Partially hydrated pollen: taxonomic distribution, ecological and evolutionary significance

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Abstract. The problem of the water content of pollen is reconsidered, especially the distinction between "partially hydrated pollen" (PH pollen), pollen with a water content greater than 30%, and "partially dehydrated pollen" (PD pollen), which has a water content of less than 30%. Both types have been found even in systematically contiguous groups or the same genus. Partially hydrated pollen, encountered in at least 40 families of angiosperms, has the advantage of germinating quickly, normally in a few minutes to less than an hour. Dispersal of highly hydrated pollen also occurs in orchids but for a different reason, i.e. to enable packaging of massulae. The disadvantage of pollen dispersed with a high water content is that water is readily lost and the pollen may desiccate and die unless it has biochemical or anatomical devices to retain water or phenological strategies, such as flowering when temperatures are not too high and when relative humidity is high. Most pollen of Gymnosperms and Angiosperms studied has, however, been found partially dehydrated.

Key words: Anther, pollen, pollen water content, anther and pollen dehydration, taxonomic distribution.

In the development of the male gametophyte of Spermatophytes, there is normally a dispersal phase in which the pollen is transported centimetres or even kilometres to reach its target, the female receptive stigma. The travel can last a fraction of a second or even days. Pollen is programmed to survive this journey, depending on the environment in which the plant grows and on the pollination circumstances (Dafni and Firmage 2000). In the final stages of maturation, pollen water content decreases, metabolism slows down and defence mechanisms are activated. This makes the pollen more resistant to the hostile environment through which it has to travel (Pacini 2000).

The water content of pollen is normally less than 10%, but higher values have been reported, as well as different values in the same species (Stanley and Linskens 1974). These values (sometimes over 50%) are generally not interpreted or explained. Other authors have occasionally reported such cases, often in terms of limited volume decrease prior to dispersal, or maintenance of high cytoplasmic metabolic rate. For example, Payne (1981) reported a smaller than usual percentage reduction in volume ($< 10\%$) in the following species: *Musa balbisiana* (Musaceae), *Ruellia ciliosa* (Acanthaceae), *Ambrosia artemisiifolia* (Asteraceae), *Crotalaria spectabilis* (Fabaceae), *Lamium amplexicaule* (Labiatae), *Sida acuta* (Malvaceae), *Lantana camara* (Verbenaceae).

Kress (1986) demonstrated that exineless pollen of *Heliconia pogonantha* (Heliconiaceae), a rainforest plant, is completely hydrated, and this is probably also true of two other species of *Heliconia*. The pollen of maize and other Gramineae is partially hydrated (Barnabás and Rajki 1981, Heslop-Harrison 1979) as shown by cytoplasmic cyclosis (Heslop-Harrison et al. 1997). Another example, of course, is the often filiform, fully hydrated pollen of marine monocots with underwater pollination (Ackerman 1995).

The problem of pollen hydration status was recently taken up in a holistic manner (Nepi et al. 2001), considering water content, correlations with carbohydrate reserves, decrease in viability in time, pollen longevity, speed of pollen tube emission, together with detection methods, in a limited number of species. In the present paper, we examine the systematic extent of the phenomenon and its various ecological and evolutionary implications. Some families with a high number of species show a certain variability, i.e. they show different pollens, both partially dehydrated and partially hydrated (e.g. Ranunculaceae, Euphorbiaceae, Compositae, Liliaceae, Orchidaceae). On the other hand, other large families are quite uniform; in this sense, certain families as Gramineae are always partially hydrated, while others as Rosaceae, Apiaceae and Lamiaceae seem always partially dehydrated, and therefore are not referred herein. Anyway, some exceptions should be possible.

Dispersal of pollen with a high water content may enable fast pollen tube emission as well as provide the possibility of transporting a large quantity of pollen in a dispersing unit, such as the pollinium (Pacini and Franchi 1996, 1999).

Materials and methods

Pollen grains of the more than one hundred species belonging to forty families listed in Tables 1 and 2 were collected just at anther anthesis from wild plants, spontaneously occurring in Tuscany or grown in the Botanical Gardens of Siena University. Pollen of two gymnosperms, *Araucaria bidwillii* and *Araucaria angustifolia*, was collected in the University Parks of Rosario (Argentina).

To determine whether pollen was partially hydrated or partially dehydrated, the "fast" method (Nepi et al. 2001) was used for the species listed in Table 1. The method measures the difference in volume of pollen in immersion oil (which is the volume at dispersal) and in water (which is the volume of rehydrated pollen). These data are listed in Table 1, which shows the wide taxonomical spread of the phenomenon. The table contains species with pollen that increases in volume by less than 50% (usually 10–40%) and that can therefore be regarded as partially hydrated (Nepi et al. 2001). Table 2 shows the values of pollen water content obtained after dehydration in an oven at 105 °C up to constant weight (Nepi et al. 2001).

Pollen samples for S.E.M. were collected from mature anthers, gold-coated in an Edwards evaporator and observed with a Philips 501 scanning electron microscope at 7.2 kV.

Observations and discussion

The research group presenting this work has been engaged with this problem for more than 10 years, first identifying differences in water content from the behaviour of spherical pollen and ovoid pollen with furrows. Spherical pollen was initially called "non dehydrated" and other pollen "dehydrated" (Pacini and Franchi 1984, Pacini 1990). Since the phenomenon was found to have different grades, it subsequently seemed more correct to call the pollen "partially dehydrated" and "slightly dehydrated" (Speranza et al. 1997). The current terminology is now "partially hydrated" (PH) and "partially dehydrated" (PD) (Nepi et al. 2001).

During its development, pollen is immersed in locular fluid, which mediates relations between the sporophyte and gametophyte. Before the anther opens, the locular fluid is reabsorbed and/or evaporates so that the pollen can be exposed to the air (Pacini 1994). At this stage, the water content of pollen with respect to its value at dispersal: a. may be the same; b. may be higher, but it will

Table 1. List of species with pollen, which when rehydrated increases in volume by 10–40% and which are therefore regarded as PH (Nepi et al. 2001). Pollen characteristics are indicated: Shape: **S** = spherical; **PH** = polyhedric; **O** = ovoidal; **SI** = subisopolar; **T** = pollen in tetrads; Pore: **P**, number of pores in brackets; **SP** = stephanoporate; **PP** = polyporate; **PL** = poreless; Furrow: **F**, number of furrows in brackets

DICOTS
Amaranthaceae
   Amaranthus retroflexus L. **S, PP**
   Celosia cristata L. **S, PP**
Anacardiaceae
   Pistacia vera L. **S, PP**
Aristolochiaceae
   Aristolochia rotunda L. **S, PL**
Betulaceae
   Alnus glutinosa (L.) Gaertner **S, P(5)**
   Betula pendula Roth. **S, P(3)**
Cactaceae
   Opuntia dillenii Mill. **PH, PP**
   Opuntia ficus indica Mill. **PH, PP**
Cannabaceae
   Humulus japonicus Sieb. & Zucc. **S, P(3)**
   Humulus lupulus L. **S, P(3)**
Caryophyllaceae
   Lychnis flos-cuculi L. **S, PP**
   Silene dioica (L.) Clairv. **S, PP**
   Stellaria media Vill. **S, PP**
Chenopodiaceae
   Atriplex hortensis L. **S, PP**
   Chenopodium album L. **S, PP**
   Spinacia oleracea L. cv. Prevital **S, PP**
Compositae
   Helianthus tuberosus L. **S, P(3)**
Convolvulaceae
   Ipomoea purpurea (L.) Roth. **S, PP**
Corylaceae
   Carpinus betulus L. **S, P(3)**
   Corylus avellana L. **S, P(3)**
   Ostrya carpinifolia Scop. **S, P(3)**
Cucurbitaceae
   Cucumis melo L. cv. Bush Star **S, P(3)**
   Cucumis sativus L. **S, P(3)**
   Cucurbita argyrosperma Hort. ex L.H. Bail. **S, PP**
   Cucurbita foetidissima Kunth. **S, PP**
   Cucurbita maxima Duchesne ex Lam. cv. Pomme d'Or, cv. Table Gold **S, PP**

Table 1 (continued)

   Cucurbita moschata (Duchesne ex Lam.) Duchesne ex Poir. cv. d'Albenga **S, PP**
   Cucurbita pepo L. various cultivars **S, PP**
   Lagenaria vulgaris Ser. **S, F(3), P(3)**
   Sechium edule Sw. **S, PP**
Droseraceae
   Drosera rotundifolia L. **T, S, PL**
Euphorbiaceae
   Croton sp. **S, PP**
Hamamelidaceae
   Liquidambar styraciflua L. **S, PP**
Juglandaceae
   Carya olivaeformis Nutt. **S, P(3)**
   Juglans regia L. **S, PP**
Lauraceae
   Laurus nobilis L. **S, PL**
   Persea americana Mill. **S, PL**
Leguminosae Caesalpinioideae
   Bauhinia forficata Link. **S, P(3)**
Malvaceae
   Alcea rosea L. **S, PP**
   Althaea officinalis L. **S, PP**
   Gossypium arboreum L. **S, PP**
   Hibiscus rosa-sinensis L. **S, PP**
   Hibiscus syriacus L. **S, PP**
   Lavatera arborea L. **S, PP**
   Malva sylvestris L. **S, PP**
   Pavonia hastata Cav. **S, PP**
Nyctaginaceae
   Mirabilis jalapa L. **S, PP**
Onagraceae
   Fuchsia coccinea Dryand. **SI, P (3)**
   Oenothera organensis Munz. **SI, P (3)**
Papaveraceae
   Papaver orientale L. **S, P (3)**
Passifloraceae
   Passiflora coerulea L. **S, PP**
Plantaginaceae
   Plantago lanceolata L. **S, PP**
   Plantago major L. **S, PP**
   Plantago media L. **S, PP**
Portulacaceae
   Portulaca oleracea L. **S, PP**
   Portulaca grandiflora Hooker. **S, PP**
Ranunculaceae
   Thalictrum flavum L. **S, PP**
Salicaceae
   Populus tremula L. **S, PL**

Table 1 (continued)

Ulmaceae
  Celtis laevigata Willd. **S, P(3)**
  Ulmus minor Mill. **S, SP**
Urticaceae
  Parietaria judaica L. **S, P(3)**
  Urtica dioica L., **S, PP**
  Urtica pilulifera L. **S, PP**
MONOCOTS
Araceae
  Arum italicum Mill. **S, PL**
  Arisarum vulgare Targ. Toz. **S, PL**
  Zantedeschia aethiopica (L.) Spreng. **S, PL**
Cannaceae
  Canna indica L. **S, PL**
Gramineae
  Alopecurus myosuroides Hudson **S, P(1)**
  Anthoxanthum odoratum L. **S, P(1)**
  Avena fatua L. **S, P(1)**
  Brachipodium pinnatum (L.) Beauv. **S, P(1)**
  Bromus hordeaceus L. **S, P(1)**
  Coix lachrima jobi L. **S, P(1)**
  Dactylis glomerata L. **S, P(1)**
  Elymus repens (L.) Gould **S, P(1)**
  Euchlaena mexicana Schrad. **S, P(1)**
  Festuca arundinacea Schreber **S, P(1)**
  Holcus lanatus L. **S, P(1)**
  Lolium multiflorum Lam. **S, P(1)**
  Molinia caerulea (L.) Moench **S, P(1)**
  Phalaris brachystachys Link **S, P(1)**
  Phleum pratense L. **S, P(1)**
  Poa annua L. **S, P(1)**
  Poa pratensis L. **S, P(1)**
  Secale cereale L. **S, P(1)**
  Sorghum bicolor (L.) Moench **S, P(1)**
  Stipa bromoides (L.) Dörfler **S, P(1)**
  Triticum aestivum L. various cultivars **S, P(1)**
  Zea mays L. various cultivars **S, P(1)**
Heliconiaceae
  Heliconia sp. **S, PL**
Iridaceae
  Crocus sativus L. **S, PL**
  Hermodactylus tuberosus Mill. **O, F(1)**
Lemnaceae
  Lemna gibba L. **S, P(1)**
Liliaceae
  Smilax aspera L. **S, PL**
  Trillium kamtschaticum Ledeb. **S, PL**
Musaceae
  Musa basjoo Siebold **S, PL**

Table 1 (continued)

Orchidaceae
  Cymbidium eburneum Lindl. **PH, PL**
  Cymbidium insigne Rolfe **PH, PL**
  Loroglossum hircinum L.C. Rich. **PH, PL**
  Phalaenopsis amabilis (L.) Bl. **PH, PL**
  Phalaenopsis equestris (Schauer)
   Reichb. f. **PH, PL**
  Serapias vomeracea (Burm.) Briq. **PH, PL**
  Stanhopea tigrina Batem. ex Lindl. **PH, PL**
Typhaceae
  Typha latifolia L. **T, S, PL**
Zingiberaceae
  Hedychium coccineum Buch.-Ham. ex Sm. **S, PL**

diminish in order to achieve an equilibrium with the environment, in the presence of wall mechanisms to adjust volume reduction (harmomegathic effect) (Wodehouse 1935); c. may be higher but without special mechanisms for water retention or for harmomegathy (Pacini 2000). In cases a. and b. the pollen survives longer than in case c. (Pacini et al. 1997, Nepi et al. 2001). Mechanisms for water retention can be cytoplasmic (presence of carbohydrates) or structural (thick intine, continuous exine, thick exine). The preferential site of water storage is the cytoplasm. The wall may also act as a water reserve by mechanisms different from that of the cytoplasm, especially in pollen with thick intine, such as that of the Lauraceae and Musaceae, which has a high water content, and that of certain gymnosperms (e.g. Cupressaceae, Taxaceae).

From a systematical point of view, there are few correlations among species with partially hydrated pollen (Tables 1 and 2); indeed, members of the same family or even genus may have either type of pollen (Fig. 1, Table 3).

Characteristics of species with partially hydrated pollen

Species with partially hydrated pollen have a high water content; it is considered "high" in comparison with the spermatophyte dispersal structures, which generally have a low water

Table 2. List of Angiosperm and Gymnosperm species tested for pollen weight loss, heated to constant weight at 105 °C. A loss of more than 30% indicates PH pollen (Nepi et al. 2001)

Species	Family	Water loss (%)
Gymnosperms		
Araucaria angustifolia (Bertol.) Kuntze	Araucariaceae	54.3 ± 0.5
Araucaria bidwillii Hook.	Araucariaceae	44.5 ± 1.1
Cupressus sempervirens L.	Cupressaceae	22.6 ± 0.02
Pinus brutia Ten.	Pinaceae	17.9 ± 4.3
Taxus baccata L.	Taxaceae	9.3 ± 0.1
Torreya nucifera (L.) Sieb. & Zucc.	Taxaceae	8.8 ± 1.2
Angiosperms		
Dasylirion acrotrichum (Schiede) Zucc.	Agavaceae	1.7 ± 2.0
Zephyranthes candida (Lindl.) Herb.	Amaryllidaceae	5.4 ± 2.3
Zantedeschia aethiopica (L.) Spreng.	Araceae	58.8 ± 0.04
Campsis radicans (L.) Seem.	Bignoniaceae	8.3 ± 0.8
Humulus japonicus Sieb. & Zucc.	Cannabaceae	65.5 ± 2.2
Helianthus annuus L. cv. Titan	Compositae	16.7 ± 0.4
Helianthus annuus L. cv. Violet Queen	Compositae	21.6 ± 1.0
Helianthus tuberosus L.	Compositae	43.7 ± 1.2
Convolvulus arvensis L.	Convolvulaceae	17.2 ± 1.6
Ipomoea purpurea (L.) Roth.	Convolvulaceae	53.6 ± 7.1
Citrullus lanatus (Thumb.) Matsum. & Nak. var. *citroides* (L.H. Bail.) Mansf. cv. d'Espagne	Cucurbitaceae	17.4 ± 4.3
Cucumis sativus L. cv. Tortarello barese	Cucurbitaceae	25.0 ± 6.2
Cucurbita argyrosperma hort. ex L.H. Bail.	Cucurbitaceae	51.5 ± 0.5
Cucurbita foetidissima Kunth.	Cucurbitaceae	33.2 ± 0.1
Cucurbita maxima Duchesne ex Lam. cv. Pomme d'Or	Cucurbitaceae	39.8 ± 1.1
Cucurbita maxima Duchesne ex Lam. cv. Table Gold	Cucurbitaceae	34.9 ± 4.8
Cucurbita moschata (Duchesne ex Lam.) Duchesne ex Poir. cv. d'Albenga	Cucurbitaceae	50.4 ± 0.8
Cucurbita pepo L. cv. Blackjack F1	Cucurbitaceae	46.2 ± 1.0
Cucurbita pepo L. cv. Coucourzelle	Cucurbitaceae	49.5 ± 2.1
Cucurbita pepo L. cv. Cou-tors Hâtive	Cucurbitaceae	49.2 ± 1.2
Cucurbita pepo L. cv. Goldzini F1	Cucurbitaceae	44.6 ± 0.9
Cucurbita pepo L. cv. Jaune Sunburst	Cucurbitaceae	50.0 ± 5.0
Cucurbita pepo L. cv. Ronice	Cucurbitaceae	50.0 ± 0.7
Cucurbita pepo L. cv. Storr's Green F1	Cucurbitaceae	46.8 ± 1.3
Cucurbita pepo L. cv. Summer Satellite F1	Cucurbitaceae	45.2 ± 6.5
Thladianta dubia Bunge	Cucurbitaceae	7.9 ± 5.3
Ricinus communis L.	Euphorbiaceae	11.0 ± 3.8
Coix lachryma jobi L.	Gramineae	31.3 ± 1.2
Euchlaena mexicana Schrad.	Gramineae	32.2 ± 0.8
Zea mays L. cv. Amero (yellow anthers)	Gramineae	31.6 ± 0.1
Zea mays L. cv. Amero (red anthers)	Gramineae	34.9 ± 0.1
Zea mays L. cv. Fraise (yellow anthers)	Gramineae	38.9 ± 0.1
Zea mays L. cv. Majeur F1	Gramineae	35.6 ± 0.2
Bauhinia forficata Link.	Leguminosae Caesalpinioideae	40.1 ± 18.1
Gossypium arboreum L.	Malvaceae	26.8 ± 2.8
Hibiscus rosa-sinensis L.	Malvaceae	57.4 ± 2.2

Table 2 (continued)

Hibiscus syriacus L.	Malvaceae	45.6 ± 0.4
Pavonia hastata Cav.	Malvaceae	51.5 ± 1.2
Feijoa sellowiana O. Berg.	Myrtaceae	9.9 ± 1.8
Fuchsia coccinea Dryand.	Onagraceae	67.7 ± 1.6
Oenothera organensis Munz.	Onagraceae	22.7 ± 2.1
Cymbidium eburneum Lindl.*	Orchidaceae	24.1 ± 0.5
Cymbidium insigne Rolfe	Orchidaceae	32.9 ± 0.1
Phalaenopsis amabilis (L.) Bl.*	Orchidaceae	16.8 ± 0.6
Phalaenopsis equestris (Schauer) Reichb. f.*	Orchidaceae	10.6 ± 6.9
Serapias vomeracea (Burm.) Briq.*	Orchidaceae	18.2 ± 6.1
Stanhopea tigrina Batem. ex Lindl.	Orchidaceae	56.8 ± 1.2
Stanhopea tigrina Batem. ex Lindl.*	Orchidaceae	34.3 ± 5.7
Chamaerops humilis L.	Palmae	15.3 ± 0.9
Trachycarpus fortunei (Hook.) H.A. Wendl.	Palmae	11.9 ± 0.4
Papaver orientale L. orange flowers	Papaveraceae	43.2 ± 1.0
Papaver orientale L. red flowers	Papaveraceae	47.4 ± 2.0
Papaver rhoeas L.	Papaveraceae	23.8 ± 0.2
Passiflora caerulea L.	Passifloraceae	46.3 ± 11.0
Urtica pilulifera L.	Urticaceae	33.3 ± 5.5
Hedychium coccineum Buch.-Ham. ex Sm.	Zingiberaceae	65.6 ± 4.2

*Pollen collected from flowers at least a week after beginning of anthesis.

content. Additionally, there are other recurring features, which though not found in all species, are fairly common (Table 4). The main features concern the pollen grain and are: spherical form, while furrows (=colpi, colpori) should be absent (Table 1, Fig. 1), fast pollen tube emission (except in orchids and some other species as *Parietaria judaica*) and rapid loss of viability, especially under conditions of low relative humidity. The speed of pollen tube emission is also related to the presence of a callosic wall in mature pollen; this is the case in *Cucurbita pepo*, *Hibiscus rosa-sinensis*, *Lavatera arborea*, *Spinacia oleracea* pollen, which germinates in 15 minutes, but not in *Parietaria judaica*, which only germinates once this wall

has formed, i.e. after about 120 minutes. Size, structure of walls and number of cells in the male gametophyte at dispersal do not seem to be related to the pollen hydration status (Table 1).

With regard to environment, there are no preclusions because species with partially hydrated pollen may also be plants that live in dry environments (e.g. Cactaceae); anthesis, however, occurs at night, which may limit water loss. The life of the flower also varies from a few hours (*Cucurbita*) to several weeks (Orchidaceae).

Before anther dehiscence, the locular fluid is absorbed and/or evaporated, the pollen dehydrates to a greater or lesser extent (Pacini

Fig. 1. Morphological diversity of pollen of certain Cucurbitaceae. **a** *Bryonia dioica*; **b** *Luffa aegyptiaca*; **c** *Citrullus lanatus* cv. Charleston Gray; **d** *Cyclanthera pedata*; **e** *Lagenaria vulgaris*; **f** *Cucumis melo* cv. Piel de sapo; **g** *Cucumis sativus* cv. Piccolo di Parigi; **h** *Sechium edule*; **i** *Cucurbita pepo* cv. Tonda di Nizza. Pollen **a**, **b**, **c** and **d** are PD with furrows and pores, the others are PH but **e** have three pores and furrows that do not close, **f** and **g** have only three pores, **h** and **i** have many pores covered by a thick layer of pollenkitt. As **e**, **f** and **g** have thin walls, they lose water during preparation for SEM, causing them to collapse. In the PH pollen **e**, **f**, **g**, **h** and **i**, the pores protrude, exposing the pectocellulose part; this is difficult to observe in **h** and **i** because of abundant pollenkitt. Bar 15 μm

2000); this is a species-specific process that depends on the environment and pollen internal mechanisms. This explains why PH pollen of maize obtained from the anther the day before anthesis germinates *in vitro* but PD pollen of *Lilium* does not, as it has already
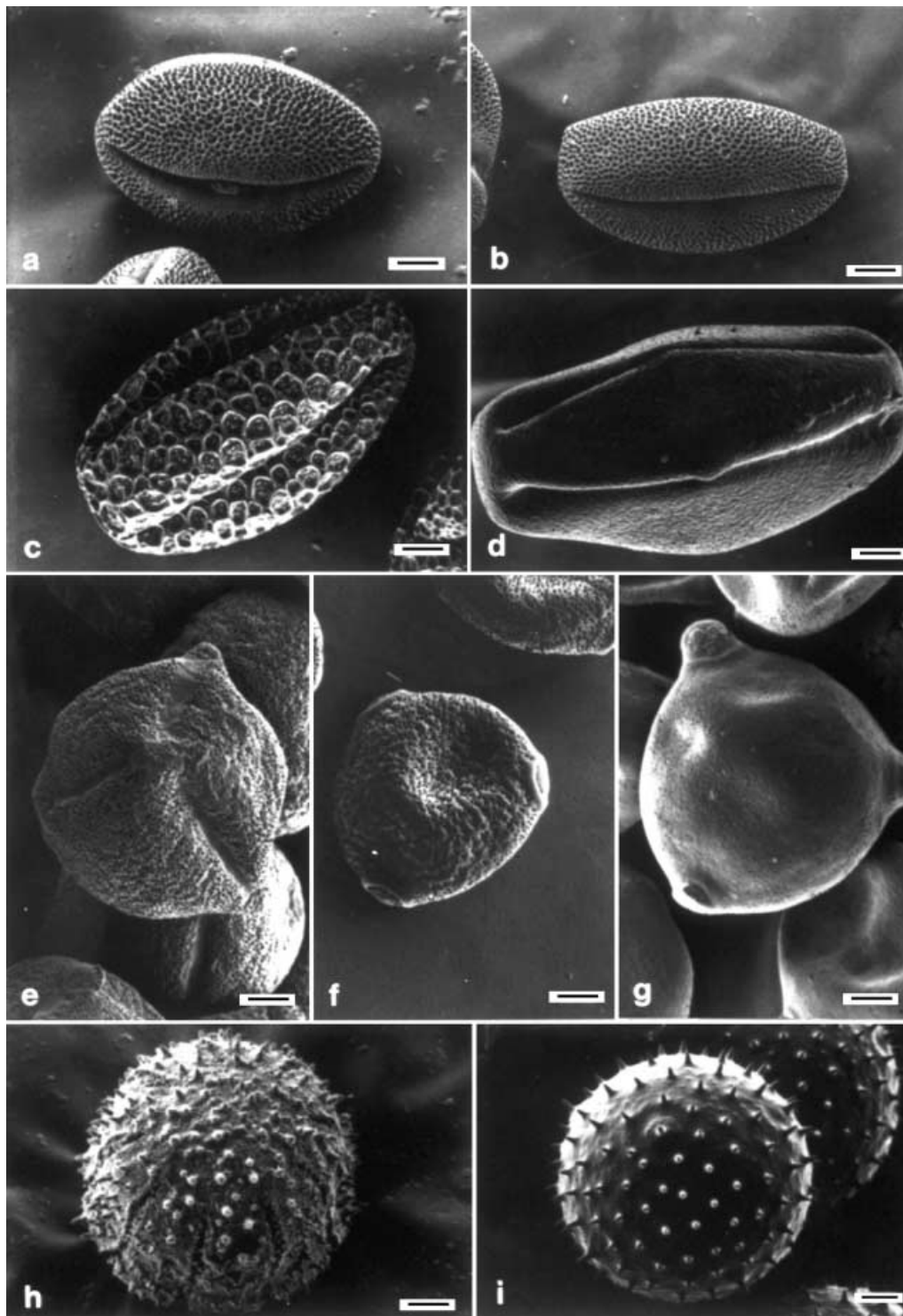
Table 3. Species in the same family or genus having both PH and PD pollen. Pollen characteristics are indicated as in Table 1

Family	Partially hydrated or not dehydrated	Partially dehydrated
Anacardiaceae	*Pistacia vera* L.	*Rhus typhina* L.
Compositae	*Helianthus tuberosus* Mill.	*Helianthus annuus* L.
Cucurbitaceae	*Cucurbita pepo* L., *Lagenaria vulgaris* Ser., *Cucumis sativus* L., *Sechium edule* Sw.	*Bryonia dioica* Jacq., *Luffa aegyptiaca* Mill., *Citrullus lanatus* (Thunb.) Matsum. & Nak. Cv. Charleston Gray
Euphorbiaceae	*Croton* sp.	*Euphorbia helioscopia* L.
Papaveraceae	*Papaver orientale* L.	*Papaver rhoeas* L.
Ranunculaceae	*Thalictrum flavum* L.	*Clematis vitalba* L.
Salicaceae	*Populus tremula* L.	*Salix caprea* L.
Araceae	*Arum italicum* Mill., *Arisarum vulgare* Targ. Toz.	*Dieffenbachia* sp.
Iridaceae	*Hermodactylus tuberosus* Mill.* Some species of the genus *Crocus***	*Iris* genus
Liliaceae	*Smilax aspera* L., *Trillium kamtschaticum* Ledeb.	*Lilium* spp.
Orchidaceae	*Loroglossum hircinum* L.C. Rich. and all species with pollinia	*Pterostylis plumosa* Cady and all species with monad pollen with a single furrow

* Data from Grilli Caiola et al. (2000)
** Data from Chichiriccò (1999 and 2000)

begun to dehydrate; the latter only germinates after a desiccation process whereas maize pollen is not programmed for this process (Lin and Dickinson 1984).

In some groups, the decrease in viability is slight while the pollen remains in the anther, but is rapid if the pollen is removed (e.g. *Cucurbita*, orchids, *Asclepias* spp. – Wyatt and Broyles 1994). In the case of *Cucurbita pepo*, we are investigating a special mechanism that seems to keep pollen humid during presentation. A similar mechanism may also exist in Orchidaceae with pollen dispersal units of the pollinium type (Pacini and Hesse 2002), where anthesis may last for more than a month (Clifford and Owens 1988).

Shape and size

Partially hydrated pollen may be large (100–200 µm, as in *Cucurbita*, Gramineae and *Silene*) or small (less than 50 µm, as in *Parietaria*, *Spinacia* and massulate orchids) (Table 1). In the absence of mechanisms to retain water, large pollen survives longer than small due to its high surface area/volume ratio which reduces the desiccation risk.

Another characteristic is spherical pollen without furrows or other infolding wall mechanisms allowing volume changes (Table 1). In *Humulus lupulus*, *Carpinus betulus*, *Corylus avellana* and *Ostrya carpinifolia* the poral intine is of the oncus type, namely pectinized and very thick, with a high water content (Punt et al. 1994) and the Gramineae and *Cucurbita* have a Zwischenkörper (Heslop-Harrison and Heslop-Harrison 1980, Nepi et al. 1995).

A cytological feature common to most PH pollen is a very thick exine (*Cucurbita*, *Lavatera*, *Mirabilis*, *Opuntia*), although PH pollen with thin (all Gramineae), discontinuous or no exine (e.g. *Heliconia*) does exist. Pollen with thin exine combined with a high water content tends to irregularly collapse during preparation for scanning electron microscope observation because water is lost; see many photos of PH pollen in the monograph of Zavada (1983) on monocot pollen.

Table 4. Diversity of certain morphological and cytophysiological characteristics of PH pollen. The only characteristic in common is a water content greater than 30%. This diversity influences capacity to retain water during presentation and dispersal and to adhere to the stigma and germinate promptly. Orchidaceae with pollinia are a special case: the pollen cannot loose too much water because the inner pollen grains would be crushed. Tubes are emitted only after separation of tetrads and creation of spaces between them for pollen tubes. Data from Franchi et al. 1996, Nepi et al. 2001, Pandolfi and Pacini 1995, Speranza et al. 1997, and original data

Characteristics	Examples
Sexual expression	Monoecy: *Cucurbita pepo, Corylus avellana, Zea mays* Dioecy: *Populus tremula, Silene dioica, Spinacia oleracea* Hermaphrodite: *Hibiscus rosa-sinensis, Althaea officinalis, Mirabilis jalapa*
Plant's habitat	Environments with high RH (certain Musaceae and Lauraceae), or with high RH when pollen is presented and dispersed (certain Cactaceae and Onagraceae that bloom at night)
Flower longevity	6 h (*Cucurbita*), 2 days (*Hibiscus*), >1 month (certain Orchidaceae)
Pollen presentation	a. launched by sudden movement of anther (*Parietaria, Urtica*); b. dispersed by wind as soon as anther opens (Gramineae, *Spinacia*); c. "primary presentation" – remains in anther awaiting pollinator or wind (*Hibiscus, Silene*); in this case pollen is often disposed in single layer; d. "secondary presentation" – presented to dispersing agents in another part of flower (*Helianthus annuus, Arum italicum, Zantedeschia aethiopica*)
Pollen dispersing units	Isolated monads (Gramineae), monads held together by pollenkitt (*Cucurbita pepo*), monads held together by viscin threads (*Oenothera*), tetrads united by common walls (*Drosera*), various types of pollinia (Orchidaceae)
Pollen size (diameter)	100–200 μm (*Cucurbita pepo, Oenothera*) 30–100 μm (*Helianthus annuus, Parietaria*) <30 μm (*Plantago* sp., Orchidaceae)
Form	Normally spherical (*Cucurbita, Laurus, Silene*, Musaceae), occasionally flat or trimerous (*Oenothera*), polyhedral (Orchidaceae with pollinia)
Pores	Without pores (certain Lauraceae, some Araceae, Musaceae), 1 (Gramineae), 3 (*Oenothera, Betula, Parietaria*), 5 (*Alnus*), many (*Chenopodium, Cucurbita, Plantago, Silene, Juglans*)
Exine	Discontinuous, as in certain Magnoliaceae, Lauraceae and Musaceae; thin, as in Gramineae (collapsing with water loss); thick, as in *Helianthus annuus* (decreasing in volume but maintaining form with water loss)
Intine	Thin, as in Gramineae, thick as in certain Lauraceae and Musaceae
Callosic wall	Present in *Cucurbita pepo* and *Lavatera arborea*, i.e. species with fast germination; absent in *Parietaria* and slow germinating species
Water content	30–50% (e.g. *Urtica pilulifera, Helianthus tuberosus, Zea mays* cv. Majeur F1), >50% (e.g. *Humulus japonicus, Ipomoea purpurea, Hedychium coccineum, Zantedeschia aethiopica*)
Number of cells	Two: *Cucurbita*; three: *Helianthus annuus*

Table 4 (continued)

Characteristics	Examples
Reserve carbohydrates	Starch: present (*Cucurbita,* Gramineae); absent (*Helianthus annuus*) Cytoplasmic polysaccharides: present (*Lavatera*); scarce or absent (*Cucurbita*, Gramineae) Callose: present (*Cucurbita, Lavatera*), absent (*Parietaria*) Pectin: present (*Cucurbita*); absent (*Parietaria*) Sucrose: present (*Lavatera*); scarce or absent (*Cucurbita*)
Time for pollen tube emission after pollen adhesion to the stigma	Seconds to minutes: *Cucurbita, Lavatera, Portulaca,* Gramineae 10 minutes–1 h: *Hibiscus rosa-sinensis, Oenothera organensis* A few hours: *Parietaria judaica* 24 h or more: Orchidaceae with pollinia
Pollen longevity	Decreases rapidly because pollen cannot conserve water away from anther (*Cucurbita*, Gramineae); remains high for 1–2 days if water retention devices exist; may be conserved for several weeks, as in Orchidaceae
Gametophytic competition	Strong competition is associated with large pollen load and long distance to ovary. Also depends on stigma surface

Table 5. Features that allow distinguishing partially hydrated pollen grains (PHP) from partially dehydrated ones (PDP). Data from Lisci et al. 1994; Nepi and Pacini 1993, 1999; Nepi et al. 2001; Pacini 1990, 1996; Pacini et al. 1997; Speranza et al. 1997

	Partially hydrated pollen (PHP)	Partially dehydrated pollen (PDP)
Definition	Pollen contains $>30\%$ water	Pollen contains $<30\%$ water
Size	Small, medium and large (from 15 to 200 μm)	Commonly mean (from 50 to 120 μm)
Pores	Many, few, none	Commonly 3
Harmomegathic devices (furrows)	Absent	Present
Sucrose content	Low as *Cucurbita pepo* 1.9 μg/mg High as *Opuntia dillenii* 180 μg/mg	Always higher than 50 μg/mg
Pollen germination	Rapid (less than 30 minutes) with the exception of massulate orchids and *Parietaria judaica*	Slow, more than one hour
Response to desiccation	Water is easily lost, especially if sucrose content is low	Water loss is controlled
Pollen longevity under natural conditions	Short, especially if pollen has a big size, a very high water content and a low amount of sucrose	Long (few days)
Pollen storage and conservability at low temperature below 0 °C	Possible only if pollen has machinery for osmotic (turgor) pressure fluctuations	Possible for long times according to temperature

The possibility of conserving water and thus viability depends on the natural environment of the plant (Dafni and Firmage 2000). Plants that grow in moist environments, such as tropical rain forests (many Cannaceae, Heliconiaceae and Zingiberaceae), are not

subjected to the risk of rapid desiccation. This pollen has a thick intine with a high pectin content that could be a water reserve (Kress and Stone 1982, Theilade and Theilade 1996). Plants that live in habitats subjected to high chances of desiccation need devices to reduce water loss. The number of pores is not correlated with water content but the pores often protrude, which means that the poral intine is partly exposed becoming a preferential site for water loss (as in *Cucurbita*, *Mirabilis*, *Oenothera*). The morphology of PH and PD pollen of nine Cucurbitaceae species is compared in Fig. 1. Four of these species (*Bryonia dioica*, *Luffa aegyptiaca*, *Citrullus lanatus* and *Cyclanthera pedata*) have PD pollen, and the others (*Lagenaria vulgaris*, *Cucumis melo*, *Cucumis sativus*, *Sechium edule* and *Cucurbita pepo*) have PH pollen. The first four have three pores and three furrows, *Lagenaria vulgaris* has furrows but they are not involved in harmomegathy because the pollen is not PD. The five species with PH pollen all have protruding pores; air-dehydration caused by preparation for SEM deformed the pollen with thinner exine (Fig. 1e, f, g).

Pollen dispersing unit

Almost all types of pollen dispersing units are found among PH pollen (Pacini 1997): pollen in monads (Gramineae, Urticaceae), pollen clumped together with pollenkitt (*Lavatera arborea*, *Cucurbita pepo*, *Zantedeschia aethiopica*), monads held together by viscin threads (*Fuchsia* and *Oenothera*), pollen joined in tetrads (*Typha latifolia*), and pollen in pollinia (Orchidaceae).

The lack of a distinct dehydration phase may have many explanations, depending on the type of pollen dispersing unit. Monads are always PD, except in the species discussed in this paper. Tetrads are usually PD and rarely PH; however, their morphology is different. In the first case, common walls are abundant and the external form of the tetrad may even be spherical and the grains tetrahedral. In the second case, there are few common walls and the tetrad is four-lobed (e.g. *Typha*, *Drosera*, *Epilobium*). Polyads composed by a maximum of 16 grains are dehydrated, and their morphology suggests that there are no exceptions because harmomegathy is always possible (each grain in such a polyad has at least one external face with harmomegathic devices – see Guinet 1986). In loose and compact pollinia, on the other hand, the pollen grains cannot change in volume. If this occurred the grains would separate and the inner ones would be damaged by compression. During dispersal, the pollinia of some orchids decrease slightly in volume, but this reduction is adapted for insertion of the pollinium in the stigma (Borba and Semir 1999). This decrease in volume may be correlated with some water loss, as indicated in Table 2 for various Orchidaceae, the pollinia of which were collected at least a week after the start of anthesis.

It seems that there is a trade-off between the absence of dehydration and transporting pollen in large clumps (400,000 to 4,000,000 in orchids), according to Wolter and Schill (1986). Also in this case, there are special mechanisms to safeguard the pollen. The pollen of Orchidaceae and Asclepiadaceae with pollinia is not directly exposed to pollinators, but remains inside the anther where atmospheric agents cannot dehydrate it. This is presumably possible, due to the development of specialised structures (the viscidium or corpusculum) to stick the pollinium to insect bodies.

Types of carbohydrate reserves and their significance

Neither starch nor callose, PAS-positive cytoplasmic polysaccharides or sucrose are correlated with pollen hydration status (compare lists in Tables 1 and 2 with list of reserve carbohydrates in Franchi et al. 1996) (Pacini 1996, Speranza et al. 1997, Nepi et al. 2001). At most, callose may be related to faster germination, as a precursor of pollen tube formation, and PAS-positive cytoplasmic polysaccharides (the chemical nature of which

is still unclear) and soluble sugars may be related to capacity to prolong viability adjusting turgor pressure and protecting membranes (Franchi et al. 1996, Pacini 1996, Speranza et al. 1997, Dafni and Firmage 2000, Nepi et al. 2001).

Some orchids, such as *Stanhopea tigrina*, have pollen with a high sucrose content (our unpublished data). Although the longevity of pollinia has been measured in only a few cases (Neiland and Wilcock 1995), it is presumably long because anthesis lasts several weeks and once the pollinium reaches the stigma it has to await maturation of the female gametophyte. In the meantime, the tetrads packed in the pollinium loosen, creating space for pollen tube emission (Pandolfi and Pacini 1995).

Pollen presentation

Among the species that have PH pollen, all types of presentation occur, namely: a. the pollen is launched by a sudden movement of the anther filament (*Parietaria judaica*); b. the pollen is not retained in the anther by pollenkitt and is dispersed as soon as the anther opens, facilitated by movement of the anther on its long hanging filament (Gramineae); c. the pollen is retained in the anther by pollenkitt until collected incidentally by an insect (*Cucurbita* and *Lavatera*), or collected actively by insects e.g. bees from *Lychnis* ss.pp. and *Laurus*, or entrained by wind (*Pistacia vera*); d. pollen in a pollinium remains in the anther and is not exposed until the viscidium, a modified part of the anther, attaches to an insect (Orchidaceae); e. pollen is presented in parts of the flower other than the anther (secondary pollen presentation in Compositae and Araceae) (Howell et al. 1993).

Pollenkitt and pollination syndrome

The presence/absence of pollenkitt is related to pollen water content in zoophilous and anemophilous species. In Gramineae and other herbaceous or woody anemophiles (e.g. *Juglans regia*), pollenkitt is very reduced or absent,

the pollen leaving the anther as soon as it opens. The flight of the pollen is short and fast, so little water is lost, even in the absence of mechanisms to retain it. In this case, the pollen reaches the stigma quickly and with less stress chances, before desiccation may occur. In the case of *Cucurbita pepo* and other entomophiles, pollenkitt is present and keeps the pollen in the anther until the pollinator arrives, when it sticks the pollen to the insects body (Pacini 1996). Moreover, PH pollen is often presented on the anther in a single layer, being removed all at once when the insect touches the anther (Nepi and Pacini 1993).

In some cases, such as *Cucurbita*, there is no efficient mechanism to retain water, especially during dispersal, and the pollen is only exposed for 6 h. Pollen is exposed for a day in *Convolvulus arvensis* and up 2 days in *Hibiscus rosa-sinensis* according to environmental parameters. In these three cases, the flower closes at the end of anthesis, preventing dispersal of the pollen left on the anther, which has lost water and is no longer viable.

Pollen consumers

PD pollen, which has more sucrose and is thus more nutritional than PH pollen, should be more common in flowers that offer pollen as a reward than in those that offer only nectar. Hence we expect high prevalence of PH pollen in cases in which pollen is not consumed as a reward (birds, bats, flies).

Detailed information on animals that consume pollen is mainly available for various bees (Roulston and Cane 2000). Bees actively collect PD pollen: however, when preferred pollen is not available, they also collect pollen of Gramineae, such as *Zea mays* (Ricciardelli D'Albore and Persano Oddo 1978). The honey may also contain a small percentage of pollen of species with PH pollen, e.g. in southern Tuscany, *Lavatera arborea*, *Malva sylvestris*, *Opuntia ficus-indica*, *Plantago lanceolata*, but this pollen is collected incidentally during the search for nectar (Ricciardelli D'Albore 1998, Ricciardelli D'Albore and Persano Oddo

1978). PH pollen is rarely collected, probably because of its lower nutritive value according to the higher water content and the consumer necessities and digestive abilities (Roulston and Cane 2000). In fact, when pollen grains are experimentally digested (Franchi et al. 1997), insoluble carbohydrate content of PD pollen was partially digested according to digestion times, whereas insoluble carbohydrate content of PH pollen was practically undigested at any time.

An interesting case is that of the Cucurbitaceae, in which *Bryonia* and *Citrullus* pollen, which is PD, is collected by bees and may account for up to 30% of the pollen in honey, whereas *Cucurbita* pollen, which is PH (Fig. 1a, c, i), is not attractive to bees: indeed, after visiting the flowers for nectar, they remove any pollen adhering to their bodies (Ricciardelli D'Albore and Persano Oddo 1978, Nepi and Pacini 1993). Bees behave in the same way also after visiting some Malvaceae, when pollen grains stick to their bodies (Ricciardelli D'Albore and Persano Oddo 1978).

Self-incompatibility

In species with PH pollen, self-pollination is likely as germination occurs as soon as the pollen lands on the stigma. To prevent self-pollination, plants may have mechanisms causing a spatial or temporal shift between male anthesis and female receptivity. If there is self-incompatibility, it cannot be due to failure of pollen to rehydrate, but the pollen tube must be arrested at some point after germination.

Stigma adhesion

Adhesion of pollen to the stigma may occur because the pollen is coated with pollenkitt (*Lavatera arborea*) and/or because the stigma is covered in exudate, or because the two surfaces have opposite electrostatic charges (Gramineae) (Heslop-Harrison 1987). Adhesion is faster and easier for PH than PD pollen, because the difference in water potential between the cytoplasm of the vegetative cell and that of the stigma papillae is presumably less (Heslop-Harrison 1979).

In the Malvaceae, with their pantoporate pollen, the phenomenon of polysiphony occurs, namely, formation of multiple pollen tubes, only one of which continues to grow and reaches the ovary. It was recently shown in *Lavatera arborea* that this phenomenon is linked to pollen hydration status, because only a PH pollen can emit up to 15 pollen tubes after a few minutes of adhesion to the stigma (Nepi and Pacini 1999). In *Lavatera arborea* it was demonstrated that polysiphony enables the pollen to anchor to the stigma, which has minute and widely spaced papillae (Nepi and Pacini 1999).

Pollen storage

Pollen is stored for scientific as well as agricultural purposes. It has long been known that not all species have pollen that stores well (Stanley and Linskens 1974). Some of the pollen listed in Table 1 cannot be stored, e.g. pollen of Gramineae, *Spinacia*, *Cucurbita* and *Gossypium*.

Loss of viability of PH pollen has only been demonstrated in a few cases; in *Heliconia*, all the pollen was dead 24 h after anthesis (Kress 1986); in *Cucurbita pepo*, only 10% of grains were still viable after the same period (Nepi and Pacini 1993). PD pollen has been found to survive longer (Pacini et al. 1997, Dafni and Firmage 2000).

PD pollen is more stable in time, in the sense that if conditions do not vary it remains viable longer than PH pollen. If hydration status is not known, species with PH pollen should not be used to compare different methods of measuring viability (Mayer and Gottsberger 2000).

Germination

Rehydration of frozen pollen is a very sensitive stage before germination (Barnabás and Rajki 1981). Germination is profoundly affected by the mode of rehydration and the relative

humidity at which it occurs. This explains why PH pollen, such as that of *Persea* and *Juglans*, germinate better if rehydrated at 100% RH (Loupassaki et al. 1997, Luza and Polito 1987).

The absence of dehydration does not always mean fast germination of pollen grains. In Orchidaceae with pollinia, pollen tubes are not normally emitted for 12 h because the tetrads must separate to create spaces for pollen tube emission (Pandolfi and Pacini 1995). Partially hydrated pollen may germinate in the anther (Pacini and Franchi 1982, Grilli et al. 2000). This phenomenon is more common when RH is high or if the plant was selected and reproduced non-sexually and has pollen of different sizes with a low viability (Pacini and Franchi 1982).

Partially hydrated pollen and competition

The higher the chances for pollen competition, the greater the selective pressure to favour PH pollen with rapid germination.

In general pollen competition is related to differential growth rate in the style in relation to reaching an available ovule (Mulcahy et al. 1996). The opportunity to germinate earlier than other grains may confer an advantage in this kind of competition: for instance almost all PH pollen grains germinate more or less at the same time after landing on the stigma (Nepi and Pacini 1993, 1999; Nepi et al. 2001). Viceversa PD pollen grains take longer to germinate and their germination is asynchronous depending on the pollen load size, on pollen packaging, on the arrival schedule and on aperture position in respect to stigma surface (Heslop-Harrison 1979).

Evolutionary significance

Almost all studied Gymnosperms existing today have PD pollen, adapted for a full rehydration on the micropyle (Pacini et al. 1999). Two exception were found in the present study, *Araucaria bidwillii* and *Araucaria angustifolia* (Table 2). In Angiosperms, there is no single evolutionary trend, as PH

and PD pollen are found in primitive and advanced families, in different genera of the same family and even in the same genus (Tables 1 and 2). The transition from PD to PH pollen seems to occur in response to certain pollination and environmental constraints as chances of desiccation and reaching of the target stigma. The only discernible evolutionary significance seems to be in the ancestors of marine monocots that had spherical (probably PH) pollen as the starting point for the trend leading to species having underwater pollination (Ackerman 1995).

Conclusions

The morphology, structure and geometry of pollen are therefore not related only or partly to type of pollination, but rather to the degree of dehydration at dispersal. When PD pollen rehydrates on the stigma it changes form from oval to spherical with an increase in the minor axis; PH pollen, which is spherical, changes in volume only if it is adapted for harmomegathy.

Partially dehydrated pollen is parallel to recalcitrant seeds (Roberts 1973) because there is no partial dehydration/rehydration phase and germination occurs at once.

The fact that PH and PD pollen may be found in the same family or genus suggests that the transition from PD to PH pollen is an easy one (as of polyphyletic origin), though the genes involved in either case are probably many. This raises the question whether some species have both types of pollen at different times or shift, producing one or the other type of pollen according to changing environmental conditions. If such species exist, the cleistogamous ones are a good example. In chasmogamic flowers, the anthers and pollen dehydrate, anthers open, and the pollen is exposed and dispersed. Viceversa cleistogamic flowers and anthers do not open; a mechanical layer does not form; the anthers and pollen do not dehydrate; the pollen germinates in the anther; the pollen tube is emitted and seeks the ovary (Lord 1981, Rebdo-Torstensson and

Berg 1995). This means that both sets of genes act in the same species, though not at the same time or in the same flower. If the two sets of genes are found in cleistogams it suggests that all angiosperms have the sets of genes of both PD and PH pollen. Activation of only one set is normal; sequential expression of both sets, which is rarely simultaneous could be a characteristic restricted to cleistogamic species.

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