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Pollen: Cytology
and Development

Preface

Pollen and pollen biology are fundamentals of the plant breeding process. At a time when genetic engineering and other novel means of genetic manipulation are being highlighted, it would seem appropriate to consider the conventional means by which variability has been introduced into plants and the mechanisms by which it has been refined in the development of productive crop varieties. Pollen is the vehicle for the transfer of genetic traits in conventional breeding programs and will as such remain a central feature of present and future variety development.

This review covers aspects of pollen physiology, germination, and cytology. Pollen nutrition is discussed in the chapters dealing with the structure and physiology of the tapetum. Pollination mechanisms are described, and the interaction of events leading to self-incompatibility and its genetic control are presented. A chapter is included on *mentor* pollen techniques in overcoming incompatibility mechanisms and extending the crossing range of pollen. An account of the control and biochemistry of meiosis and the ultrastructural changes within pollen during these and later germination events is also presented. The culture of immature pollen for the production of doubled haploid plants for use as inbred lines in plant breeding is reviewed. Cultural techniques and the overall application of this technology are described in detail. Current developments taking place in the genetic manipulation of pollen are also in the volume, since these developments mark one important area in the future of pollen biology.

Pollen and its use in controlled crossing programs is the fundamental tool in the science of plant breeding. The opportunities presented by developments in biotechnology, including the culture of pollen, the genetic manipulation of pollen, and the manipulation of incompatibility mechanisms, present new opportunities for the plant breeder and geneticist alike.

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Pollen Germination and Pollen-Tube Growth

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I. Introduction: A Preliminary Conspectus

The essential function of the pollen grain and the tube emerging from it is to deliver two male gametes into the embryo sac as a prelude to that characteristic event of angiosperm reproduction, the double fertilization. The haploid male gametophyte that produces and conveys the gametes is structurally simple, but the attainment of its functional objective demands considerable physiological sophistication, expressed in a complex of adaptations associated with dispersal, interaction with the stigma and style, nutrition, growth, and target finding.

The train of events in a normal pollination leading to a successful fertilization is outlined in Table I. The summary emphasizes the interactions of the male gametophyte with the female-acting sporophyte, and also—in the final step—with the female gametophyte. These interactions determine, in large measure, the behavior of the male gametophyte in the postdispersal phase of its active life, a period during which it exists in close association with living tissues of a different genotype. These tissues may themselves respond to the presence of the invading pollen tube by changes in metabolism, secretion, membrane properties, and even structure. The pollen tube thus encounters a complex and changing environment during its growth—an environment that differs not only along the length of the pathway to the embryo sac, but also in response to the presence of the tube itself. It follows from this that investigations of pollen and pollen-tube behavior in artificial media cannot always be expected to offer a reliable guide to the detail of events in the natural system. This caution should be borne in mind in appraising many of the results reviewed in the ensuing sections.

While the sequence set out in Table I is common to all flowering plants, forming so to speak the irreducible core of the pollination process, the angiosperm families differ among themselves in many respects, and not only in matters of detail. Thus far-reaching differences arise from variation in the timing of various developmental events. A striking example concerns the phasing of the development of the male gametophyte—whether certain processes occur before or after the imposition of the temporary dormancy in the anther, the prelude to pollen dispersal. If generative cell division

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TABLE I

SEQUENCE OF INTERACTIONS BETWEEN THE MALE GAMETOPHYTE AND THE FEMALE-ACTING
SPOROPHYTE AND FEMALE GAMETOPHYTE[a]

Fate of the male gametophyte	Interaction with
1. Capture	Stigma surface materials
2. Hydration	
3. Germination	Stigma surface and underlying cells
4. Tube penetration	
5. Tube growth through the style	Secretions in the transmitting tissue of stylar canal
6. Entry into the embryo sac and gamete discharge	Female gametophyte

[a]Modified from J. Heslop-Harrison (1975b).

occurs before this stage, the pollen is dispersed in a tricellular condition; if after, it is released in a bicellular state. This variation in the timing of the generative cell division serves as a marker for more profound differences, for certain physiological properties tend to be correlated with the condition of the pollen at the time of anthesis. One important correlate is the type of incompatibility control whereby the tissues of the female-acting sporophyte discriminate against self-pollen (for review, see De Nettancourt, 1977). The state of development before dispersal also determines the physiological behavior of the male gametophyte in the immediate postdispersal period. The contrast between two monocotyledonous families, the phylogenetically primitive Liliaceae and the advanced Gramineae, illustrates this. Lily pollen is released in the bicellular state, and much of the development of the gametophyte is left until after the pollen is rehydrated on the stigma. Germination in this case may take up to an hour, and the full tube growth rate is not attained immediately. In the Gramineae, a family with tricellular pollen, the gametophyte is developmentally more advanced at the time of anthesis, even to the extent that much of the precursor material of the pollen-tube wall is already synthesized at this time. Germination may occur in as short a period as 90 seconds after the pollen arrives on the stigma, with the emerging tube achieving near to maximal extension rate almost at once.

In several respects the growth of the pollen tube in the pistil is unusual for an angiosperm cell, showing some features comparable with those of an invading filamentous pathogen. The distance between the point of arrival on the stigma and the embryo sac is traversed by elongation of the vegetative cell of the male gametophyte, which extends by apical growth of the cell wall (Fig. 1), but after the initial period living cytoplasm occupies no more than a part of the tube so formed. The protoplast moves contin-
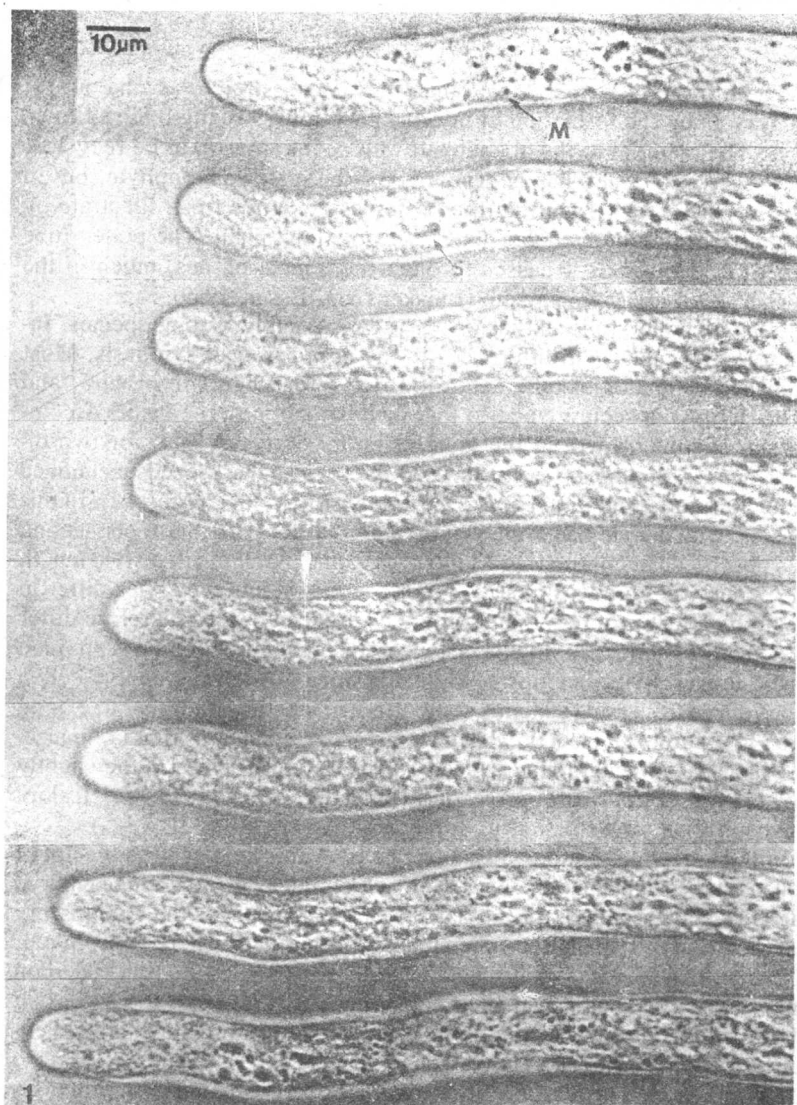
FIG. 1. Time lapse sequence showing the tip of a pollen tube of rye (*Secale cereale*) growing at a rate of approximately 0.56 μm per second on the surface of semisolid culture medium. The exposures were made by flash photomicrography at intervals of ~ 7 seconds. The cytoplasm shows the characteristic zonation of an actively growing tube. The larger organelles are excluded from the apical 15 μm, which is occupied by the wall precursor bodies (cf. Fig. 27). Forward cyclotic streams carry mitochondria (M) to within 15–20 μm of the tip, and amyloplasts (S, distinguishable by their starch content) to within 30–35 μm, before they are carried back into the tube by reverse streams. The sequence shows that the tube tip does not extend linearly, but executes more or less regular, low amplitude, nutation-like migrations as it grows.

uously into the distal part, the vacated proximal stretches being cut off at intervals by the formation of internal partitions, usually referred to as plugs. The material of the abandoned wall never appears to be recycled, so it represents a net loss of reserves for the male gametophyte, but in some species it is known that the protoplast acquires fresh substrate by uptake from the intercellular material of the host pistil. The pollen tube therefore "feeds" as it passes through the female tissues, much in the manner of an invasive parasite (Linskens and Esser, 1959).

Rates of pollen-tube extension vary considerably among species, indicative presumably of differences in the speed of wall synthesis. Most published estimates of tube extension rate refer to growth *in vitro*, and so do not give a secure guide to the likely situation in the pistil. A comparison of tube growth in the style given by Stanley (1971) for two dicotyledons is of interest since it records one of the fastest speeds mentioned in the literature, that for *Oenothera organensis* (evening primrose). In this species, with a style length of 55 mm, a growth rate of 1.8 μm per second was observed. In contrast, in *Malus communis* (apple) with a style length of 7.7 mm, the rate was 0.097 μm per second. While the styles differ in length by a factor of less than 8, the tube extension rates therefore differ by a factor of almost 20. In this instance, the higher growth rate is attained in the longer style, but this relationship does not always apply. Pollen tubes of rye traversing a stigma some 4 mm in length were found to extend at up to 1.5 μm per second, while in comparable conditions those of maize, in which stigma length may exceed 50 cm, actually achieved the slightly slower rate of 1.14 μm per second (Shivanna *et al.*, 1978; Y. Heslop-Harrison *et al.*, 1984b).

In the following sections the sequence of events between the receipt of pollen on the stigma and the final approach of the pollen tube to the embryo sac is traced essentially in the natural order, except for the anticipatory interpolation of explanatory chemical and other data where appropriate. The treatment is necessarily selective, and many topics are touched upon only cursorily. Thus no detailed consideration is given to the extensive literature dealing with the requirements for pollen germination and pollen-tube growth in artificial culture, nor to various special metabolic aspects of germination. Furthermore, the treatment of pollen–pistil interactions is restricted to a review of certain aspects of pollen-tube guidance; and incompatibility phenomena, a vital part of breeding-system control in angiosperms, are not considered in any detail. These topics have been covered adequately in recent texts and reviews (e.g., Stanley and Linskens, 1974; De Nettancourt, 1977; Shivanna and Johri, 1985; chapters in Johri, 1984).

II. The Hydrodynamics of Germination

A. THE DEHYDRATION PHASE

During the final period of maturation in the anther, the pollen together with the anther tissues undergo rapid dehydration (Linskens, 1967a,b), and further water loss may follow after anther dehiscence and during dispersal, depending on temperature and the humidity of the atmosphere. Pollen water content has generally been expressed as percentage weight loss on heat drying, and published figures range from less than 6%, for example from species of *Populus* (Dumas *et al.*, 1984) to 35% and higher for grass species (J. Heslop-Harrison, 1979b). However, estimates based on weight loss do not necessarily provide a satisfactory basis for comparison. The dry weight of pollen is contributed mainly by storage reserves in the vegetative cell and by the various polymers of the pollen walls. These components vary widely between species in relation to the overall mass of the grain, so dry weight provides an equivocal base line for estimating the water content of the protoplasm of the gametophyte, which would presumably be better assessed by measurement of protein–water ratios. The use of NMR spectroscopy for measuring pollen water offers considerable promise (Dumas and Gaude, 1983; Dumas *et al.*, 1984; Duplan and Dumas, 1984), and reliable estimates of the degree of hydration of the vegetative cell protoplast, and ultimately of the distribution of water in different states within the whole pollen grain, may now be expected from this source.

Notwithstanding the uncertainties of the evidence, there is no doubt that in general during dispersal the male gametophyte has a substantially lower water content than a normal somatic cell. The consequences of this are manifold, expressed both in metabolism and structural organization. As in other desiccated plants and plant organs the metabolic rate of dormant pollen is low, as measured, for example, by respiratory rate and protein synthesis (Rosen, 1968; Mascarenhas, 1975; Hoekstra and Bruinsma, 1975, 1979). This is no doubt attributable in part to changes in membrane organization, enzyme conformation, and rates of substrate movement consequent on dehydration, much as has been suggested in explanation of the metabolic decline in desiccating seeds. In respect to protein synthesis, however, Bashe and Mascarenhas (1984) have pointed to another effect likely to be of considerable significance, namely the change in intracellular ion concentrations, notably of K^+, brought about by the withdrawal of water. These authors suggest that when K^+ concentration passes beyond the optimum range of 100–130 mM, binding of

mRNA to ribosomes is progressively inhibited, preventing the formation of initiation complexes and thus bringing protein synthesis to a halt. In their experimental material, dehydrated pollen of *Tradescantia paludosa* with a water content of 8%, the measured concentration of K^+ was ~280 mM, well beyond the level required entirely to inhibit protein synthesis.

B. HYDRATION

Reactivation of the pollen occurs on the stigma surface, or, in the case of stigmas bearing fluid secretions, partly or wholly in the secretion product. It has long been appreciated that the essential prerequisite is hydration (strictly, rehydration), which takes place mainly through water transfer from the stigma (Kerner and Oliver, 1904). Research during the last two decades has thrown increasing emphasis on this step as a vital one in pollen performance, and has led to a growing appreciation of the complexity of the process. Apart from environmental and other factors influencing the microclimate at the stigma surface, the most important regulatory influences are the control of water passage into the vegetative cell by structural and physiological adaptations of the pollen itself on the one side, and the properties of the stigma surface and its secretions on the other.

Von Mohl (1834) and various other early authors appreciated the importance of pores and slits in pollen grain walls as devices accommodating the grain to volume change. However, it was the perceptive work of Wodehouse (1935) that first brought out the sophistication of some of the regulatory functions of pollen walls. Mainly from his consideration of the Compositae, Wodehouse (1935) concluded that exine structure was often adapted in quite precise ways to accommodate the volume changes associated with dehydration and hydration of the vegetative cell, and introduced the term *harmomegathy* to describe the features concerned. Subsequent work has amply justified Wodehouse's conclusions (Payne, 1972, 1981; Blackmore, 1982).

In many pollens, the regulatory function of the walls in governing water movement in and out of the grain is associated with adaptations of the aperture sites, the regions of the wall forming the potential points of exit for the pollen tube (J. Heslop-Harrison, 1971). Characteristically, the apertures are areas where the more or less impermeable sporopollenin of the outer wall layer, the exine, is reduced or virtually absent. The inner layer, the pectocellulosic intine, is more permeable to water and water vapor, so the apertures form preferred pathways for water loss and gain. However, the movement is regulated by various devices which are themselves dependent on the changes in volume of the grain related to water

TABLE II

EXAMPLES OF POLLEN APERTURAL MECHANISMS REGULATING WATER MOVEMENT IN AND
OUT OF THE GRAIN[a]

Exine type	Sealing devices
With slits (colpate, sulcate)	Infolding, with buckling, folding, or interleaving sporopollenin shutters; lipid seal (e.g., *Lilium*)
Porate	
Single pore	Operculate; no lipid seal (Gramineae)
Multiple pores	(a) Operculate; with or without lipid seal (e.g., Caryophyllaceae)
	(b) Occlusion by sporopollenin granules; lipid seal (e.g., Malvaceae)
Colporate; triaperturate	Infolding, with simple folding or buckling shutters or interleaving sporopollenin plates; with or without lipid seal (commonest dicotyledonous types)
Inaperturate (or omniaperturate)	Granulate or irregularly incised exine; lipid seal

[a] Modified from J. Heslop-Harrison (1979c).

movement. Control is imposed through variation in the area of exposed intine, and in some species also by lipidic surface materials derived from the tapetum in the later stages of pollen maturation. Some examples of regulatory apertural mechanisms are listed in Table II, and various details are discussed more fully by J. Heslop-Harrison (1979c).

Pollen reaching a receptive stigma will normally have equilibrated with the atmosphere, and this will often mean that it will have near to its minimal water content, with the apertures closed by the sealing devices where these are present. Hydration on the stigma can be measured by volume change, and time curves for three species with approximately spherical grains but different apertural types are shown in Fig. 2.

The initial ingress of water resulting in the dilation of the grain stretches the exine and brings about changes in the shape and area of the apertures. Examples from two species with different aperture types are illustrated in Figs. 3 and 4. In general, the consequence of the onset of hydration is to increase the area of exposed intine, and so to lessen the hydraulic resistance to further inflow of water. Uptake is therefore governed by a positive feedback mechanism acting to facilitate further inward flux as the water content increases. In species with lipidic apertural seals (Table II), dispersal of the lipids as the grain dilates similarly decreases the impedance to water ingress. Interestingly, in some species the process
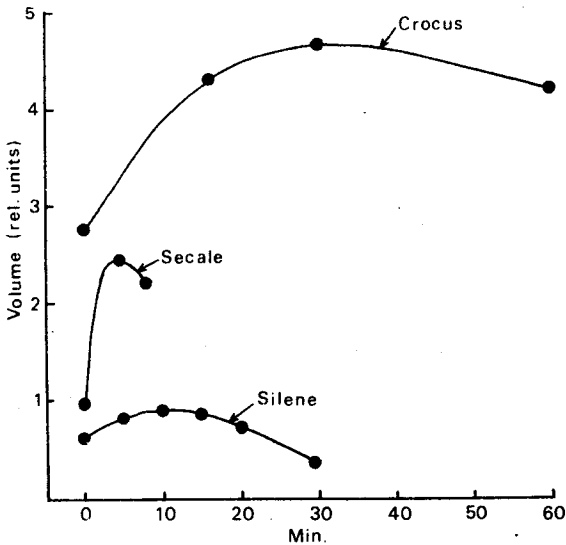
FIG. 2. Volume changes during hydration and germination in pollen grains of *Crocus chrysanthus*, *Secale cereale*, and *Silene vulgaris* recorded photographically on the stigma. In each case the pollen tube emerged at the point of maximum volume. From J. Heslop-Harrison (1979c).

can be arrested and reversed in experiment, providing a glimpse of how pollen in the natural environment of the stigma surface may adapt to changing water balance without necessarily losing the competence for germination when conditions ultimately do become favorable (Fig. 5).

No consideration of pollen rehydration, metabolic reactivation, and germination can be complete without reference to the conditions encountered on the stigma surface. For the successful attainment of fertilization, pollen and stigma must be closely coadapted, the stigma providing not only a suitable chemical milieu for pollen function but also the appropriate physical conditions for attachment and water transfer. The variation in the requirements of different pollens for germination *in vitro*—and indeed the fact that some cannot be grown at all on or in artificial media—reflects in part the substantial differences among angiosperm species in the stigma environment. A broad distinction can be made between species where the stigma is "dry" in the sense that it carries no free-flowing secretion, and those where the stigma surface is "wet," bearing just such a secretion during the receptive period (Y. Heslop-Harrison and Shivanna, 1977).

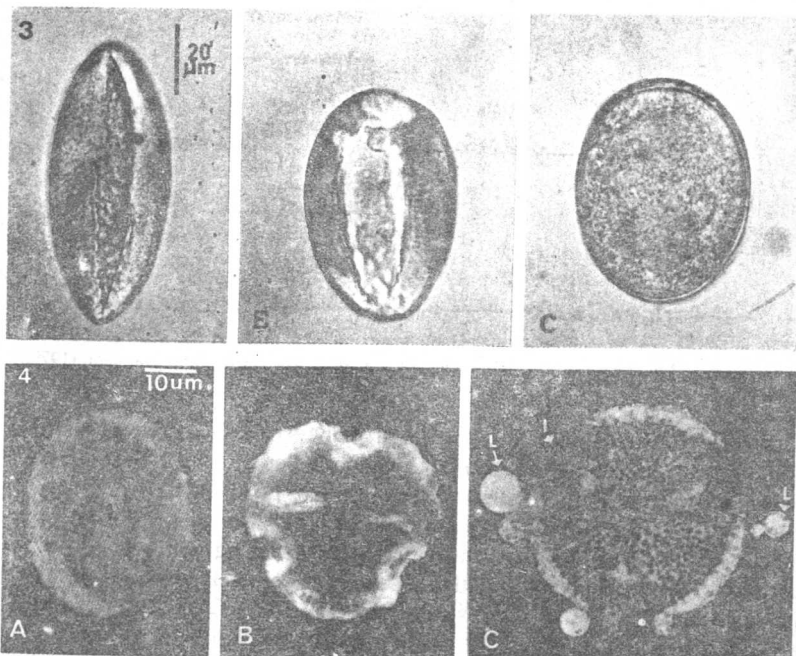The receptive surface cells of dry-type stigmas invariably bear a thin

FIG. 3. Pollen grains of *Iris* sp. suspended in media of osmotic pressures allowing varying degrees of hydration. (A) Medium permitting virtually no expansion; the surface lipid (L) is held firmly in the colpial slit. (B) Medium allowing some hydration; colpial lipid beginning to disperse. (C) Fully hydrated grain in germination medium.

FIG. 4. Tricolporate pollen grains of *Tagetes patula* (Compositae). (A) Suspended in a medium of an osmotic pressure preventing hydration; equatorial view showing one colpus, closed and sealed by lipid (L). (B) As A, but in polar view, showing all three colpi. (C) Grain in a medium allowing full hydration, polar view. The aperture sites are now gaping, revealing the intine (I), and the lipids (L) are dispersing into the medium. Fluorescence micrographs, auramine O staining for the sporopollenin of the exine and the surface lipids.
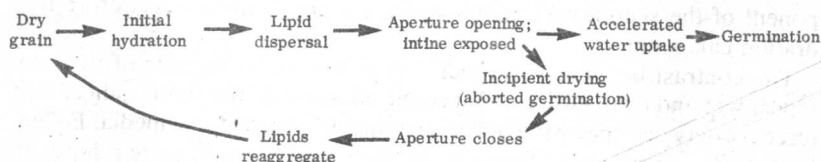

FIG. 5. Pollen hydration sequences on the "dry" type of stigma. The upper track shows an uninterrupted progression from the desiccated grain to germination. Should circumstances prevent full hydration, germination may be aborted and the apertures may close again, as in the lower track. From J. Heslop-Harrison (1979c).

nonapertural
exine

pectocellulosic
wall   cuticle   intine

plasma-
lemma   meniscus   apertures   plasma-
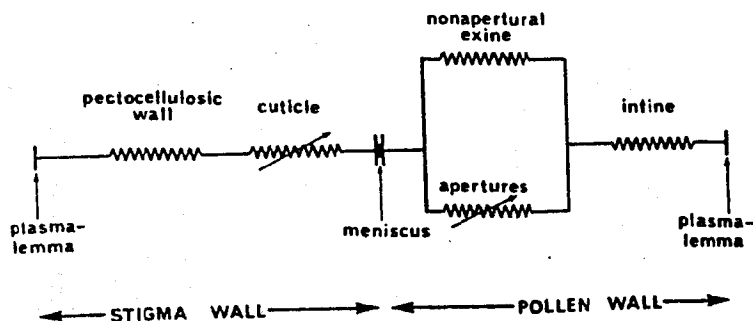lemma

◄——— STIGMA WALL ———►   ◄——— POLLEN WALL ——►

FIG. 6. The resistances encountered by water passing through the stigma and pollen walls expressed in the form of a simple electrical analog. From J. Heslop-Harrison (1979a).

surface secretion or pellicle, which is proteinaceous and often with a lipid component (Mattsson *et al.*, 1974), to which pollen attaches. The secretion overlies a discontinuous cuticle. This is made up of islets or rodlets of cutin in the outer part of the pectocellulosic cell wall, a structural organization which could provide a means for regulating water transfer according to the turgidity of the stigma cells (J. Heslop-Harrison *et al.*, 1975; J. Heslop-Harrison and Y. Heslop-Harrison, 1982c). Taking into consideration the adaptations of the pollen grain itself for controlling water movement, the system over all may be summarized in the simple electrical analog of Fig. 6.

With stigmas of the "wet" type, the pollen is received immediately into a fluid matrix, or it becomes inundated with fluid after the rupture of the stigma surface cuticle and the release of the underlying secretion, as in the papilionoid Leguminosae (J. Heslop-Harrison and Y. Heslop-Harrison, 1983). When the secretion is watery, or when it constitutes a lipid–water emulsion with water forming the continuous phase, hydration is likely to begin immediately and proceed with expedition. Uptake may be less rapid when lipid is the continuous phase, or when, as in *Petunia* (Konar and Linskens, 1966a,b) the lipid layer forms a shield over the aqueous component of the secretion, through which the grains must pass before hydration can begin.

The contrast between the conditions presented by stigmas of the two types, wet and dry, suffices to account for some of the more radical differences between species in pollen germinability in artificial media. Pollen from plants of the wet-stigma type is often found to germinate readily in liquid media with the appropriate osmotic balance, whereas that from dry-stigma species is often refractory, requiring special conditions to establish something near a natural hydration rate (Bar Shalom and Mattsson, 1977):

These comments refer to the general trends; the correlation is by no means perfect, and there are in any event no special rules for species with intermediate stigma types.

Not all of the regulation of pollen hydration on the stigma surface can be attributed to control of the water flux by nonspecific physicochemical factors. Earlier indications that differences in hydration rate could be involved in some types of self-incompatibility response have been amply confirmed by recent work (Dumas and Gaude, 1983). In the genus *Brassica,* the self-incompatibility system is of the sporophytic type, the reaction being governed by the genotype of the pollen parent with the inhibition of incompatible pollen occurring at or near the stigma surface. Zuberi and Dickinson (1985) found that the rejection of incompatible pollen is positive and effective in conditions of relatively low atmospheric humidity, but less so in humid conditions when water can be taken up more readily from the atmosphere. Moreover, these authors showed that, tested *in vitro,* pollens of different compatibility genotypes hydrate from the atmosphere at different rates, presumably indicating different water affinities, perhaps determined by the pollen surface materials. In a system of quite another type in the heterostylous genus *Primula,* the control of pollen hydration on the stigma surface is also a major factor in the self-incompatibility response (Shivanna *et al.,* 1983). In *Primula vulgaris* both pollen and stigma are dimorphic, and hydration rates differ in compatible and incompatible pollinations. In this instance, removal of surface materials from viable pollen reduced the rate of uptake of atmospheric water in the thrum morph, indicating that these fractions, derived originally from the tapetum of the parent plant, are in some, as yet obscure, manner involved in determining the response.

C. FORCES CONCERNED IN WATER PASSAGE

The analog given in Fig. 6 presupposes that water will pass steadily from left to right, that is, from the stigma into the pollen. For this to happen, a water potential gradient must be maintained between the stigma and the vegetative cell throughout the period of hydration and subsequent germination. However, Watanabe (1955) observed what appeared to be an unusual phenomenon in grass pollens, one not readily accounted for by the assumption that the vegetative cell acts as a simple osmotic system throughout the hydration period. He found that hydration of wheat and other pollens on the stigma surface is not accompanied by a continuous dilation of the grain, for the initial period of enlargement is followed by an interval when liquid is actually exuded from the grain surface, subsequently to be resorbed as hydration continues. From a study of 28 spe-