

Seed Morphology of *Vitis vinifera* and Its Relationship to Ecogeographical Groups and Chlorotypes

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Abstract

Grapevine (*Vitis vinifera* L.) comprises over 10,000 different cultivars and cultivar groups. These show morphological diversity and genetic polymorphism that follow geographical gradients W to E and N to S. This has led many authors to conclude that grapevine is polyphyletic.

Grapevine biogeographical and ecogeographical patterns of variation were first described by Negrul in terms of “Proles”, which were characterized by the type of indumentum on leaves and shoots, and grape shape as main descriptors. Recent studies have shown the existence of eight different chlorotypes (seven in cultivars and wild grapevines and one exclusively in wild grapevines) or six haplotypes. We compare the geographical patterns and relationships of chlorotypes/haplotypes with proles and morphological descriptors. We studied the indumentum type in nearly 1,000 grapevine cultivars and wild grapevine populations extending from Western Europe and North Africa to Central Asia. Chlorotypes and ecogeographical groups show similar patterns of variation in a transect W to E. However, low correlation was found between chlorotypes and ecogeographical groups, e.g. chlorotype A (characteristic of many cultivars from Spain) is mainly found in accessions of the Proles *Occidentalis* (with arachnoid indumentum on the abaxial surface of adult leaves). However, cultivars morphologically belonging to Proles *Orientalis* Subproles *Caspica* (with erect hairs) and Proles *Pontica* (with mixed indumentum) also show chlorotype A. Alternative approaches for interpreting this divergence are discussed in this paper.

INTRODUCTION

There have been several different approaches to elucidating relationships between wild and cultivated Eurasian grapevines, and various theories: monophyletic, polyphyletic-multispecific and hybrid theories offer conflicting interpretations for grapevine diversity (Fig. 1). In the monophyletic- monospecific theory proposed by De Candolle, Engler, Hegi, Planchon, Negrul and Baranov (Rivera et al., 2004) local populations of cultivated grapevine descend from local wild populations, both being conspecific. In the monophyletic-bispecific theory, populations of cultivated grapevine have a common extinct ancestor, which might also be the ancestor of wild grapevine; they are thus two different species. Occasional hybridization may have produced some cultivars or cultivar groups as proposed by Khorszhinzkii (1910) and Sosnovszky (1949, 1974). According to the polyphyletic- multispecific theory the different local populations of cultivated grapevine descend from different independent wild ancestors, extinct or not. Thus, each cultivated grapevine species has a corresponding set of wild relatives, with primary species producing new cultivar groups through hybridization (Andrasovszky, 1925; Vassylichenko, 1970). Finally, the hybrid theory proposes hybridization of wild European and Central Asiatic grapevine species in the origin of cultivated grapevine (Terpó, 1978). Independently of these theories, all authors recognize some regular geographical patterns within the complex of cultivars.

Biogeographical groups of grapevine cultivars were delimited by Troshin et al. (1990) and Troshin (1999), which followed previous approaches by Negrul (1938, 1946) in terms of ecogeographical groups (Table 1, Fig. 2).

Chlorotype distribution in populations of *Vitis sylvestris* C.C. Gmel (synonym

Vitis vinifera L. subsp. *sylvestris* (C.C. Gmel.) Hegi) and *V. vinifera* L. subsp. *sativa* was analyzed by Imazio et al. (2006) and Arroyo et al. (2006). Their frequencies follow a geographical pattern (Table 2, Fig. 3). The putative occurrence of western and eastern domestication events is, according to Arroyo et al. (2006), consistent with the morphotype classification of cultivated grapes proposed by Negrul (1938, 1946).

Rivera and Walker (1989) proposed, using archaeological and biogeographical data, the theory of independent domestication events. The independent origin of the ecogeographical groups does not necessarily mean that cultivation of wild grapevine started simultaneously in different zones in the area of the present *Vitis vinifera* complex. It presumably occurred during different periods and in distant localities, where local wild plants with particular genotypes were taken into cultivation and domesticated in such a way that these genotypes were inherited by their descendants and persisted in terms of particular features in cultivated populations. This assumes the previous existence of regionally differentiated specific or infraspecific taxa. These may correspond to the differentiation into:

- Eastern populations of Central Asia and eastern Caucasus (*V. trichophylla*, *V. nuristanica* Vassilcz., *V. caucasica*)
- Pontic populations of the Balkans and the Black Sea (*V. vinifera* subsp. *balcanica*)
- Western and central European (*V. sylvestris*).

Grapevine cultivation led to a wide germplasm exchange throughout the main area in which grapes are grown for wine making, as a fruit or for raisin production. Therefore geographical limits became disguised and, usually, when looking for the occurrence of *Vitis* types in a certain place, we are going to find a more or less heterogeneous mixture of the different main geographical groups, although with one of these more or less clearly dominant.

MATERIAL AND METHODS

For seed morphology we studied 142 grapevine seed samples belonging to five *Vitis* species, 92 cultivars of *V. vinifera*, 12 feral/wild populations ('lambusques' in Levadoux (1956)) and four hybrid rootstocks. The seeds were deposited in two repositories in Spain: CIDA (Centro de Investigación y Desarrollo Agrario) of La Rioja at Mendavia (Navarra) and Casa de las Vides/CAPA (Conselleria de Agricultura Pesca y Alimentación) at Agullent (Valencia). Seeds of wild species also came from botanical gardens, or were collected in wild populations. The samples with their names, type, biogeographical group, code, country of origin and the repository or place of deposition are listed in Rivera et al. (2007).

For leaf and shoot indumentum (as markers of grapevine ecogeographical groups, particularly the Office International de la Vigne et du Vin (OIV) characters 84 and 85 (OIV, 1984)) we studied 822 cultivars from the Institut für Rebenzüchtung Geilweilerhof, the CIDA collections of La Rioja (Spain), the Istituto Agrario San Michelle all'Adige (Italy), and Casa de las Vides collection in Agullent (Valencia). The descriptors used followed OIV (1984) and GENRES (2006).

Chlorotypes/haplotypes were putatively ascribed to each cultivar according to the lists of Imazio et al. (2006) or the complementary data of Arroyo et al. (2006). We assumed that chlorotype and ecogeographical features were constant for all accessions of the same cultivar.

RESULTS

Working with seed morphology, Rivera et al. (2007) ascribed the different samples to biogeographical groups according to the vegetative and reproductive morphology of the cultivars using OIV (1984) descriptors. The Occidentalis group is present in all seed 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 well represented in groups 1 (54%) and 2 (53%), and less in groups 7 (35%) and 3 (30%). Group 8, and 4 do not include any Pontica cultivars. Caspica cultivars are rare and more

or less uniformly distributed amongst all groups (Table 3). Thus, seed morphology does not seem to support the ecogeographical groups.

We have also found no correlation between the seed clusters and chlorotypes (Table 4), i.e. cultivars with chlorotype A present a wide range of seed morphologies.

All ecogeographical groups (Proles) are widespread and are present within most of the area of *Vitis vinifera*. Only relative frequencies of groups indicate geographical differences (Fig. 2), with Proles *Occidentalis* linked to the western part of the area. Chlorotypes show partial geographical patterns. Cultivars with chlorotype A are highly abundant in Western Europe while they were not observed in Eastern Mediterranean and Central Asian samples. Similarly, haplotypes 6 and 3 are frequent in accessions from Western Europe. Chlorotypes C and D, which are very common among Near and Middle Eastern cultivars, are less frequent among Iberian Peninsula cultivars. Haplotype 1 is strongly represented in Caucasian accessions.

Only minor partial correlations are found between chlorotypes and major-ecogeographical groups, e.g. 60% of *Occidentalis* cultivars (Table 5) present chlorotype A, and 46 % of cultivars with chlorotype A are ascribed to Proles *Occidentalis*. Furthermore, 65% of *Occidentalis* accessions (Table 6) present haplotype 6 and 39% of accessions with haplotype 6 are ascribed to Proles *Occidentalis*.

However, Proles *Pontica*, which accounts for over 50% of all grapevine cultivars (Table 7), shows the whole range of chlorotypes and haplotypes (Tables 5 and 6).

DISCUSSION

Subgenus *Vitis* comprises 10 sections and 60 species in America and Asia that can hybridize with Eurasian grapevine, therefore specific limits are obscure. At present, over 10,000 cultivars of Eurasian grapevine are recorded. Most are of either recent or ancient hybrid origin.

Seed morphology is related to taxonomy, geography and selection for main use, but cannot be used definitively to determine wild and cultivated grapevine and is related only partially with the ecogeographical groups.

Ecogeographical groups, such as the Proles *sensu* Negrul, are helpful for rationally organizing diversity within cultivated grapevine. However, all groups are widespread and are present within most of the area of *Vitis vinifera*. Only the relative frequency of groups indicates geographical differences.

Molecular techniques contribute to determining grapevine phylogeny and can be applied to the study of particularly well preserved archaeological materials. Molecular data challenge the accepted systematics of *Vitis* and particularly the ecogeographical groups within *V. vinifera*. Combined analysis of classical descriptors and molecular markers will help to determine the validity of ecogeographical groups and discriminate between the alternative hypotheses for grapevine origin and systematics. However, if chlorotypes/ haplotypes are good geographical markers, proles are not.

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Tables

Table 1. Major ecogeographical groups of *Vitis vinifera* cultivars (Troshin et al., 1990; Troshin, 1999).

| Group (Convar) | Subgroup (Subconvar) | Main area and status | Examples |
|------------------------------|---|--|--|
| pontica Negr. | georgica Negr. | Georgian Black Sea Basin | Saperavi, Pletchiski |
| pontica Negr. | balcanica Negr. | Northern part of the Balkans | Alimshak, Plavai, Furmint |
| pontica Negr. | meridionali-balcanica Trosh | Crimea, Don and Kuban regions, Greece, Albania | Limberger, Kadarka, Kabassia |
| pontica Negr. | georgica-caspica Gram. | Georgian Black Sea Basin | Rkatsiteli, Goroula, Sirgoula, Tchinouri |
| occidentalis Negr. | gallica Nem. | Northern part of France | Aligoté, Merlot, Cabernet Sauvignon, Chardonnay, Sylvaner, Sémillon, Riesling |
| occidentalis Negr. | pyrenaica Gram. | Spain, Portugal and the southern part of France | Bicane, Vermentino, Gros Vert, Mourvedre, Morastel, Sersial, Gouveia, Ugni Blanc, Verdejo, Touriga |
| orientalis Negr. | caspica Negr. var. trans-caucasica Gram. & Trosh. | Trans Caucasia | Arenii Tchernii, Baian Shirei, Tavkeri |
| orientalis Negr. | caspica Negr. var. medii-asica Gram. & Trosh. | Central Asia | Terbash, Rasmi, Bishti |
| orientalis Negr. | antasiatica Negr. var. trans-caucasica Gram. & Trosh. | Trans Caucasia | Ag Iuzium, Ararati, Shabash |
| orientalis Negr. | antasiatica Negr. var. medii-asica Gram. & Trosh. | Central Asia | Katta Kurgan, Nimrang, Taifi Rosovii, Halili Belii |
| boreali-africana Gram | - | Morocco, Algeria, Tunisia | Ahmar bou Ahmar, Farrana |
| orientali-mediterranea Gram. | - | Eastern Mediterranean, earlier origin, primitive | Muscat flavoured are common, Muscadine, Chasselas |

Table 2. Frequency of the grapevine haplotypes / chlorotypes (**a**: sensu Imazio et al., 2006; **b**: sensu Arroyo et al., 2006) in the major geographical zones. Chlor.= Chlorotype.

a

| | Haplotype 1 | Haplotype 2 | Haplotype 3 | Haplotype 4 | Haplotype 5 | Haplotype 6 | Totals |
|-----------------------|-------------|-------------|-------------|-------------|-------------|-------------|--------|
| Central Asia | 1 | 1 | 0 | 0 | 0 | 0 | 2 |
| Caucasus | 25 | 0 | 0 | 1 | 0 | 0 | 26 |
| Eastern Mediterranean | 7 | 5 | 1 | 4 | 0 | 1 | 18 |
| Eastern Europe | 2 | 1 | 3 | 1 | 0 | 2 | 9 |
| Central Europe | 7 | 11 | 3 | 3 | 1 | 7 | 32 |
| Western Europe | 9 | 4 | 6 | 3 | 4 | 28 | 54 |
| North Africa | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | 51 | 22 | 13 | 12 | 5 | 38 | 141 |

b

| | Chlor. A | Chlor. B | Chlor. C | Chlor. D | Chlor. E | Chlor. F | Chlor. G | Totals |
|-----------------------|----------|----------|----------|----------|----------|----------|----------|--------|
| Central Asia | 0 | 5 | 36 | 19 | 0 | 0 | 0 | 60 |
| Caucasus | - | - | - | - | - | - | - | - |
| Eastern Mediterranean | 9 | 8 | 61 | 62 | 1 | 0 | 0 | 141 |
| Eastern Europe | 8 | 27 | 6 | 36 | 0 | 0 | 0 | 77 |
| Central Europe | 15 | 1 | 7 | 39 | 6 | 0 | 0 | 68 |
| Western Europe | 50 | 2 | 25 | 18 | 0 | 0 | 3 | 98 |
| North Africa | 7 | 7 | 13 | 7 | 0 | 0 | 0 | 34 |
| Totals | 89 | 50 | 148 | 181 | 7 | 0 | 3 | 478 |

Table 3. Presence of the major ecogeographical groups of grapevine cultivars (sensu Troshin et al., 1990) in the eight clusters of the seed multivariate analysis (Rivera et al., 2007).

| Group (Convar) | Subgroup (Subconvar) | I1 | I2 | I3 | I4 | I6 | II5 | II7 | II8 | Total |
|--------------------|----------------------|----|----|----|----|----|-----|-----|-----|-------|
| pontica Negr. | | 17 | 7 | 8 | 0 | 3 | 8 | 6 | 0 | 49 |
| occidentalis Negr. | | 9 | 3 | 9 | 3 | 9 | 14 | 7 | 2 | 56 |
| orientalis Negr. | caspica Negr. | 1 | 0 | 1 | 1 | 1 | 0 | 1 | 0 | 5 |
| orientalis Negr. | antasiatica Negr. | 2 | 1 | 4 | 0 | 2 | 3 | 0 | 1 | 13 |
| wild / feral | | 1 | 2 | 0 | 0 | 0 | 5 | 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 4. Presence of the grapevine chlorotypes (sensu Arroyo et al., 2006) in the eight clusters of the seed multivariate analysis (Rivera et al., 2007).

| | I1 | I2 | I3 | I4 | I6 | II5 | II7 | II8 | Totals |
|--------------|----|----|----|----|----|-----|-----|-----|--------|
| Chlorotype A | 8 | 4 | 8 | 2 | 3 | 9 | 1 | 0 | 35 |
| Chlorotype B | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Chlorotype C | 2 | 0 | 2 | 1 | 0 | 3 | 1 | 0 | 9 |
| Chlorotype D | 6 | 0 | 1 | 0 | 0 | 2 | 3 | 0 | 12 |
| Chlorotype E | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| Totals | 16 | 4 | 11 | 3 | 3 | 14 | 5 | 0 | 56 |

Table 5. Presence of the grapevine chlorotypes (sensu Arroyo et al., 2006) in the major ecogeographical groups of grapevine cultivars (simplified from Troshin et al., 1990). Within Glabrous are those cultivars ascribed to subconvar. Antasiatica and others with very low trichomes density.

| | Glabrous | Occid. | Caspica | Pontica | Totals |
|--------------|----------|--------|---------|---------|--------|
| Chlorotype A | 8 | 26 | 4 | 19 | 57 |
| Chlorotype B | 1 | 1 | 1 | 6 | 9 |
| Chlorotype C | 7 | 10 | 2 | 33 | 52 |
| Chlorotype D | 14 | 6 | 1 | 33 | 54 |
| Chlorotype E | 0 | 0 | 0 | 6 | 6 |
| Chlorotype G | 1 | 0 | 0 | 1 | 2 |
| Totals | 31 | 43 | 8 | 98 | 180 |

Table 6. Presence of the grapevine haplotypes (sensu Imazio et al., 2006), in the major ecogeographical groups of grapevine cultivars (simplified from Troshin et al., 1990). Within Glabrous are those cultivars ascribed to subconvar. Antasiatica and others with very low trichomes density.

| | Glabrous | Occid. | Caspica | Pontica | Totals |
|-------------|----------|--------|---------|---------|--------|
| Haplotype 1 | 2 | 2 | 1 | 16 | 21 |
| Haplotype 2 | 2 | 1 | 0 | 5 | 8 |
| Haplotype 3 | 3 | 2 | 1 | 5 | 11 |
| Haplotype 4 | 0 | 1 | 0 | 4 | 5 |
| Haplotype 5 | 0 | 2 | 1 | 3 | 6 |
| Haplotype 6 | 4 | 15 | 4 | 16 | 39 |
| Totals | 11 | 23 | 7 | 49 | 90 |

Table 7. Relative numbers of cultivars ascribed to each major ecogeographical group. Elaborated using data from the GENRES (2006) database.

| Ecogeographical group | Percentage of grapevine cultivars |
|---|-----------------------------------|
| Proles Occidentalis | 25% |
| Proles Pontica (sp. Balcanica & sp. Georgica) | 58% |
| Proles Orientalis sp. Caspica | 5% |
| Glabrous | 12% |

Figures

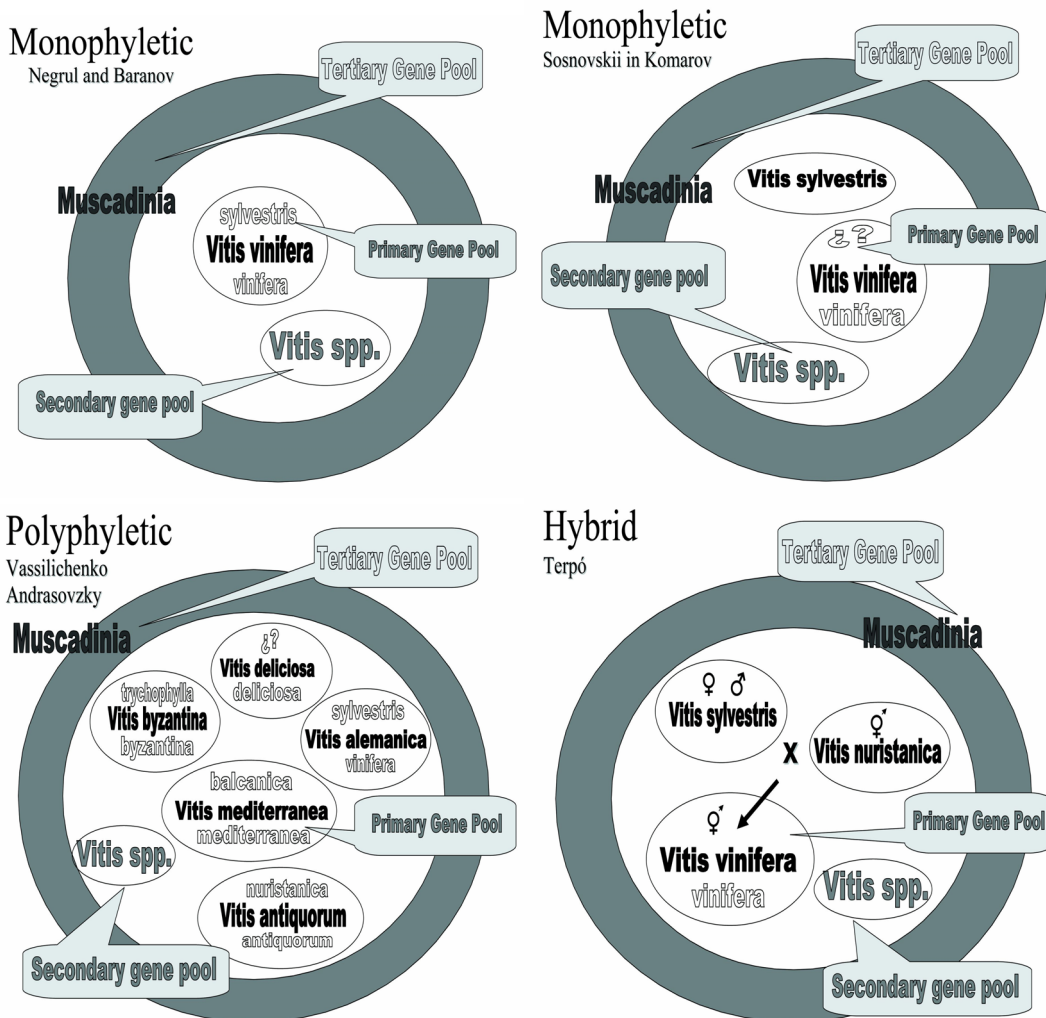


Fig. 1. Major conflicting interpretations of phylogeny and systematics of grapevine cultivars (*Vitis vinifera* L.): monophyletic monospecific or bispecific, polyphyletic multispecific and hybrid multispecific (from Rivera et al., 2004).

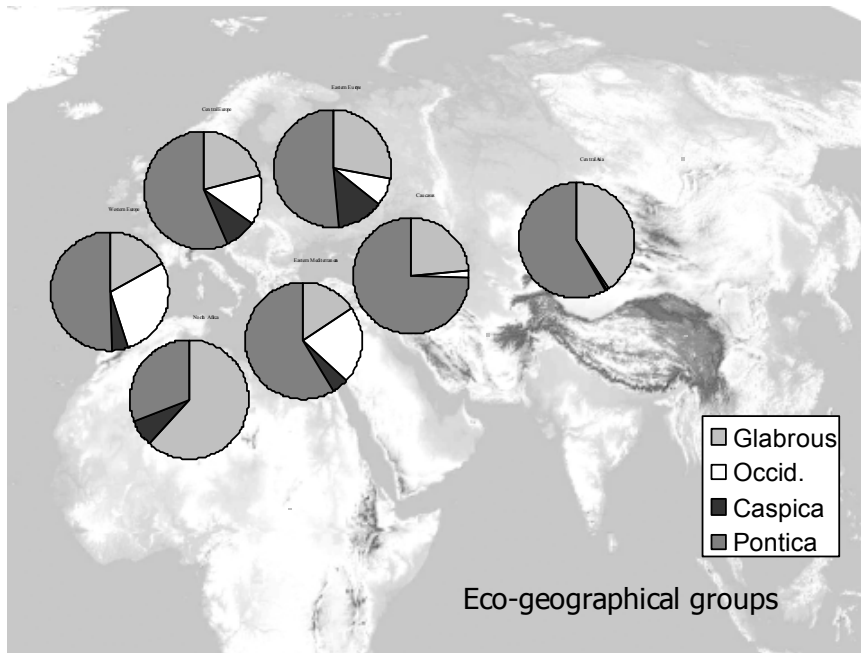


Fig. 2. Eco-geographical groups' frequencies in *Vitis vinifera* subsp. *sativa* cultivation zones (calculated with the 820 cultivars analysed). From west to east: Western Europe, Northern Africa, Central Europe, Eastern Europe, Eastern Mediterranean, Caucasus and Central Asia.

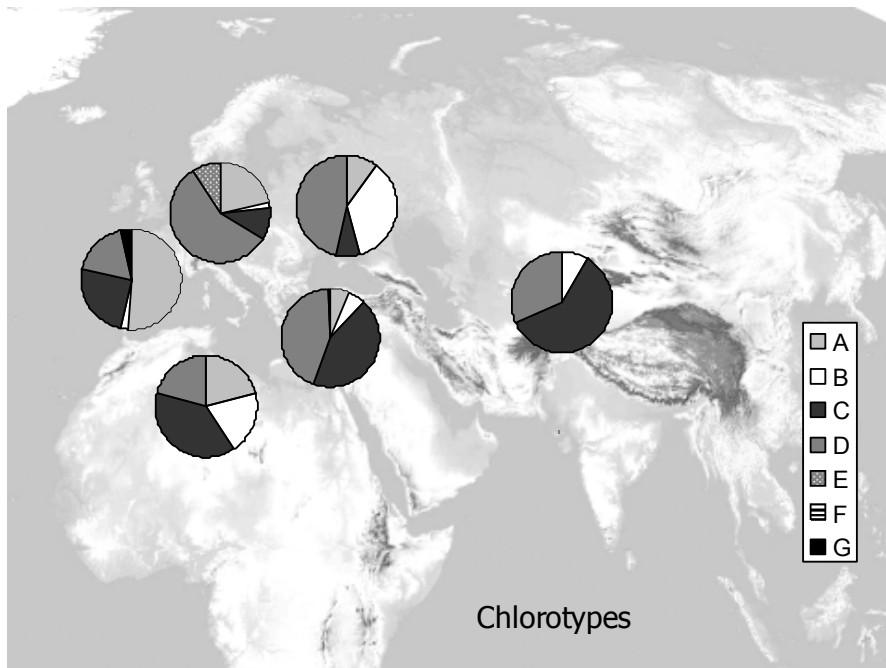


Fig. 3. Chlorotype distribution in *Vitis vinifera* subsp. *sativa* population groups (adapted from Arroyo et al., 2006). From west to east: Western Europe, Northern Africa, Central Europe, Eastern Mediterranean, Eastern Europe, Caucasus (not analysed) and Central Asia.

