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# Geometric Morphometry and Archaeobotany: Characterisation of Grape Seeds (*Vitis vinifera* L.) by Analysis of Form

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## Abstract

**The external profile of seeds is a good phenotypic descriptor, indicative of the origin and diffusion of grape vines (*Vitis vinifera* L.) over the centuries. Archaeological seeds conserved in an anaerobic environment after discovery were compared with modern seeds, belonging to eleven autochthonous cultivars, sampled in Tuscan vineyards. To study seed profiles we used geometric morphometric analysis (elliptical Fourier analysis) combined with multivariate statistical methods. When the values obtained were entered in a database, they produced a cladogram based on Euclidean distances between varieties of archaeological and modern vines, showing that seeds belonging to the same group had phenotypic affinities.**

## Keywords

**Archaeobotany, Grape Seeds, Elliptic Fourier Analysis, Etruscan, Viticulture History**

**Subject Areas:** **Anthropology, Archaeology, History**

## 1. Introduction

Archaeobotany involves disciplines such as palynology (study of pollen), anthrocology [anthracology is more frequent] (study of charcoal), phytolith analysis (study of silica bodies of higher plants) and study of diatoms

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(unicellular siliceous algae). Carpology (study of seeds and fruits) occupies a special place due to an abundance of economic and ecological information. Carpological form is a good phenotypic descriptor that can reveal climatic, ecological and sociocultural changes [1] and provide indications about evolution, history and the domestication of plants useful to humans [2]. As reported by [3], quantification and description of the form of fruits can help describe and characterize an agricultural cultivar, while in the study by [4], an analytical approach similar to the one used here made it possible to study differences in the form of olive endocarps.

During excavations in the period 2011-2013, new well-conserved specimens including archaeological grape seeds were found. With respect to the analysis described by [4], the specimens included an additional three new archaeological seeds found in Florence (Italy) in spring 2011, three seeds from the excavations conducted at Miranduolo castle (Chiusdino, Siena, Italy) in April-May 2012 and 17 seeds from Poggio Bacherina (Chianciano Terme, Siena, Italy). Four archaeological seeds from Poggio la Croce (Radda in Chianti, Siena, Italy) found in 2009 and four seeds from Shahr-i Sokhta (Iran) found in 2007 were also included in the analysis.

To ensure their conservation, the seeds found were immediately placed in sterile capsules sealed in vacuo in an anaerobic environment (absence of oxygen) and stored in the refrigerator (0˚C - 4˚C).

Later they were characterized morphologically by elliptical Fourier analysis combined with multivariate statistical methods to obtain the results presented in this paper.

## 2. Analysis of Form

Analysis of the form of archaeological and modern seeds was conducted analysing the frontal external profile of all specimens as described by [5]. The number of seeds analysed was 813 and they consisted of Tuscan archaeological seeds [Firenze (43˚768591'N, 11˚254714'E, 13th century AD, seven seeds), Miranduolo castle (43˚120348'N, 11˚079410'E, 11th century AD, six seeds), Poggio Bacherina (43˚039420'N, 11˚815203'E, 2nd century BC, twenty seeds), Poggio la Croce (43˚39'45.25"N, 11˚23'45.25"E, 6th century BC, four seeds)], Iranian seeds [Shahr-i Sokhta (30˚6500003'N, 61˚399999'E, 23rd century BC, four seeds)] and modern Tuscan seeds [70 seeds per cultivar: Sangiovese Casciano, Sangiovese Montalcino, Cabernet Bossi, Merlot Bossi, Canaiolo Monti in Chianti, Canaiolo Montalbuccio, Trebbiano Montalbuccio, Trebbiano Tognazza, Malvasia Monti in Chianti, Albano Montalbuccio and Silvestris (selected in September 2013 in the Mediterranean bioclimatic area between 43˚02' - 43˚25'N and 11˚25' - 11˚28'E)]. The first name of the modern seeds indicates cultivar and the second provenance, except for *Vitis vinifera* ssp. sylvestris. They were selected from specimens of autochthonous varieties of ancient Tuscan vineyards, having the highest morphological affinities (size, length and form) with the archaeological varieties. The term *autochthonous* refers to varieties that have adapted to the host area to the point of belonging, and have their origins in the ancient history of the area. To analyse form, the seeds were disposed on a back-lighted surface to enhance their profile (**Figure 1(a)**) and photographed with a digital camera at high resolution. An automated procedure was used to transform the photos into black and white (**Figure 1(b)**), from which the profiles of the seeds were extracted (**Figure 1(c)**—green).

The forms of all seeds were then processed in sequence by elliptical Fourier analysis, extracting the coefficients of the harmonic equations of each to enable comparison of samples of different origins and age, and ex-

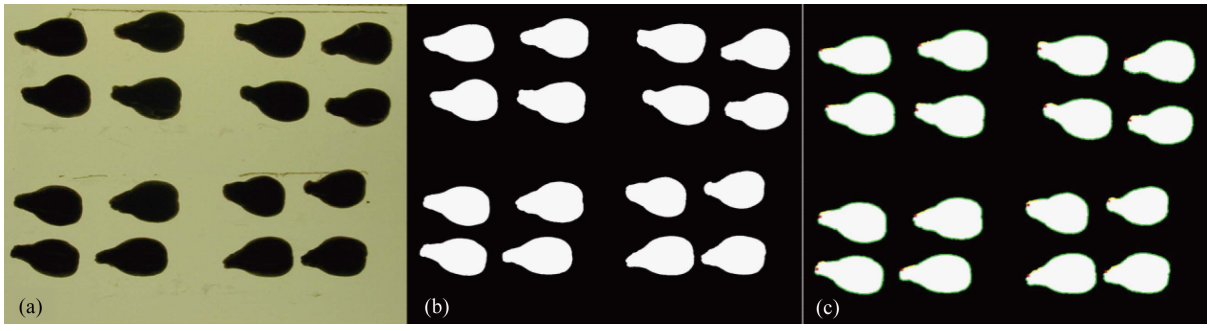


**Figure 1.** (a) Original frontal images of grape seeds (*Vitis vinifera*) on a back-lit surface. (b) The same image converted automatically into black and white. (c) Profiles (green) extracted automatically in a clockwise direction starting from the seed apex (red). The coordinates were then processed by elliptical Fourier analysis.

tracting a cladogram based on Euclidean distances that revealed four groups (**Figure 2**).

**Figures 3-6** show details of the four groups generated by the cladogram. The mean forms of seeds belonging to each cultivar are shown on the right hand side. It is evident that seeds of the same group have similar forms. Statistical analysis involved 770 modern seeds (77 per cultivar) which is a good statistical basis for testing hypotheses of relationship.

## 2.1. Group 1

The forms of Group 1 (**Figure 3**) are roundish with a slight piriform depression in the posterior part. Two culti-

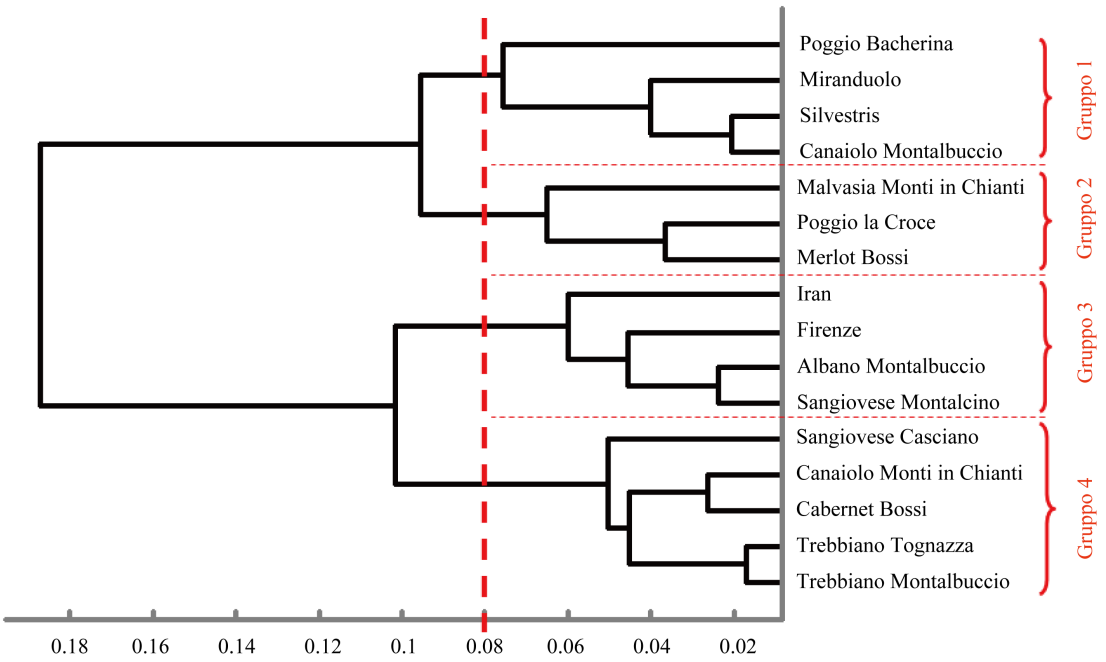


**Figure 2.** Cladogram based on Euclidean distances of the coefficients of harmonic equations extracted by elliptical Fourier analysis from profiles of archaeological and 770 modern grape seed specimens. The cladogram expresses the degree of mutual similarity between specimens: the shorter the distance of the lines between two cultivars, the greater their similarity. The bold dashed red line represents the level at which the cladogram is cut to generate the four groups shown on the right.

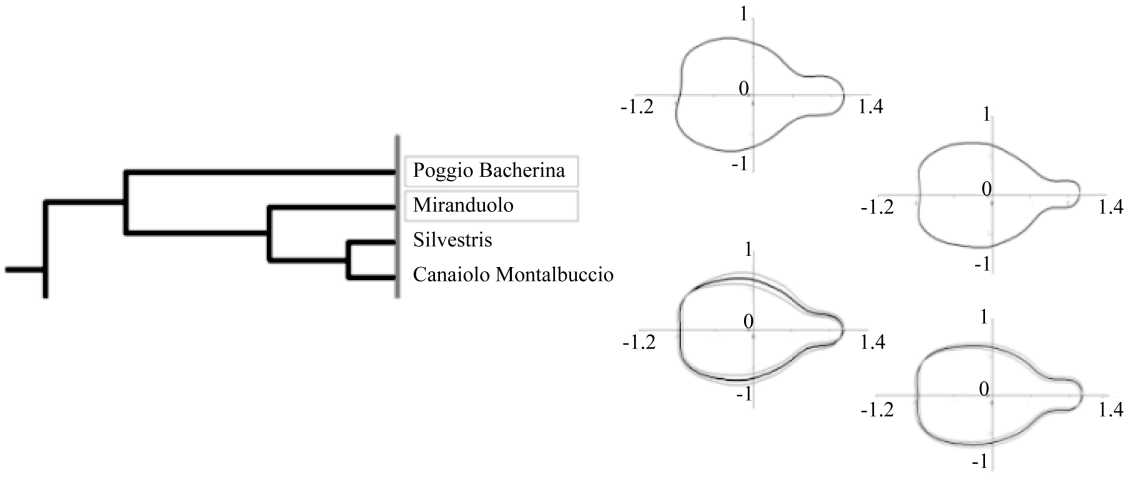


**Figure 3.** Group 1 with the mean forms of seeds of the cultivars Poggio Bacherina, Miranduolo, Silvestris and Canaiolo Montalbuccio shown on the right. The archaeological specimens are indicated in rectangles.

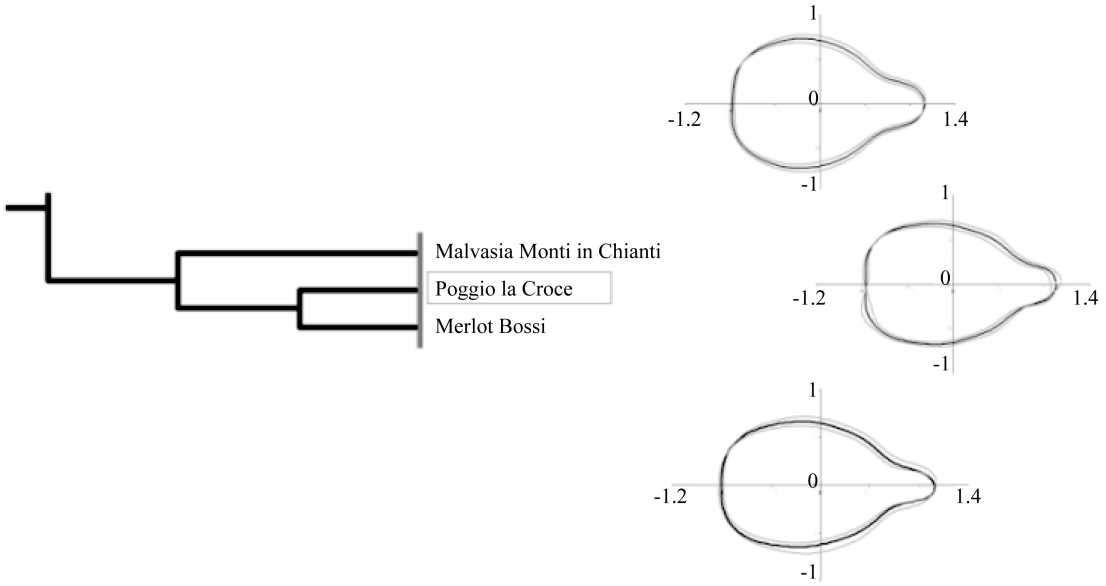


**Figure 4.** Group 2 with the mean forms of seeds of the cultivars Malvasia Monti in Chianti, Poggio la Croce and Merlot Bossi shown on the right. The archaeological specimens are indicated in rectangles.

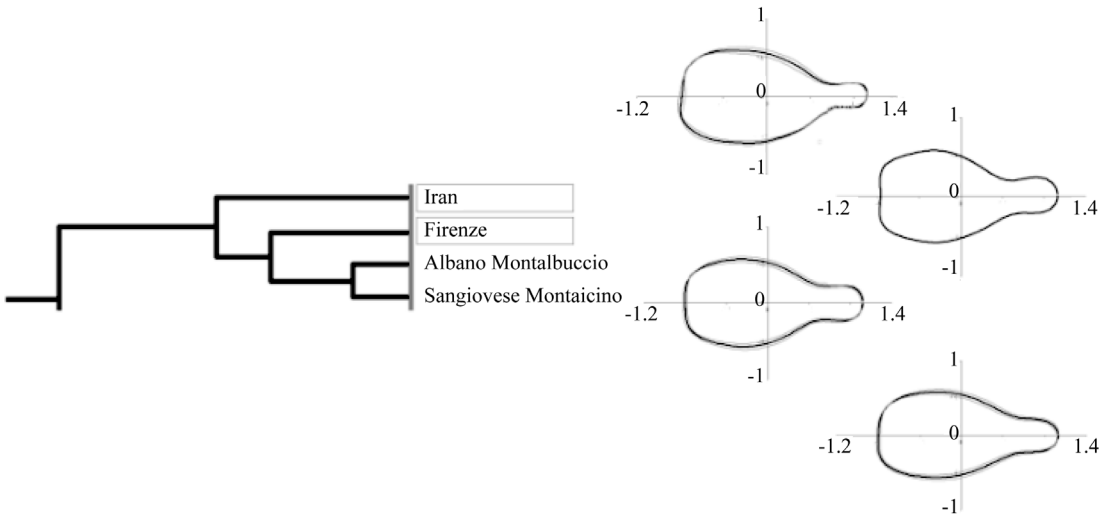


**Figure 5.** Group 3 with the mean forms of seeds of the cultivars Iran, Firenze, Albano Montalbuccio and Sangiovese Montalcino shown on the right. The archaeological specimens are indicated in rectangles.

vars from archaeological sites (Poggio Bacherina and Miranduolo) and two modern cultivars (Silvestris and Canaiolo Montalbuccio) belong to this group. The archaeological seeds are further apart than the modern seeds and also distanced from them (longer cladogram distances between cultivars).

## 2.2. Group 2

The forms belonging to Group 2 (**Figure 4**) are the roundest. Again the group included archaeological (Poggio la Croce) and modern seeds (Malvasia Monti in Chianti and Merlot Bossi).

## 2.3. Group 3

The forms belonging to Group 3 (**Figure 5**) are more elongated. The remaining two cultivars from archaeological sites (Iran and Firenze) and two modern cultivars (Albano Montalbuccio and Sangiovese Montalcino) belong

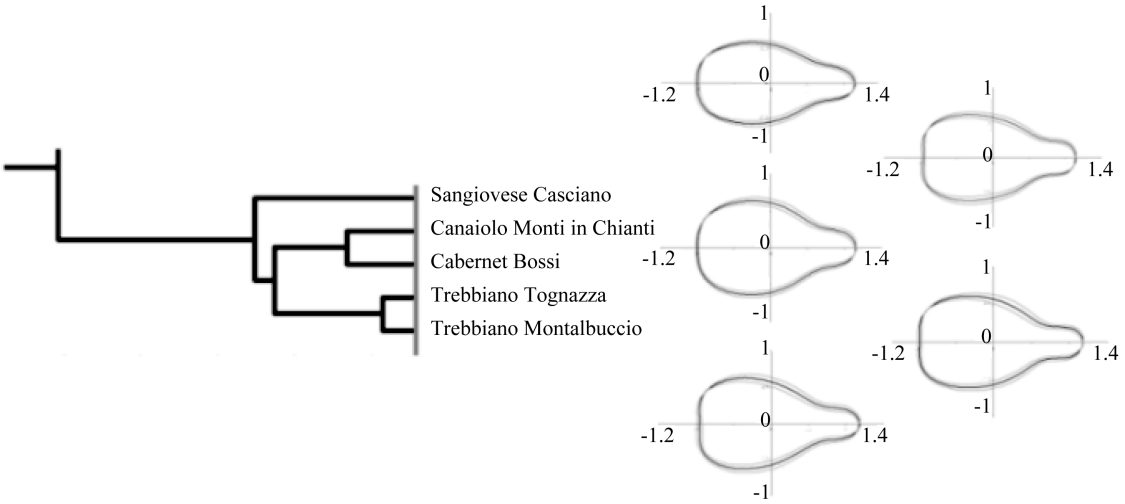


**Figure 6.** Group 4 with the mean forms of seeds of the cultivars Sangiovese Casciano, Canaiolo Monti in Chianti, Cabernet Bossi, Trebbiano Tognazza and Trebbiano Montalbuccio shown on the right.

to this group. As in Group 1, the archaeological seeds are further apart than the modern seeds and also distanced from them (longer distances between cultivars in the cladogram).

### 2.4. Group 4

The forms belonging to Group 4 (**Figure 6**) are slightly elongated and triangular. No archaeological seeds belonged to this group.

## 3. Conclusions

In this study we examined more than 800 grape seed specimens. The varietal characterisations proved to be particularly descriptive, efficacious and economical. Specifically:

- In Group 1, Etruscan archaeological seeds from Poggio Bacherina (2nd century BC) and medieval seeds from Miranduolo (11th century) showed compatibility with modern seeds Canaiolo and Montalbuccio. Canaiolo is an ancient Tuscan variety mentioned in "Dè Opera di agricoltura" by Pietro de Crescenzi (1233-1320). Besides carpological similarities between modern seeds Canaiolo Montalbuccio and Silvestris, we also observed pomological similarities (small leaf, loose bunch, vine trunk up to 40 cm in diameter).
- In Group 2, the affinity between Poggio la Croce (6th century BC) and Merlot Bossi (modern) seeds from Gironde in France is in line with the hypothesis that French wines had origins in Etruscan wines. This is shown by research conducted on Etruscan amphorae from 6th century BC found in the harbour site of Lattara in the south of France [6]. There is a fascinating affinity between archaeological Etruscan seeds from Poggio la Croce and modern seeds of Malvasia Monti in Chianti. The Etruscans loved light, aromatic, sparkling white wines that they drank with rich foods. Etruscan tombs and vases (8-4th century BC) depict vines strung from tall trees that acted as living trellises. In Tuscany there are still pergolas of Malvasia that produce light, sparkling, thirst-quenching, low-alcohol white wines.
- In Group 3, the affinity of Iranian archaeological seeds (23rd century BC) and Florentine medieval seeds (12th century) with the modern cultivar Sangiovese Montalcino, the basis of all Tuscan red wines, including Brunello and Rosso di Montalcino, Chianti and Nobile di Montepulciano, adds weight to scientific sources showing that in prehistoric times, domestication of the vine originated in the area between the Black Sea and Iran ([7]-[10]). This hypothesis may be confirmed by the affinity of archaeological seeds with modern seeds of Albano Montalbuccio, a variety of the Po valley and the area between the Apennines and the Adriatic coast, where the vine presumably entered in Italy in antiquity, imported from the east, and from whence it spread.

Finally, the method of morphometric analysis applied to palaeobotanical specimens is immediate and economical, but is not enough in itself to establish the descent of modern material from ancient specimens. Genetic

studies could provide better correlations, but in our experience the genetic profiles of older palaeobotanic material entered in databases show tenuous affinities that can in most cases be calculated at 1% - 3%. Moreover, a DNA studies are destructive of specimens, which precludes the possibility of using seeds from archaeological museums. In future, phylogenetic comparisons of palaeobotanic and modern materials will be sustained by improved methods, larger databases and inter disciplinary studies.

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