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MONOGRAPH

Creation of a pollen database for Mediterranean flowering plants

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ABSTRACT Palynology is the science that studies pollen grains (size, morphology, structure, function, ornamentation, physical and chemical properties), the carriers that transport the male gametes to the pistil (more precisely to the stigma) allowing the fertilization of the eggs. In seed plants pollens represent an extra generation (haploid generation), the widely reduced male gametophyte. During the pollen release phase, the pollen grains separate completely from the plant (diploid generation, or sporophyte) in an attempt to reach the female flower to allow the release of genetic material and, therefore, the fertilization of the egg. Pollens possess many varieties of shapes, sizes, designs, ornamentations, openings with variable shapes and numbers that can be observed by optical microscopy and that have a high systematic value. Each botanical species has pollens with unique characteristics that allow their identification. Palynology is widely used as an extremely important tool in various types of studies and investigations, such as paleobotany, forensic investigations, melissopalynology, studies on the biodiversity of precise geographical areas, identification of cases of introduction of non-native species and identification of hybridization between species. For these reasons the creation of a pollen database could be a particularly efficient and useful tool.

KEY WORDS Palynology; biodiversity; pollen database.

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INTRODUCTION

One of the consequences of the decline of biodiversity is the reduction in the quantity, quality and diversity of pollen, which is a fundamental food source for bees, dependent on environmental changes. Bees have also been affected with unprecedented worldwide declines. Today we are witnessing a true 'eco-drama'.

Biodiversity is the whole and the variety of all living things, plant and animal forms, present in the ecosystems of the planet. The concept of biodiversity is closely linked to the life of each living form: greater biodiversity provides a greater chance of survival. Even for pollen, diversity of pollen is key.

For the purpose of the pollen database, various pollen species were investigated by performing microscopic analysis (Erdtman, 1943; Moore et al., 1991).

Pollen structure

Generally mature pollen grains are circular or elliptical, have variable dimensions, and are cov-

ered by a layer of lipids and carotenoids and other substances which facilitate their adhesion on the stigma surface (pollenkitt) (Fig. 1).

Pollen has sizes ranging from 10 μm to 100 μm. This variety of dimensions is considered a diagnostic character. It may also depend on the degree of hydration or dehydration of the pollen or the method of preparation.

The use of the following categories could be helpful: very small (<10 μm), small (10–25 μm), medium (26–50 μm), large (51–100 μm) and very large (> 100 μm).

Pollen grains have openings from which the genetic material exits to fertilize the eggs: colpus (elongated) and pores (circular). A combination of porus and colpus is termed a colporus (Knox & McConchie, 1986; Blackmore & Crane, 1998; Banks, 2003).

The presence of colpus, pores (or both), their number, size and disposition is another character to make a classification of the different pollen forms, referable to specific botanical species.

The pollen wall

The pollen has a protective wall (sporoderm) composed of two layers: intine and exine (Blackmore et al., 2007; Rowley & Skvarla, 2000). The intine is constituted by polysaccharides such as cellulose, pectine and hemicellulose, the exine is constituted by sporopollenin. Commonly, the pollen wall in apertural regions is characterized by the reduction of exinous structures or by a deviant exine, and a thick, often bilayered intine.

Exin can be divided into: endexine and ektexine (Bor, 1979).

The ektexine consists of a basal foot layer, an infratectum and a tectum, the endexine is a mainly unstructured, single layer (Fig. 2).

In the regions of openings the pollen wall is characterized by a different exine construction.

The terms sexine for the outer, structured, and nexine for the inner, unstructured exine layer are widely used in light microscopy, but do not fully correspond to ekt- and endexine, respectively.

The angiosperm pollen wall

In angiosperms, ektexin is generally made up of tectum, infra tectum and the foot layer (Knox, 1984). The tectum, more or less continuous, can be covered by supertectal elements. The infratectum can be columellate or granular. The foot layer can be continuous, not continuous or totally absent. Endexine can be continuous, not continuous, compact or spongy, present only in the openings or absent (Erdtman, 1952; Doyle, 2005).

The gymnosperm pollen wall

The pollen wall of the gymnosperms is different from that of the angiosperms in two characters: 1. the endexine is always lamellatE in the mature pollen; 2. the infratectum can be granular or alveolate but it is never columellate (Gullvåg, 1966; Van Campo, 1971).

The main stratification (ektexine, endexine and

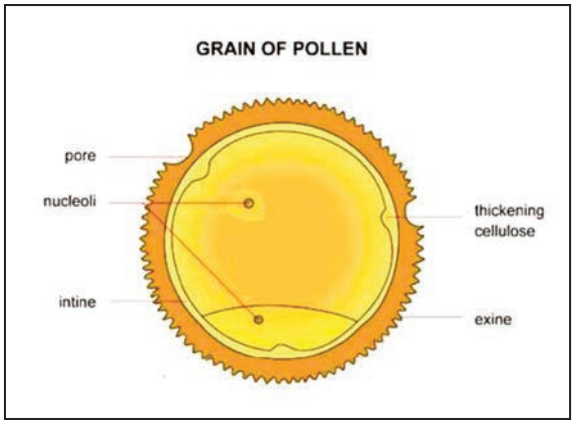

Figure 1. Pollen structure.

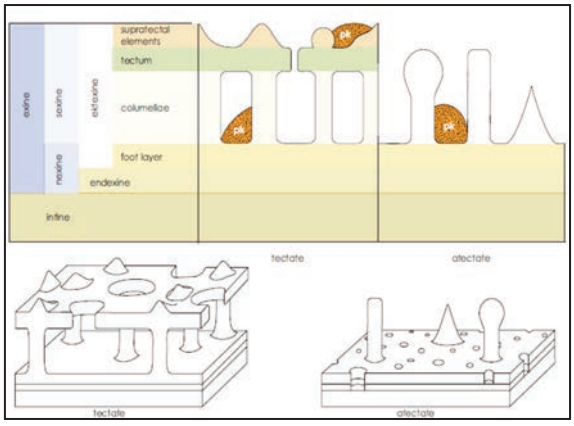

Figure 2. Pollen wall stratification.

intine) is the same in gymnosperms and angiosperms.

Dispersal units

Mature pollen is shed in dispersal units. The post-meiotic products either remain permanently united or become partly or usually completely disintegrated.

In the latter case the dispersal unit is a single pollen grain, a monad; if the post-meiotic products remain united, dyads (a rare combination), tetrads or polyads are the result.

MATERIAL AND METHODS

The presence of structures and ornamentations on the outer portion of the exine, allows to attribute a certain pollen morphology to a well-defined botanical species.

Also important for recognition are the openings that may be present in pollen grains, having very variable shapes and sizes (Pozhidaev, 2000).

Pollens without openings are called inaperturate, while pollens with openings are called aperturate.

Depending on the morphology, the openings are classified as colpus and pores. Pollens that have pores are called porates, pollens that present colpus are called colpates (Erdtman, 1945, 1957; Iversen & Troels-Smith, 1950; Jerković et al., 2010).

Also, the number and position of colpus and pores is useful for recognition as it varies from species to species.

The first step of the present work was the collection of the flowers, choosing those with pollen-filled anthers.

The collection was carried out in various sites near Palermo (Sicily, Italy), such as the Botanical Garden of Palermo, the Ficuzza wood, Mezzojuso and Piana degli Albanesi.

Since this study is also preliminary to a second work in which we deal with melissopalynology and variability of pollen representation in unifloral honeys, we have given much importance to those floral species used for honey production (Table 1).

The second step is the palynological analysis, which consists in the observation, by optical microscope, of the pollen collected directly from the anthers of the flowers.

Place of collection	Species collected	Honey species
Piana degli Albanesi	4	2
Botanical Garden of Palermo	3	0
Ficuzza wood	7	5
Mezzojuso	6	3

Table 1. Places of collection and species collected.

The pollen is collected by washing the anthers with ethyl ether, $(C_2H_5)_2O$, and then placed on a microscope slide to proceed with microscopic observation.

Observation by optical microscope can be carried out naturally or with the use of a dye such as fuchsin, that enhances the morphological characteristics the exine. This gives the pollen a magenta color with different intensity, depending on the permeability of the pollen and the concentration of the dye. Glycerine gelatin was also used to fix the pollen to the glass slide. Once the slides have been prepared with the collected pollen, we passed to the observation with the optical microscope. Based on the size of the pollen grains, different magnifications (10x, 40x and 100x) were used.

The two different observation methods, with fuchsin and without fuchsin, allow us to study the various pollen morphologies that are photographed, examined, cataloged and included in a palynological database that can be consulted for various purposes (botanical, paleobotanical, forensic, melissopalynology, biodiversity) (Figs. 3–8).

The observation without fuchsin highlights the shape, size and color of the pollen; the use of fuchsin allows us to study in more detail the structure of the exine with its various ornamentations and structures. The observation without fuchsin allows to study other natural characteristics of pollen, such as their color.

RESULTS AND CONCLUSIONS

In order to make the microscopic analysis more

accurate, observations were also made using fuchsin, a dye that enhances the morphological characteristics of the pollen, especially the exine, the outer layer of the pollen grain.

Through natural observation and with fuchsin, we observed, studied and cataloged the various pollen morphologies belonging to different floral species present in the Mediterranean area, such as Rosemary, Sulla, Dandelion, Asphodel, Thistle, *Ailanthus*, Almond, Chestnut, Carob tree, etc.

The data collected through our observations were compared with data from other palynological encyclopedias and, then, we proceeded to create a palynological database of the main floral species of the Mediterranean. The steady development and updating of a palynological database could be very important for the study of biodiversity, because it would provide an additional tool to identify, for example, cases of introduction of non-native species, cases of speciation or hybridization.

Species	Pollen unit	Pollen class	Aperture number	Aperture type	Size
Ailanthus altissima	monad	colporate	3	colporus	26 - 60 µm
Asphodelina lutea	monad	sulcate	1	sulcus	51 - 100 µm
Asphodelus ramosus	monad	sulcate	1	sulcus	51 - 100 µm
Castanea sativa	monad	colporate	3	colporus	10 - 25 µm
Ceratonia siliqua	monad	colporate	4	colporus	26 - 50 µm
Citrus aurantium	monad	colporate	4	colporus	26 - 50 µm
Eucalyptus camaldulensis	monad	colporate	3	colporus	10 - 25 µm
Euphorbia dendroides	monad	colporate	3	colporus	26 - 50 µm
Ferula communis	monad	colporate	3	colporus	26 - 50 µm
Galactites tomentosa	monad	colporate	3	colporus	26 - 50 µm
Hedysarum coronarium	monad	colpate	3	colpus	10 - 25 µm
Malva neglecta	monad	colporate, inaperturate	>6	porus	51 - 100 µm
Oxalis pes-caprae	monad	colpate	3	colpus	26 - 50 µm
Pinus pinea	monad	saccate	1	leptoma	50 - 100 µm
Pyrus pyraster	monad	colporate	3	colporus	26 - 50 µm
Prunus dulcis	monad	colporate	3	colporus	26 - 50 µm
Quercus ilex	monad	colpate	3	colpus	26 - 50 µm
Rosmarinus officinalis	monad	colpate	6	colpus	26 - 50 µm
Rubus ulmifolius	monad	colporate	3	colporus	10 - 25 µm
Taraxacum officinale	monad	iophate	3	colporus	26 - 50 µm

Table 2. Characteristics of analyzed pollens.

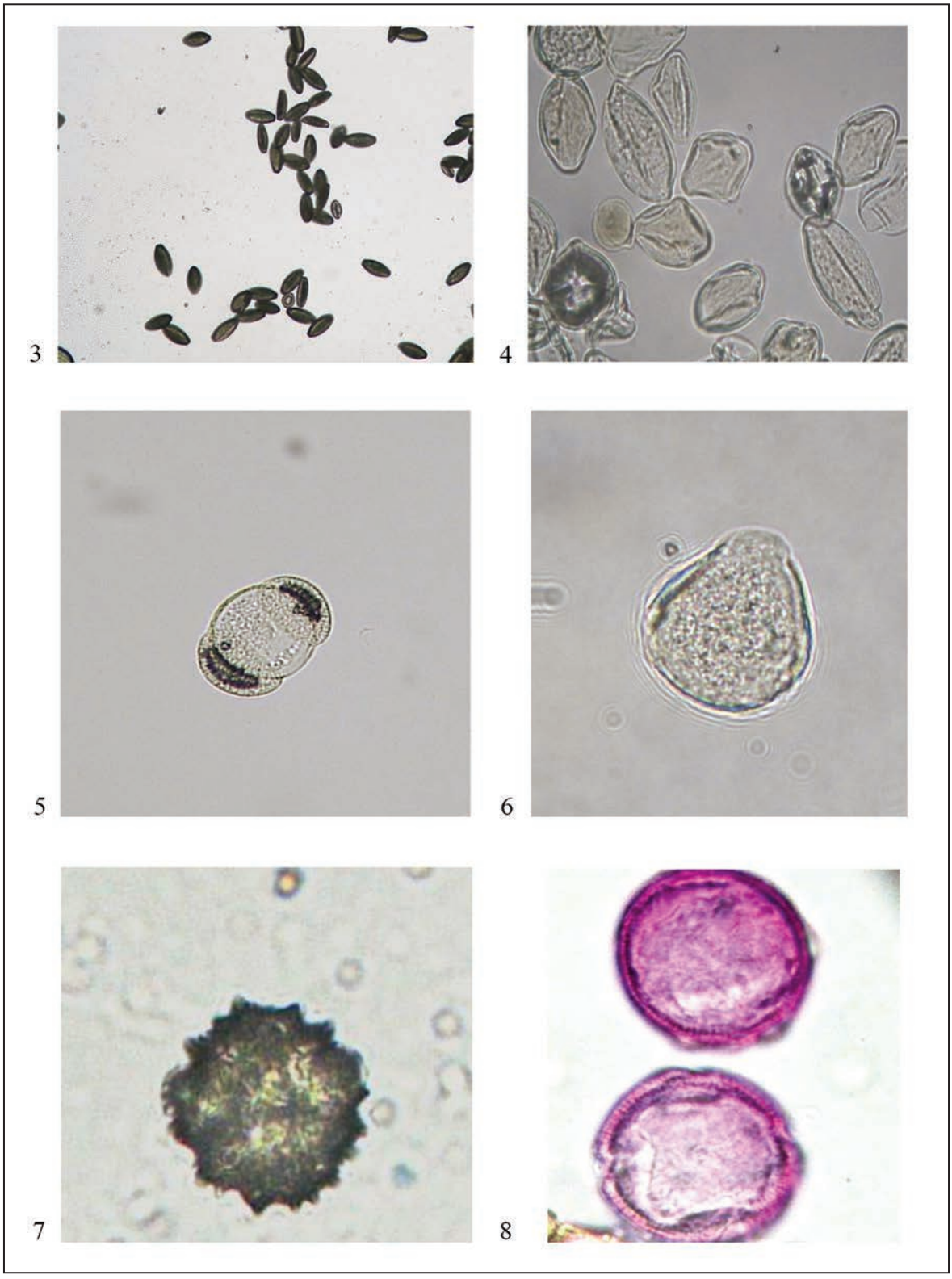

Figure 3. Pollen of *Pyrus pyraster* (10x). Figure 4. Pollen of *Castanea sativa* (40x). Figure 5. Pollen of *Pinus pinea* (40x). Figure 6. Pollen of *Ailanthus altissima* (40x). Figure 7. Pollen of *Galactites tomentosa* (40x). Figure 8. Pollen of *Citrus aurantium* with fuchsin (40x).

This study was also necessary for another more complex work, which consists of melissopalynological analysis of the honeys both through the use of optical microscopy, for the recognition of pollen present in honey through comparison with the data present in our database, and through the use of Molecular Biology techniques, such as DNA extraction, PCR and real-time PCR, which will allow a more precise analysis (Loveaux et al., 1978; Simonetti et al., 1989; Persano Oddo et al., 1995; Schievano et al., 2013; Mannina et al., 2015).

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