering instead of a matrix of 710 seeds as rows, one of 142 sample codes as rows. The median value was used in each one of the variables to represent the cluster of 5 scores from each sample.

SEM photographs, both dorsal and ventral view, were made with a SEM JEOL 6100, at a voltage of 10 kV. Seeds were previously covered with a thin layer of gold. Optical Digital images were made using an Agfa Arcus 1200 desktop scanner set at 2400 pp.

Results and Discussion

Seed polymorphism: Seeds show a wide range of variation in body dimensions 3.7-8.1 x 1.8-5.2 x 1-3.7 mm, and in other parameters. Tab. 3 presents the different minimum and maximum values, the mean, median, standard deviation, as well as the 1st and 3rd quartile, for each parameter.

Major clusters and relevant characters: The distribution of frequencies for the seed characters does not allow a clustering of the cultivars using each parameter separately. However the combined intervention of all characters allows the clustering of cultivars. The transformed dendrogram shows hierarchical cophenetic relationships between objects with two largely differentiated clusters (I and II) divided in 8 smaller clusters. Cluster I includes large, long and wide seeds, which present a large and prominent beak. This corresponds in part with the slender, elongate, seed group reported by TERPÓ (1977) that comprised altogether obovate and elliptical seeds. Cluster II corresponds with the rounded seed group reported by TERPÓ (1977). We have analyzed the distribution of FACSAR (1970, 1972 a) seed outline-classes in the different clusters obtained with the multivariate analysis (Tabs 1 and 5). Cluster I contains a relatively high proportion of pentagonal seeds (about 50 %), triangular seeds are significantly represented (23 %), the proportion of rounded seeds is much less (only 14 %)

Table 3

Descriptive statistics of the parameters (mm) for the 142 samples. L: total length; W: maximum breadth; W/L: ratio maximum breadth/total length; T: maximum thickness; LD: length of the beak, dorsal view; LPV: Length of the beak, ventral view; AA: breadth of the beak at the apex; AT: breadth of the beak at the base; EP: thickness of the beak at the base; LMC: maximum length of the chalaza; AMC: maximum breadth of the chalaza; DBC: distance from the apex of the chalaza to the apex of the seed; Min.: minimum; 1st Q.: first quartile; Med.: median; Mean: arithmetic mean; 3rd Q.: third quartile; Max.: maximum; Sd.: standard deviation

Par./Stat.	Min.	1 st Q.	Med.	Mean	3 rd Q.	Max.	Sd.
L	3.73	5.34	5.81	5.8	6.2	8.1	0.71
W	1.79	3.56	3.75	3.77	3.97	5.23	0.37
W/L	0.43	0.61	0.65	0.66	0.70	1.06	0.07
T	0.98	2.50	2.70	2.71	2.91	3.77	0.33
LD	0.38	1.32	1.63	1.63	1.9	3.66	0.45
LPV	0.27	1.11	1.3	1.34	1.56	2.74	0.36
AA	0.11	0.76	0.87	0.89	1.01	2.02	0.23
AT	0.28	1.43	1.59	1.6	1.78	2.78	0.29
EP	0.80	1.43	1.56	1.57	1.72	2.18	0.22
LMC	0.80	1.39	1.58	1.6	1.78	3.14	0.30
AMC	0.40	0.95	1.1	1.1	1.26	2.10	0.23
DBC	0.01	0.78	0.89	0.89	1.01	1.71	0.20

and quadrangular are also scarce. The proportions change in Cluster II, rounded seeds are in higher proportion (over 50 %) and pentagonal (38 %) seeds while quadrangular and triangular here account altogether only for 10 % of the samples.

Cluster I, with 85 samples, comprises large, long and wide seeds with a prominent long beak. It is divided in turn into 5 smaller clusters (4-8) (Tab. 4). Group 1: The seeds of the cultivar 'Semillon', 5.5-6 mm x 3.5-4 mm, are representative of the cluster (Plate 1). The chalaza scutellum is ovate and very well differentiated. The fossettes are, also, very prominent, the raphe is not prominent. The beak is long and distinct. Group 2: Group of 'Cayetana Blanca' and 'Graciano' (Plate 2). Group 3: 'Albariño', 'Bobal' and 'Bobal'-related cultivars (Plate 2). Group 4: Large, very long seeds of 'Airén' and 'Kadin Barmak' ('Cornichon') (Plate 2). Group 6: Some of the Muscat and Muscat-related cultivars (Plate 2).

Cluster II comprises 57 samples. It is divided in turn in three sub-clusters (5, 7, 8) (Tab. 4). Group 5: Globose small seeds with a short beak. The seeds of 'Pinot Noir' are representative; they are small (5-5.5 x 3.5-4 mm), rounded, rather cordate, (Plate 1). The chalaza scutellum is small and not very prominent. The seed has a short tapering beak. Within this cluster are seeds less rounded and with a short

Table 4

Descriptive statistics of parameters selected (mm), for the groups 1-8. L: total length; W/L: index maximum breadth/total length; LD: length of the beak, ventral view; EP: thickness of the beak at the base; AA: breadth of the beak at the apex; AT: breadth of the beak at the base; Min.: minimum; 1st Q.: first quartile; Mean: arithmetic mean; 3rd Q.: third quartile. Max.: maximum

L	Min.	1 st Q.	Median	Mean	3 ^r Q.	Max.	W/L	Min.	1 st Q.	Median	Mean	3 ^r Q.	Max.
1	5.09	5.70	5.97	5.96	6.15	7.02	1	0.57	0.62	0.64	0.64	0.66	0.70
2	5.03	5.78	6.05	5.97	6.19	6.6	2	0.64	0.65	0.67	0.67	0.69	0.71
3	4.98	5.37	5.83	5.8	6.16	6.58	3	0.55	0.58	0.61	0.61	0.64	0.70
4	6.21	6.25	6.49	6.81	7.06	8.05	4	0.47	0.56	0.60	0.57	0.61	0.62
5	4.61	5.07	5.34	5.29	5.48	5.92	5	0.63	0.66	0.70	0.69	0.72	0.80
6	6.15	6.65	6.85	6.87	7.16	7.39	6	0.57	0.59	0.65	0.63	0.66	0.70
7	5.50	5.74	5.96	5.94	6.1	6.39	7	0.54	0.62	0.64	0.63	0.65	0.68
8	3.90	4.30	4.67	4.69	5.01	5.62	8	0.56	0.72	0.76	0.76	0.81	1.03

LD	Min.	1 st Q.	Median	Mean	3 rd Q.	Max.	EP	Min.	1 st Q.	Median	Mean	3 rd Q.	Max.
1	1.16	1.30	1.46	1.49	1.67	1.93	1	1.45	1.51	1.56	1.62	1.73	1.87
2	1.06	1.22	1.26	1.29	1.3	1.77	2	1.54	1.67	1.73	1.72	1.78	1.92
3	1.24	1.46	1.52	1.57	1.66	2.04	3	1.24	1.45	1.53	1.55	1.65	1.79
4	2.05	2.15	2.23	2.27	2.36	2.59	4	1.47	1.64	1.74	1.72	1.82	1.93
5	0.90	1	1.11	1.1	1.2	1.30	5	1.27	1.35	1.45	1.44	1.50	1.70
6	1.19	1.41	1.54	1.5	1.6	1.83	6	1.63	1.68	1.75	1.78	1.86	1.99
7	0.94	1.04	1.13	1.15	1.25	1.35	7	1.32	1.48	1.54	1.53	1.60	1.7
8	0.42	0.72	0.75	0.8	0.9	1.25	8	0.92	1.06	1.22	1.2	1.29	1.60

AA	Min.	1 st Q.	Median	Mean	3 rd Q.	Max.	AT	Min.	1 st Q.	Median	Mean	3 rd Q.	Max.
1	0.79	0.91	0.98	1.01	1.06	1.82	1	1.42	1.62	1.73	1.72	1.81	1.99
2	0.81	0.85	0.96	1.02	1.15	1.69	2	1.53	1.63	1.79	1.74	1.84	1.97
3	0.65	0.81	0.93	0.91	1.02	1.25	3	1.28	1.5	1.65	1.62	1.73	1.85
4	0.9	1.09	1.19	1.14	1.25	1.30	4	1.59	1.66	1.89	1.90	2.12	2.23
5	0.55	0.76	0.81	0.78	0.84	0.96	5	1.25	1.38	1.46	1.45	1.54	1.63
6	0.74	0.86	0.95	1.02	1.04	1.85	6	1.47	1.67	1.85	1.83	2	2.06
7	0.65	0.76	0.78	0.79	0.83	0.96	7	1.37	1.47	1.5	1.51	1.54	1.76
8	0.20	0.49	0.57	0.55	0.66	0.75	8	0.81	1.04	1.1	1.11	1.25	1.27

Table 5

Frequencies of the FACSAR's Shape groups in the clusters resulting of the multivariate analysis

Type/Group	Selected cultivars	Frequency in each group							
		1	2	3	4	5	6	7	8
Pillar-like beaked	Aragonés, Bonicaire	3	0	1	1	1	1	1	0
Triangular	Bobal, Folle Blanche	7	1	9	1	2	2	2	0
Rounded	Chardonnay Blanc, Merlot, Monastrell, Viura	2	5	4	0	15	1	6	8
Pentagonal	Airén, Flame Tokay, Tempranillo	19	7	8	2	12	11	8	2

but more abruptly distinct beak. 'Malvasia Bianca' has medium-sized seeds (3.2-3.9 x 4.5-5.2) mm, pyriform; they have a small but very distinct beak, the chalaza scutellum is large and very prominent. Group 7: Comprises small-seeded cultivars ('Silvaner Grün', 'Viura', 'Tempranillo') together with some "lambrusques" presumably escaped from cultivation (Plate 2). Group 8: comprises small rounded seeds of Japanese *V. thunbergii*, American *V. riparia* (Plate 2) and Chinese *V. bryoniifolia*, altogether with *V. berlandieri* hybrids and *V. vinifera* cultivars with markedly rounded and/or very small seeds ('Korinthiaki', 'Cabernet Franc', 'Planta Nova'). The seeds of cv. 'Korinthiaki' are representative of this cluster. They are small (4-4.3 x 2.5-3 mm) (Plate 1). The chalaza scutellum is hardly prominent. The beak is well differentiated.

The total length of the seeds is an excellent character ($R^2 = -0.875$ with axis 1). TERPÓ (1976) recognized three length groups within *V. sylvestris*: small < 4.5 mm, medium 4.5-5.5 mm and long > 5.5 mm. We found a high overlapping in groups 1, 2, 3 and 7 (5-6.2 mm). However, seeds of groups 4 (6.2-8 mm) and 6 (6.1-7.3 mm) are significantly longer and those of groups 8 (3.5-5.6 mm) and 5 (4.6-5.9 mm) shorter (Tab. 4, Fig. 2). Only groups 8 and 5 fall within the range of Terpó (1976) for wild grapevine seed length. The smaller seeds belong to wild American and Asiatic species. This is important because within group 5 are 5 samples collected in the wild, lambrusques in LEVADOUX' (1956) terms, each one closely similar with different cultivar samples. This similarity points towards either these lambrusques are feral individuals escaped from cultivation or are wild relatives (ancestors) of the cultivars. The maximum breadth shows a lower correlation with axis 1 ($R^2 = -0.671$), only separating group 6 (4-4.7 mm) from groups 8 (2.8-4.4 mm) and 3 (3.1-3.8 mm). The length of the beak in ventral view is useful. TERPÓ (1976) recognised three beak length classes: short

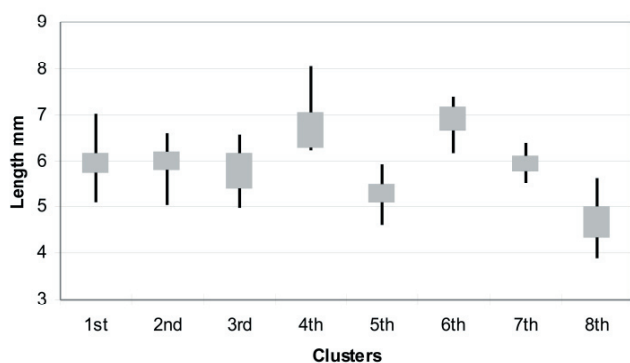


Fig. 2: Variation of seed length (mm) in the 8 groups.

(< 1 mm), medium (1-2 mm), long (> 2 mm). Although the different groups overlap (Tab. 4), clusters 8 (0.4-1.6 mm) and 5 (0.9-1.6 mm) show shorter beaks. In accordance beaks between 0.4 and 1.6 mm are characteristic for Extra-European and European wild grapevines. Longer beaks are found in clusters 3 (1.6-2.2 mm), 6 (1.6-2.4) and specially 4 (2.3-3.2 mm). NEGRUL (1960) reported from archaeological data that under the effect of cultivation and selection the beak grew unproportionately faster than seed length.

The breadth of the beak in the hilum or seed base shows high correlation with axis 1 ($R^2 = -0.826$). Cluster II shows lowest values (0.2-1 mm) (Tab. 4), cluster I shows the highest (0.7-1.8 mm). Though a promising character, its use would be restricted to recent seeds since most archaeological samples lack the hilum due to fracture.

The group 8 shows smaller breadth of the beak at the joint with the seed body (0.8-1.27 mm), it is wider in the rest of groups (1.25-2.2 mm).

The distance from the apex of the chalaza-scutellum to the apex of the seed is not different between groups. But it is not exactly equal to the position of the chalaza in the seed, because it is not given in rapport to the total length. It is possible that further studies show more consistent relationships as proposed by PLANCHON (1887), VIALA and PÉCHOUTRE (1910) and NEGRUL (1960). The length and breadth of the chalaza show also low relevance.

The typology of fossettes (TERPÓ 1977) (parallel, furcate, turning inwards, turning outwards) has been studied in the different samples (Tab. 2). The more frequent type is furcate (74 samples). It is slightly more often found in Cluster II (59 % of the samples) vs. 48 % in Cluster I. The parallel type follows (50 samples), 32 % of samples in Cluster II vs. 27 % in Cluster I. The Turning-outwards type of fossettes is found in 17 samples. Only one sample shows Turning-inwards fossettes. Therefore we have not found correlations between type of fossettes and clusters resulting from the multivariate analysis.

We have also plotted the main purpose of use of each sample cultivar or species (wine, table, raisin or others) (Tab. 2). Wine cultivars are predominant in all groups (114 out of 142 samples) except group 8, in which wild and rootstocks account for 70 % of samples. Table grapes (7) and raisins (only 1) are scarcely represented in the sample. Notwithstanding it is remarkable that among the 7 table grape cultivars only one ('Perle von Csaba') is included in Cluster II, within group 5.

STUMMER'S INDEX: STUMMER (1911) proposed the index breadth/length to discriminate *Vitis vinifera* cultivars and wild forms of *Vitis sylvestris*. Cultivated grapevines

shows pear-shaped seeds, with long beak while the wild ones show a relatively short beak. STUMMER's index values from 0.44 to 0.55 are exclusive for *V. vinifera* cultivars, while > 0.76 are exclusive for the feral/wild of Austria (Tab. 6). These limits were later defined by SCHIEMANN (1953) (> 0.7 for the wild) and TERPÓ (1976) (> 0.73 for the wild in Hungary and > 0.8 in Moldavia and Crimea).

Table 6

Different approaches to the STUMMER's Index (breadth/length) values. C = cultivars, W = wild, min = minimum, max = maximum

	C			W		
	min	mean	max	min	mean	max
STUMMER 1911 (Austria)	0.44	0.55	0.76	0.55	0.65	0.83
SCHIEMANN 1953 (Germany)	0.54	0.61	0.70	0.64	0.73	0.83
MARTÍNEZ DE TODA and SANCHÁ 1990 (Spain)	0.45	0.65	0.80	0.45	0.75	1.05
TERPÓ 1976 (Hungary)	0.48	0.55	0.73	0.53	0.70	0.85
TERPÓ 1976 (Moldavia)	0.48	0.58	0.80	0.60	0.76	0.95
TERPÓ 1976 (Crimea)	0.49	0.59	0.80	0.65	0.76	0.91

MARTÍNEZ DE TODA and SANCHÁ (1990) studied seeds of supposed *Vitis sylvestris* Gmel. and from 'Tempranillo', 'Garnacha' and 'Viura'. The breadth/length value was always < 0.80 in the three cultivars, while 24 % of the seeds of *Vitis sylvestris* Gmel. had higher values. Seeds, about 16 % of the seeds from cultivars, and 69 % from *Vitis sylvestris* Gmel., have breadth/length ratios > 0.7. However the index breadth/length shows a low correlation coefficient with axis 1. In 6 groups 0.7, the limit of the STUMMER's index, is reached (Fig. 3). All groups show values below the limit therefore belonging to cultivars. Seven groups display values above the line 0.7 that may correspond to the wild. Only groups 4 and 7 fall exclusively within the range of cultivars but no one was exclusively within the wild, although most values of groups 8 and 5 are above the limit.

The index breadth/length (STUMMER's index) does not allow to separate wild grapevines and cultivars, although it may help to define the "wild" syndrome. Values above 0.8, however, correspond mainly to extra-European *Vitis* species in our analysis. Therefore, nowadays, wild plants with

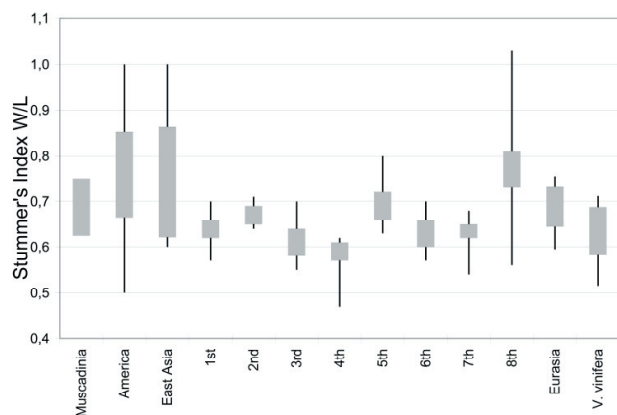


Fig. 3: Variation of STUMMER's index values in different *Vitis* groups. From left to right: 1: *Muscadinia* species; 2: American species of subgenus *Vitis*; 3: East Asia species of subgenus *Vitis*; 4-11: clusters 1 to 8 of this analysis; 12: Eurasian wild grapevine; 13: *Vitis vinifera* cultivars.

STUMMER's index above 0.8 collected in Europe may likely be rootstocks escaped from cultivation. However, in a very low proportion, may correspond to wild relatives of cultivars.

The major problem we have dealt here is finding true *Vitis sylvestris* specimens, because most of the reported wild grapevine populations in riparian forests are composed of feral individuals proceeding from cultivars or rootstocks, sometimes mixed with "true" wild grapevine and widely hybridizing with it. Ferals tend to have smaller berries and plumper pips than their cultivated relatives therefore "looking wild" but the multivariate analysis helps to group ferals and cultivars and therefore to distinguish between ferals and true *Vitis sylvestris*. However those in cluster 5 are closer to the prototype of wild grapevine. Another interpretative approach considering that *V. sylvestris* is polytypic and that the STUMMER's index is valid only for the Danube area or Central Europe and that in the Mediterranean true wild populations are more variable in seed shape and index ratio than in the Danube areas neighboring Vienna.

Geographical origin and clusters: Biogeographical groups of grapevine cultivars have been delimited by TROSHIN *et al.* (1990) and TROSHIN (1999) who followed eco-geographical groups by NEGRUL (1960). We ascribed the different samples to the groups according to the vegetative and reproductive morphology of the cultivars using OIV descriptors (Tab. 2). The Occidentalis group is present in all clusters, predominant in groups 5 (40 %), 7 (41 %), 6 (60 %) and 4 (75 %) and relatively scarce in groups 8 (20 %), 2 (23 %) and 1 (29 %). 'Pontica' cultivars are specially represented in groups 1 (54 %) and 2 (53 %), and less in groups 7 (35 %) and 3 (30 %). Groups 8 and 4 do not include any 'Pontica' cultivar. 'Caspica' cultivars are rare (Tab. 7).

The different cultivars are supposed to have originated in different countries. Tentative attribution of country of origin for each sample is presented in Tab. 2 following ALLEWELDT and DETTWEILER-MÜNCH (1992). Spanish cultivars are predominant in all clusters (53-77 %) except number 8 (Tab. 8). French cultivars are frequent in group 1 (35 %) and group 5 (20 %). This is interesting because some French cultivars show closest similarity to the prototype of wild ancestral grapevine (RIVERA and WALKER 1989).

Cultivars and cultivar groups: Pairwise correspondences were found with samples of 'Airén' (1, 2), 'Merlot' (81, 82, but not 80), 'Viura' (50, 87), 'Pardillo' (= 'Mary Sancho') (75, 76), 'Calop Blanco' (31, 117), 'Beba' (= 'Eva') (46, 65), 'Bobal' and 'Bobal Negro' (21, 24, 20, 152, but not 22, possibly due to intra-varietal heterogeneity), 'Servant' (116, 133), *V. berlandieri* hybrids (12, 15, 11), 'Viura' (70, 88), 'Alphonse Lavallée' (85, 127).

Other cultivars show close resemblance: 'Cabernet Sauvignon' (27, 29), 'Jaén Blanco' (= 'Cagazal') (28, 30), 'Frasco' (50, 51), 'Moscato Giallo' (97, 98), 'Tempranillo' and 'Tempranillo Blanca' (135, 138, but not 136), 'Mazuelo' (10, 78 but not 77).

Related cultivars that fall close or together in the clusters are: (1) 'Malvasía Blanca' (126), 'Parraleta' (107) and 'Malvasía' (134); (2) 'Tempranillo' (29) and 'Tempranillo' ('Cencibel', a common synonym, 36); (3) 'Palomino' (112)

Table 7

Presence of the major eco-geographical groups of grapevine cultivars (*sensu* TROSHIN *et al.* 1990) in the clusters of the multivariate analysis

Group (convar)	Subgroup (subconvar)	1	2	3	4	5	6	7	8	Total
pontica Negr.		17	7	8	0	8	3	6	0	49
occidentalis Negr.		9	3	9	3	14	9	7	2	56
orientalis Negr.	caspiaca Negr.	1	0	1	1	0	1	1	0	5
orientalis Negr.	antasiatica Negr.	2	1	4	0	3	2	0	1	13
wild / feral		1	2	0	0	5	0	3	2	13
American		1	0	0	0	0	0	0	3	4
Eastern Asiatic		0	0	0	0	0	0	0	2	2

Table 8

Geographical origin of the grapevine species and cultivars (*sensu* ALLEWELDT and DETTWEILER-MÜNCH 1992) in the clusters of the multivariate analysis

Country	1	2	3	4	5	6	7	8	Total
Spain	19	10	17	3	19	8	10	1	87
France	11	1	2	0	6	0	2	1	23
Italy	1	2	2	0	3	3	4	0	15
USA	0	0	0	0	0	0	0	5	5
Japan	0	0	0	0	0	0	0	2	2
Portugal	0	0	1	0	2	1	1	0	5
Others	0	0	0	1	0	3	0	1	5

and 'Verdil' (154); (4) 'Barbera' (16) and 'Silvaner Grün' (148); (5) 'Graciano' (61) and 'Navarra' (103), in contrast with CERVERA *et al.* (2000); (6) 'Albillo' (5) and 'Cainho' (32), supporting results of VIDAL *et al.* (1999); (7) 'Loureiro Blanco' (68) and 'Touriga Nacional' (145), supporting results of FERREIRA *et al.* (2000); (8) 'Garnacha Tinta' (70 and 78) and 'Garnacha Peluda' (56).

Thus seed morphology might be useful when studying genetic relationships between cultivars in those cases where DNA analysis is not available, keeping in mind its limits and coupling it with other morphological characters based on leaves and berries.

We have found differences among samples of the same cultivar in the case of 'Albillo' (6 and 7), 'Cencibel' (36 and 38), 'Merlot' (80 vs. 81 and 82), 'Monastrell' (86 and 87), 'Moravia Dulce' (93 and 95), 'Moscatel' (94 and 96), 'Pintaillo' (121 and 122), 'Viura' (69 vs. 70 and 88). This can be due to the existence of strongly different clones of the same cultivar or to error in labeling of the samples. Mislabeling has been confirmed, reviewing voucher specimens, in 'Albillo' (6), 'Cencibel' (38), 'Merlot' (80) and 'Monastrell' (86). This is little surprising for the first three cultivars, since they were sampled in vineyards and mislabeling by non-experts is likely to occur, but it is certainly worrying to find it at a reference collection.

Alternatively when two samples have shown a high similarity a close relationship might be inferred. This is found in 'Cinsaut' (39) and 'Pintaillo' (121), 'Bonicaire' (25) and 'Gamay' (53), 'Chenin Blanc' (44) and 'Sauvignon Blanc' (131) supporting the results of SEFC *et al.* (2000), 'Garnacha Tinta' (60) and 'Semillon' (132), in contrast with SEFC *et al.* (2000), 'Monastrell' ('Mourvedre') (102) and 'Italia' (66), 'Im-

perial Napoleon' ('Don Mariano') (45) and 'Verdejo Blanco' (153), 'Folle Blanche' (48) and 'Godello' (64) in contrast with VIDAL *et al.* (1999), 'Garnacha de San Vicente' (58) and 'Albarín Negro' (3), 'Frasco' (50) and 'Monastrell' (87), 'Moravia Dulce' (93) and 'Vermentino' (155), 'Frasco' (51) and 'Listán Negro' (67), 'Tinta Grossa' (142) and 'Valenci Negro' (151), 'Flame Tokay' (47) and 'Pedro Ximénez' (115), 'Moscato Giallo' (98) and 'Königin der Weingärten' (123), 'Moravia Agria' (91) and 'Parellada' (106), 'Viura' (69) and 'Sangiovese' (130), 'Malvar' (72) and 'Pinot Noir' (120) in contrast with SEFC *et al.* (2000). This merits further investigation in order to determine the coincidences that might point to a common ancestry.

Wild, escaped and feral grapevines:

The existence of feral individuals (meaning escaped from cultivation) in natural habitats can explain the close relationship found between 'Albillo' (7) and a feral from 'Borosa' ('Jaén') (157), 'Moscato Giallo' (99) and a feral from 'Borosa' (158), 'Negramoll' (104) and a feral from 'Borosa' (160), 'Muscat of Alexandria' (101) and *V. sylvestris* from the Siena Botanic Garden (Italy) (165), 'Pearl of Csaba' (118) and a feral from Santa Cruz de Campezo ('Alava') (171) and other from 'Borosa' (170), 'Merseguera' (83) and a feral from Roncal Valley ('Navarra') (168), 'Carignan Noir' ('Mazuelo') (77) and a feral from Roncal Valley ('Navarra') (167), 'Tempranillo' ('Tinto Róriz') (143) and a feral from 'Borosa' (162), 'Torrónés' (146) and a feral from 'Rioja' ('Tinta Asilvestrada') (141), 'Garnacha Tinta' (78) and a feral from 'Abengibre' (10). Assuming that wild grapevine follows exclusively the prototype of *Vitis sylvestris*, considering the "cultigen" features of the individuals sampled, and the heterogeneity of the populations, the direct relationship between lambrusques and cultivars led us to suppose these are descendants of cultivars instead of the reverse. FORNECK *et al.* (2003) interpreted the coincidence of one accession of wild grapevine from Turkey with 'Cabernet Sauvignon' as indicating hybridization. This led us to recognize that the samples of wild grapevines found nowadays in the Iberian Peninsula and Italy may not all be descendants of the prototypic *Vitis sylvestris* but also from sub-spontaneous (feral) individuals escaped from cultivation, including both cultivars and American species used as rootstocks, which colonize natural and secondary habitats.

However, the hybrid complex population of lambrusques in the Borosa (upper Guadalquivir) shows a high diversity of seed types, included in groups 2, 5 and 7, of the

analysis. Some show close similarities with cultivars that, most likely, were never cultivated in this area. Therefore why this coincidence? CARREÑO *et al.* (2004) reported this kind of long-distance coincidence using *SSR* analysis, where wild grapevines from 'Navarra' and 'Guipuzcoa' displayed higher genetic similarity with cv. 'Jaén', from E Spain, than with local cultivars ('Calagraño', 'Garnacha' or 'Tempranillo') of the upper Ebro River basin.

An alternative hypothesis is the presence of deviating populations of *Vitis sylvestris*. The analysis of chloroplast DNA polymorphisms and *cpSSR* in cultivated grapevine led IMAZIO *et al.* (2006) and ARROYO-GARCÍA *et al.* (2006) to suggest the existence of at least two different origins for the cultivated germplasm, especially for the Iberian Peninsula cultivars. Therefore the existence of a wild Iberian grapevine genetically and morphologically distinct from the Danube and Rhine *Vitis sylvestris* prototype and from the Near Eastern populations is possible, thus with seeds deviating of the seemingly wild type.

Conclusions

Seeds show a wide range of variation in body dimensions 3.7-8.1 x 1.8-5.2 x 1-3.7 mm, and in other parameters. The total length of seeds is an excellent character, however, the distribution of frequencies for the seed characters does not allow a clustering of cultivars using each of the parameters separately. The combined intervention of all characters allows the clustering of cultivars in two large clusters.

Cluster I includes large, long and wide seeds, which present a large and prominent beak. This corresponds in part with the slender, elongate, seed group reported by TERPÓ (1977) that comprised obovate and elliptical seeds. Cluster II corresponds with the rounded seed group reported by Terpó (1977).

Only groups 8 and 5 (Cluster II) fall within the range for wild grapevine seed length of TERPÓ (1976), however, they include different cultivars together with seemingly wild samples. The smaller seeds belong to Extra-European species.

We have not found correlation between type of fossettes and clusters resulting from the multivariate analysis. We have not found a high correlation between the clusters and the groups of cultivars or "proles" in the sense of NEGRUL (1946 a).

The index breadth/length (STUMMER'S index) does not allow to separate wild grapevines and cultivars, although may help to define the "wild" syndrome. Values above 0,8 correspond to Extra-European *Vitis species* in our analysis. Lambrusques in LEVADOUX' (1956) terms, tend to display smaller berries and plumper pips than their cultivated relatives therefore "looking wild" but the multivariate analysis tend to place ferals/wild and cultivars together. Thus we suggest setting aside this criterion to identify samples collected in the wild as *Vitis sylvestris*.

Likewise, this work cast some doubts on the use of the Stummer's index in grapevine seed analysis in archaeology to separate cultivars and wild individuals. However, further multivariate analysis of seed morphology in archaeological

samples might determine synchronic patterns of variation, but also in a diachronic perspective. A wider sampling, including more cultivars from the eastern Mediterranean, the Caucasus and Asia might set light to part of the questions raised in this work.

Seed morphology thus might prove to be useful when studying relationships between species and cultivars, keeping in mind its limits, and coupling it with other morphological characters based on leaves and berries and the molecular evidence.

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References

- ALLEWELDT, G.; DETTWEILER-MÜNCH, E.; 1992: The Genetic Resources of *Vitis*. Genetic and Geographic Origin of Grapevine Cultivars, their Prime Names and Synonyms. 3rd Ed. Institut für Rebenzüchtung. Geilweilerhof.
- ANDRASOVSKY, J.; 1915: A szőlőmagvak diagnosztikus értéke (Der diagnostische Wert der Traubensamen). Borászati Lapok 47, 39.
- ANDRASOVSKY, J.; 1917: A szőlőmagvak diagnosztikus értéke (Der diagnostische Wert der Traubensamen). Ampel. Int. Évk. 6, 49-59.
- ANDRASOVSKY, J.; 1925: Szőlő. In: S. JAVORKA (Ed.): Magyar Flora, 701-708.
- ARROYO-GARCÍA, R.; RUIZ-GARCÍA, L.; BOLLING, L.; OCETE, R.; LÓPEZ, M. A.; ARNOLD, C.; ERGUL, A.; SÖYLEMEZO, G.; LU, H.; UZUN, H.; CABELLO, I.; IBÁÑEZ, J.; ARADHYA, M.; ATANASSOV, A.; ATANASSOV, I.; BALINT, S.; CENIS, J.; COSTANTINI, L.; GORISLAVETS, S.; GRANDO, M.; KLEIN, B.; MCGOVERN, P. E.; MERDINOGLU, D.; PEJIC, F.; PELS, F.; PRIMIKIROS, N.; RISOVANNAYA, V.; ROUBELAKIS-ANGELAKIS, K.; SNOUSSI, H.; SOTIRI, P.; TAMHANKAR, S.; THIS, P.; TROSHIN, I.; MALPICA, J.; LEFORT, F.; MARTÍNEZ-ZAPATER, J.; 2006: Multiple origins of cultivated grapevine (*Vitis vinifera* L. ssp. *sativa*) based on chloroplast DNA polymorphisms. Mol. Ecol. 15, 3707-3714.
- BUXÓ, R.; 1997: Arqueología de las Plantas. Ed. Crítica, Barcelona.
- CARREÑO, E.; LOPEZ, M. A.; LABRA, M.; RIVERA, D.; SANCHA, J.; OCETE, R.; MARTÍNEZ DE TODA, F.; 2004: Genetic relationship between some Spanish *Vitis vinifera* L. subsp. *sativa* cultivars and wild grapevine populations (*Vitis vinifera* L. subsp. *sylvestris* (Gmelin) Hegi): A preliminary study. Plant Genet. Res. Newslett. 137, 1-4.
- CERVERA, M. T.; CABEZAS, J. A.; SÁNCHEZ-ESCRIBANO, E.; CENIS, J. L.; MARTÍNEZ-ZAPATER, J. M.; 2000: Genetic identification of table grape cultivars (*Vitis vinifera* L.) based on AFLP markers. Vitis 39, 109-114.
- CLEMENTE, S. DE R.; 1807: Ensayo sobre las Variedades de la Vid Común que Vegetan en Andalucía. Villalpando, Madrid.
- CLEMENTE, S. DE R.; 1814: Essai sur les Variétés de la Vigne qui Végètent en Andalousie. Poulet, Paris.
- CLEMENTE, S. DE R.; 1879: Ensayo sobre las Variedades de la Vid Común que Vegetan en Andalucía. Estereotipia Perojo, Madrid.
- DELWEN, S.; 2003: International Work Group for Palaeoethnobotany (online). <http://www.socarchsci.org/9809n.htm>

- EBADI, A.; SEDGLEY, M.; MAY, P.; COOMBE, B. G.; 1996: Seed development and abortion in *Vitis vinifera* L. cv. Chardonnay. *Int. J. Plant Sci.* **157**, 703-712.
- ENGELMANN, G.; 1875: Synopsis of American Grapevines. *Bushberg Catalogue* **1875**, 4-11.
- FACSAR, G.; 1970: Habitus studies on seeds of *Vitis vinifera* L. sorts. *Acta Agron. Acad. Sci. Hungaricae* **19**, 403-6.
- FACSAR, G.; 1972 a: Makroszkopikus Szerkezeti Vizsgálatok *Vitis vinifera* L. Fajták Érő Bogyóin. *Kertész eseti Egyetem Növénytani Tanszéke* **35**, 147-158.
- FACSAR, G.; 1972 b: A Kerti Szőlő (*Vitis vinifera* L.) Fajtáinak Magtípus rendszere. *A Kertész eseti Egyetem Növénytani Tanszéke* **35**, 191-216.
- FACSAR, G.; 1975: Agricultural-botanical analysis of the medieval grape seeds from the Buda castle hill. *Mitt. Archäolog. Inst. Ungar. Akad. Wiss.* **4**, 157-173.
- FERREIRA MONTEIRO, F.; NIÑEZ, E.; MAGALHAES, R.; MARTINS, A.; BOWERS, J. E.; MEREDITH, C. P.; 2000: Fingerprinting of the main *Vitis vinifera* cultivars grown in the northern region of Portugal. *Acta Hort.* **528**, 121-127.
- FORNECK, A.; WALKER M.; SCHREIBER, A.; BLAICH, R.; SCHUMANN, F.; 2003: Genetic Diversity in *Vitis vinifera* ssp. *sylvestris* Gmelin from Europe, the Middle East and North Africa. *ISHS Acta Hort.* **603**, 549-552.
- GALET, P.; 1970: Rapport sur la viticulture en Afghanistan. *Vitis* **9**, 15-46.
- GALET, P.; 1988: Cépages et Vignobles de France. Tome I. Les Vignes Américaines. Ed. Déhan. Montpellier.
- GALET, P.; 1990: Cépages et Vignobles de France. Tome II. L' Ampelographie Française. Ed. Déhan. Montpellier.
- GENRES; 2003: International Variety Catalogue. www.genres.de/idb/vitis.
- GRAY, A.; FERNALD, M. L.; 1989: Gray's Manual of Botany: A Handbook of The Flowering Plants and Ferns of The Central and Northeastern United States and Adjacent Canada. Dioscorides Press, Portland, OR (Reprint of the 8th ed., 1950).
- IHAKA, R.; GENTLEMANT, R.; 1997: The R Project for Statistical Computing. Department of Statistic of the University of Auckland in New Zealand. Revised by "R Core Team". <http://www.r-project.org/>
- IMAZIO, S.; LABRA, M.; GRASSI, F.; SCIENZA, A.; FAILLA, O.; 2006: Chloroplast microsatellites to investigate the origin of grapevine. *Genet. Res. Crop Evol.* **53**, 1003-1011.
- JONES, G.; LEGGE, A.; 1987: The grape (*Vitis vinifera* L.) in the Neolithic of Britain. *Antiquity* **61**, 452-455.
- KOLENATI, F. A.; 1846: Versuch einer systematischen Anordnung der in Grusinien einheimischen Reben nebst einem oekonomisch-technischen Anhang. *Bull. Soc. Sci. Nat. Moscou* **19**, 283-371.
- KROCHMAL, A.; NAWABI, A.; 1961: A descriptive study of the grapes of Afghanistan. *Vitis* **2**, 241-256.
- LAGUNA, E.; 2003 a: Sobre las formas naturalizadas de *Vitis* L. (Vitaceae) en la Comunidad Valenciana. I. Especies. *Flora Montiberica* **23**, 46-82.
- LAGUNA, E.; 2003 b: Datos sobre la producción de fruto de las especies e híbridos invasores de vides (*Vitis* L.). *Toll Negre* **2**, 10-15.
- LAGUNA, E.; 2004: Datos foliares de las especies e híbridos aloctonos de vides (género *Vitis*) en el territorio valenciano. *Toll Negre* **3**, 11-25.
- LEBART, L.; MORINEAU, A.; 1984: Multivariate Descriptive Statistical Analysis, Correspondence Analysis and Related. Ed. Wiley, New York.
- LEBART, L.; MORINEAU, A.; 1985: SPAD, Systeme Portable pour l'Analyse des Donnees. CESIA, Paris.
- LEVADOUX, L.; 1948: Les cépages à raisins de cuve. *Bull. O I V (Off. Int. Vigne Vin)* **21**, 39-45.
- LEVADOUX, L.; 1956: Les populations sauvages et cultivées de *Vitis vinifera* L. *Ann. l'Amel. Plantes* **1**, 59-118.
- MUNSON, T. V.; 1909: Foundations of American Grape Culture. TV Munson and Son, Denison, Texas.
- MURTAGH, F.; 1985: Multidimensional Clustering Algorithms, COMPOSTAT Lectures 4. Physica-Verlag, Würzburg.
- MURTAGH, F.; HECK, A.; 1987: Multivariate Data Analysis. D. Reidel Publishing Company, Dordrecht.
- NEGRUL, A.; 1946 a: Proischozdenie kulturnogo vinograda i ego klassifikacia. In: A. VARANOV (Ed.): *Ampleografija SSSR* **1**, 159-212.
- NEGRUL, A.; 1946 b: Semejstvo Vitaceae Lindley. In: A. VARANOV (Ed.): *Ampleografija SSSR* **1**, 45-117.
- NEGRUL, A.; 1960: Evolucija razmera semjan i jagod u vinograda. *Izvaet. TSHA* **2**, 167-176.
- OCETE, R.; LOPEZ M. A.; PEREZ, M. A.; 1999: Las poblaciones españolas de vid silvestre, Monografías INIA, Agrícola nº 3. INIA, Ministerio de Agricultura, Pesca y Alimentación, Madrid.
- OIV; 1983: Código de los Caracteres Descriptivos de las Variedades y Especies de *Vitis*. Ed. A. Dedon, Paris.
- PALAZÓN J. A.; CALVO, J. F.; 1999: Métodos cuantitativos (online). http://www.um.es/docencia/emc/datos/funciones.R_ (accessed 05/17/07).
- PLANCHON, J. E.; 1887: Ampelideae, Monographie des Ampéidées Vraies. In: A. P. DE CANDOLLE (Ed.): *Monographiae Phanerogamarum* **5**, 305-368. Treuttel et Würtz, Paris.
- POTEBNJA, A.; 1911: Semena evropejskich sortov vinograda i ich znacenie dl'a klassifikacii. *Trudy Bjuro Prikladnoj Botanike St. Petersburg* **3**, 147-158.
- REHDER, A.; 1990: Manual of Cultivated Trees and Shrubs Hardy in North America. Second Edition. Dioscorides Press, Portland, OR.
- REYNIER, A.; 1995: Manual de Viticultura. Ed. Mundi-Prensa, Madrid.
- RIVERA, D.; WALKER, M.; 1989: A review of palaeobotanical findings of early *Vitis* in the Mediterranean and of the origins of cultivated grapevines with special reference to new pointers to prehistoric exploitation in the Western Mediterranean. *Rev. Palaeobot. Palynol.* **61**, 205-237.
- SCHIEHMANN, E.; 1953: *Vitis* im Neolithicum der Mark Brandenburg. *Züchter* **23**, 318-327.
- SEFC, K. M.; LOPES, M. S.; LEFORT, F.; BOTTA R.; ROUBELAKIS-ANGELAKIS, K. A.; IBANEZ, J.; PEJIC, I.; WAGNER, H. W.; GLÖSSL, J.; STEINKELLNER, H.; 2000: Microsatellite variability in grapevine cultivars from different European regions and evaluation of assignment testing to assess the geographic origin of cultivars. *Theor. Appl. Genet.* **100**, 498-505.
- SMITH, H.; JONES, G.; 1990: Experiments on the effects of charring on cultivated grape seeds. *J. Archaeol. Sci.* **17**, 317-27.
- STUMMER, A.; 1911: Zur Urgeschichte der Rebe und des Weinbaues. *Mitt. Anthropol. Gesellschaft Wien* **41**, 283-296.
- TERPÓ, A.; 1976: The carpological examination of wild-growing vine species of Hungary. I. *Acta Bot. Acad. Sci. Hungaria* **22**, 209-247.
- TERPÓ, A.; 1977: The carpological examination of wild-growing vine species of Hungary. II. Qualitative and quantitative characteristics of vine seeds. *Acta Bot. Acad. Sci. Hungaria* **23**, 247-73.
- TROSHIN, L.; 1999: Ampelografía i Seleksia Vinograda. Volinie Mastera, Krasnodar.
- TROSHIN, L.; NEDOV, P.; LITVAK, A.; GUZUN, N.; 1990: Improvement of *Vitis vinifera sativa* DC. taxonomy. *Vitis (Special Issue)*, 37-43. *Proc. 5th Intern. Symp. Grape Breed.*, 1989.
- VIALA, P.; PÉCHOUTRE, P.; 1910: Morphologie externe de la graine In: P. VIALA, V. VERMOREL (Eds): *Ampélographie* **1**, 156-166. Masson et Cie., Paris.
- VIDAL, J. R.; MORENO, S.; GOGORCENA, Y.; MASA, A.; ORTIZ, M.; 1999: On the genetic relationships and origins of six grape cultivars of Galicia (Spain) using RAPD markers. *Am. J. Enol. Vitic.* **50**, 69-75.
- WARD JR., J. H.; 1963: Hierarchical clustering to optimize and objective function. *J. Am. Statistical Ass.* **58**, 236-244.
- WEBB, D. A.; 1968: Vitaceae. In: T. G. TUTIN *et al.* (Eds.): *Flora Europaea* **2**, 246-247. Cambridge University Press, Cambridge.
- ZOHARY, D.; HOPF, M.; 1994: Domestication of Plants in the Old World. The Origin and Spread of Cultivated Plants in West, Asia, Europe and the Nile Valley. Clarendon Press, Oxford.

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