POLLEN-STIGMA INTERACTIONS IN BRASSICA
IV. STRUCTURAL REORGANIZATION IN THE POLLEN GRAINS DURING HYDRATION

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SUMMARY

With the aid of osmium tetroxide vapour, dry pollen and pollen at various stages of hydration has been fixed anhydrously for examination with the transmission electron microscope (TEM). In addition to establishing features characteristic of grains at different states of hydration, this technique has enabled the detection of a superficial layer investing both the exine and the pollen coating. This layer, some 10 nm in depth, binds both lanthanum and Alcian Blue and is shown to be the first component of the pollen grain to make contact with the stigmatic pellicle. The use of vapour fixation has also rendered it possible to chart the passage of water into the pollen grains with great accuracy, for each level of hydration displays a strikingly different cytoplasmic organization. For example, dry pollen is characterized by the presence of unusual structures at the protoplast surface and large numbers of spherical fibrillar bodies, whilst the protoplast of hydrating pollen is conspicuously stratified and contains a peripheral layer of membranous cisternae, subjacent to which is a fibrillar matrix derived from the spherical bodies found in the dry grains. Vapour-fixed, fully hydrated pollen resembles conventionally fixed grains.

The pollen coating appears electron-translucent after anhydrous fixation and contains discrete, slightly rounded bodies some 50 nm in diameter. The uptake of water by grains on the stigma is accompanied by conspicuous structural changes in this layer for, after a short period in contact with the papillar surface, the spherical bodies rapidly disappear and the coat becomes electron-opaque. Close examination of this 'converted' coating reveals the presence of membranous vesicles and other structural components.

INTRODUCTION

It has long been appreciated that the pollen of many angiosperms is dispersed in a highly desiccated condition (Knowlton, 1921; Vinson, 1927) and evidence is now accumulating that the low levels of water contained in the grains play an important role in the maintenance of pollen viability (Barnabas & Rajki, 1976). Thus, in addition to being an organ capable of capturing pollen grains, the stigma must also possess the machinery for ensuring a carefully regulated rehydration of the pollen grains before their germination (Heslop-Harrison, 1979). Further, it has been suggested that disturbance of the accurately metered transfer of water from the stigma to the pollen takes place during the operation of the self-incompatibility (SI) system of plants such as Brassica oleracea, where inhibition of self-pollen occurs on the stigma surface, and pollen compatibility is sporophytically determined (Roberts, Stead, Ockendon & Dickinson, 1980; Ferrari, Lee & Wallace, 1981). However,

Key words: anhydrous fixation, Brassica oleracea, pollen coating, pollen hydration.
hydration of pollen is not easily assessed in vitro since any contact with aqueous media results in the rapid assumption of an hydrated form. Notwithstanding, an accurate knowledge of the degree of pollen hydration is most important, for, while earlier studies pointed towards the complete failure of pollen to hydrate after incompatible matings (Roberts et al. 1980), more recent work has provided evidence of a partial hydration of incompatible pollen (Zuberi & Dickinson, 1985). In this connection it is also noteworthy that high levels of atmospheric humidity have long been known to overcome, wholly or partially, the operation of the SI system in Brassica (Carter & McNeilly, 1975, 1976). Although the degree of hydration of pollen grains may easily be assessed in vivo by the calculation of the ratio between the two axes of the grain (Stead, Roberts & Dickinson, 1979), conventional electron microscopic techniques have, for the reasons outlined above, failed to provide any ultrastructural information pertaining to the hydration of pollen grains on stigmas. We have therefore investigated the use of anhydrous fixation techniques for the preparation of pollen for the transmission electron microscope (TEM) in the hope that they may enable us to identify different cytoplasmic states with different degrees of hydration. This approach has proved startlingly successful and, in addition to identifying different stages of pollen hydration, both in vivo and in vitro, it has proved possible to detect elements of the pollen surface, the presence of which was entirely unsuspected.

MATERIALS AND METHODS

Two inbred lines of B. oleracea homozygous for the incompatibility alleles S63 and S25 were raised from seed kindly provided by Dr D. J. Ockendon (NVRS, Wellesbourne, Warwick, U.K.). At the onset of flowering, plants were removed from a greenhouse to a growth room maintained at 15°C. Immediately before use, freshly dehisced anthers were excised from flowers and, using a small paintbrush, the pollen was brushed into watch glasses or directly onto stigmas. All the stigmas used were of the S63 genotype. Compatible matings were S63XS25, following which pollen tubes were produced within 2h, and incompatible matings were self-pollinations of S63. The progress of the various pollinations was monitored using the Aniline Blue fluorescence method (Linskens & Esser, 1957).

The material was vapour-fixed in a large sealed jar containing an opened vial of osmium tetroxide crystals and, where appropriate, silica gel or phosphorous pentoxide as a desiccant. Pollen grains were placed in watch glasses, either dry or floating on germination medium. For staining components of the pollen wall coating, 1% lanthanum nitrate (Agar Aids, Stanstead, Essex, U.K.) or 0.1% Alcian Blue (CCI 74240, BDH Chemicals Ltd, Poole, Dorset, U.K.) was added to the germination medium (CaCl2·6H2O, 1.66×10⁻³ M; KNO3, 1.17×10⁻³ M; H3BO3, 0.085×10⁻³ M; Tris, 5.5×10⁻⁴ M and 20% (w/w) polyethylene glycol (M, 1000). Flowers were placed with their peduncles in a nutrient medium in small plastic caps well sealed with ‘Parafilm’. Material was exposed to the vapour for between two and four hours, although darkening of all tissue occurred within the first few minutes. Following removal from the jar, specimens were swiftly encapsulated in warm agar (3% containing 0.05% Triton X-100). When floating pollen was fixed, the germination medium was removed from beneath the grains in the watch glasses before encapsulation, using a Pasteur pipette. Samples of pollen were allowed to hydrate for appropriate periods in a moist atmosphere (100% relative humidity) before being vapour-fixed.

After encapsulation, the agar blocks were cut to a convenient size and transferred to 0.07 M-phosphate buffer at pH 7.2 containing 2% glutaraldehyde for 2h. Control samples of pollen were conventionally fixed (Dickinson & Lewis, 1973) after first encapsulating them in agar. The agar blocks were dehydrated in an acetone series and finally embedded in Epon 812, using 1,2 epoxy-propane as carrier. Sections from these blocks were stained with uranyl acetate and lead citrate.
(Reynolds, 1963), and examined in either a JEOL 100B TEM operating at 60 kV, or a Hitachi S-800 at 75 kV.

When using phosphotungstic acid as a plasma membrane stain, sectioned material, mounted on gold grids, was floated for 20 min on 10% (w/v) aqueous solution of hydrogen peroxide at room temperature. Following washing in distilled water, the grids were transferred to drops of the phosphotungstic acid solution (1% (w/v) phosphotungstic acid in a 10% (v/v) aqueous solution of hydrochloric acid) for 15 min at 25°C. Stained sections were rinsed twice in distilled water, dried and then examined in the electron microscope, with and without conventional post-staining.

**RESULTS**

*The structure of the pollen cytoplasm at different stages of hydration*

Using axis ratio as an indication of pollen hydration, grains were vapour-fixed at various stages of hydration, both in the anther and following exposure to high humidity. In addition, pollen was vapour-fixed late in its maturation when, presumably, water is actively pumped from the loculus. Recent work (Zuberi & Dickinson, 1985) indicated pollen to be at its most dehydrated at anthesis, and grains were also fixed at this point. The cytoplasm of vapour-fixed pollen varied strikingly in its image depending upon the degree of hydration of the grain. Features characteristic of each stage of hydration are set out below.

**Dry pollen**

The cytoplasm of pollen fixed at anthesis, and that retained in a desiccator for prolonged periods, contains many spherical, electron-opaque inclusions apparently composed of fibrillar material (see Figs 1, 2). These spherical bodies are assembled in a layer beneath the protoplast surface. While these cells are not bounded by a clearly evident plasma membrane, traces of membrane-like profiles were present at the cell periphery. These short sections of membrane were often associated with fibrillar strands, apparently extending perpendicularly from the membrane surface into the intine (see Figs 4, 5). Both the spherical bodies and these radial strands bind phosphotungstic acid (see Fig. 6). The interior of the grain is dominated by larger spherical bodies some 1 μm in diameter, invested by flattened, ribosome-encrusted, cisternae of endoplasmic reticulum (ER) (see Figs 2, 14). Dictyosomes, closely packed and generally without associated vesicles are present, as are numerous mitochondria. It is interesting that the mitochondria fixed in these dry cells differ strikingly from 'normal' morphology but this difference most probably results from changes occurring during fixation, rather than differences in the organelles in *vivo*, for mitochondria in hydrated cells (e.g. see Fig. 15) present the same image.

The cytoplasmic organization of dry pollen is depicted diagrammatically in Fig. 3A.

**Partially hydrated pollen**

The cytoplasm of pollen fixed in the anther before anthesis resembles in many ways that of pollen that has undergone partial rehydration in a moist atmosphere (see Fig. 7). In contrast to the dry pollen, the prooplasts of these cells contain...
accumulations of fibrillar material that occupy the space originally taken by the spherical bodies. This layer, and a thinner layer of electron-opaque ER cisternae between it and the protoplast surface, give the protoplast a strikingly stratified appearance. By this stage the plasma membrane itself may often be identified clearly although the activity of vesicles at the cell surface generally renders this boundary very indistinct. The radial strands seen in the dry pollen are also present in this material, and it is interesting that close examination of the protoplast surface reveals that these strands frequently possess a membrane-like structure (see Fig. 8), indicating that they may not simply be individual elements of the fibrillar intine. Further, there are some profiles (see Figs 10, 11, 12) that suggest that this 'stranded' layer may be derived from a layer of vesicles. The plasma membrane, when visible, the radial strands and the fibrillar layer all react following staining with phosphotungstic acid (see Fig. 9).

The origin of the large numbers of vesicles present in the cytoplasm subjacent to the plasma membrane is not immediately clear from the electron micrographs. Certainly vesicles resembling those at the surface may be seen in association with very active dictyosomes, but these vesicles are also observed close to the elements of ER that invest the large spherical bodies deeper in the cytoplasm and anastomose through the fibrillar layer (see Fig. 13). Indeed, profiles may also be observed indicating contact between this ER and the dictyosomes themselves.

Fig. 3B depicts the organization of partially hydrated cytoplasm.

**Fully hydrated pollen**

Once grains have achieved their maximum change in shape in a moist atmosphere they are assumed to be fully hydrated. The cytoplasmic contents of material vapour-fixed at this stage appear fairly evenly dispersed, the stratification and fibrillar appearance being lost (see Fig. 3c). However, in the final stages of rehydration, a

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**Fig. 1.** Dry pollen, vapour-fixed. Note the evenly staining pollen coating (c) and the coating superficial layer (arrows). The cytoplasm features spherical bodies (s) containing a fibrillar matrix. TEM; X27 000.

**Fig. 2.** Material as depicted in Fig. 1. Beneath the layer of fibrillar inclusions (s) there is an accumulation of electron-lucent spherical inclusions (arrows), invested by rough endoplasmic reticulum. TEM; X9500.

**Fig. 4.** Material as shown in Figs 1 and 2, but showing as area of the cell surface containing plasma membrane (arrows). The fibrillar strands extending from the plasma membrane to the intine (i) are clearly visible. TEM; X83 000.

**Fig. 5.** As Fig. 4, but showing a region of the cell surface where the plasma membrane is less well organized (arrows). Indeed, in some areas (circle), profiles reminiscent of vesicles may be seen. TEM; X46 200.

**Fig. 6.** Dry pollen, unstained, but reacted with phosphotungstic acid. The fibrillar inclusions (i) and the protoplast surface (arrows) react with the stain. TEM; X26 000.

**Fig. 7.** Protoplast surface of hydrating pollen grain. Note the stratification of the cytoplasm into a superficial layer of cisternae (c) and a subjacent fibrillar layer (f). A mitochondrion (m) is well shown. As with some of the dehydrated material the protoplast is bounded not by a plasma membrane, but by a palisade of vesicles (arrows). TEM; X35 500.
Fig. 3A. Diagrammatic representation of the cytoplasm and wall of a fully dehydrated pollen grain. Both the pollen grain coating (c) and the exine (e) are invested by a membrane-like layer (l). The cytoplasm contains spherical fibrillar bodies (s), and larger inclusions (o) bounded by rough endoplasmic reticulum (er). The plasma membrane is not easily discerned, instead a palisade of vesicles (p) occupies its normal position.
Fig. 3B. As A, but showing hydrating pollen. The cytoplasm has become stratified. The spherical fibrillar bodies have commenced to fuse into a layer of larger fibrillar vesicles (v), while the endoplasmic reticulum (er) has permeated this layer to make contact with cisternae (arrows) at the protoplast surface. The vesicular palisade (p) has become more evident, and a plasma membrane may also be seen below it. A mitochondrion (m) is also shown. See legend to A for further details.
Fig. 3c. As A, but showing fully hydrating pollen. The stratification of the cytoplasm has been lost, together with the fibrillar layer. Instead mitochondria (m) and cisternae of ER (er) populate the cytoplasm. The plasma membrane is well defined (arrows) and the vesicular palisade (p) is only present on occasions. See A for further details.
massive reorganization of the cytoplasm does take place, preceding the emergence of the pollen tube (see Fig. 15).

The pollen coating following anhydrous fixation

The pollen coat of vapour-fixed material is, apparently, relatively unaffected by the degree of pollen hydration. The layer appears electron-translucent and contains small, discrete, rounded particles some 50 nm in diameter. In contrast to the pollen fixed conventionally the coat was tightly packed between the baculae of the exine, and sealed into the pollen wall by a superficial layer some 10 nm in depth (Fig. 16A–D). The conventionally fixed coating is devoid of this layer, and bulges out from between the exine. In some cases it appears to fragment (see Fig. 17).

In a preliminary investigation into the nature of the coating superficial layer (CSL), pollen grains were vapour-fixed while floating on germination medium containing 1% lanthanum nitrate or 0–1% Alcian Blue. Figs 18, 19 show both these stains to bind strongly to the CSL. Further, phosphotungstic acid also displayed affinity for this layer. The fact that this layer is not only formed over the tops of the bacula, but also is mobile over the coating surface (see Fig. 16C, D) suggests that it is a true boundary and not an artefact of osmic vapour fixation.

Structural changes accompanying pollination

Changes in the CSL. Anhydrous fixation of stigmas reveals the cuticle as an electron-translucent layer covered by a pellicle composed of electron-opaque material (see Fig. 20). It is this pellicle that first interacts with the CSL on pollination and, while the eventual consequences of this event depend upon the compatibility of the mating, the pellicle and the CSL appear to fuse following both compatible and incompatible pollinations (see Fig. 20). In incompatible crosses the fusion is very rapid and the pellicle and CSL rapidly become indistinguishable from the surrounding matrix.

Hydration of grains on the stigma surface. Both compatible and incompatible grains hydrate. The cytoplasm of these grains may be observed to undergo the changes outlined earlier from dry, through partially hydrated, and finally to attain the cytoplasmic organization characteristic of fully hydrated grains. We are confident in our identification of the fully hydrated pollen since it is only from grains in this condition that pollen tubes are produced. Hydrating incompatible grains are indistinguishable from compatible, although there is some preliminary evidence that pollen on incompatible stigmas achieves full hydration less rapidly.

Changes in the pollen coat following pollination. The region of the pollen coat bridging the grain and the stigmatic papillae undergoes a dramatic conversion as the pollen hydrates. It is transformed from its traditional electron-lucent aspect to an electron-opaque matrix of densely packed membranous assemblies (see Fig. 21), via an intermediate ‘granular’ state. The significance of this conversion remains to be determined, but it takes place in pollen coats when in contact with stigmas of either
compatibility. Traces of coat 'conversion' were also observed in pollen grains fixed in the presence of lanthanum and Alcian Blue, but never on grains vapour-fixed in the anther, or hydrating in a moist atmosphere. The speed of conversion may differ depending upon the compatibility of the mating and the incompatibility (S) genes present, but complete conversion of the coat normally takes place within 20 min of a compatible pollination. Interestingly, conversion always starts at that surface of the coating apposed to the pellicle (see Fig. 22).

**DISCUSSION**

**The value of anhydrous fixation**

The use of osmium tetroxide vapour is by no means new, but has normally been associated with techniques for enzyme localization (Knox & Heslop-Harrison, 1971). Its use for the fixation of pollinated stigmas has permitted characterization of features that appear to be linked directly to the water content of the pollen at the time of fixation. It is well appreciated that vapour fixation may not result in the best presentation of all features of the pollen cytoplasm, and this is particularly the case for membranes that appear 'negatively' stained. Nevertheless, the results obtained are adequate to provide an accurate impression of changes in pollen cytoplasm as it imbibes water, and in the case of hydrated cells, may easily be compared with the 'conventional' morphology.

**The nature of the CSL and its role in pollination**

The discovery of the CSL investing both the exine and the pollen coat was unexpected. This bounding layer is only detected after vapour fixation and must be involved in the initial contact and adhesion between the grain and the stigmatic

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Fig. 8. Detail of vesicular layer at the surface of a hydrating protoplast. In some places (circle) membranous profiles may easily be seen. TEM; ×51 200.

Fig. 9. Material as shown in Fig. 7 but reacted with phosphotungstic acid. Note the affinity of the subjacent fibrillar layer (f) and the protoplast surface (arrows) for the stain. TEM; ×28 000.

Fig. 10. Vesicular activity at the surface of a hydrating protoplast. Note the population of small vesicles (arrows) apparently discharging at the cell surface. TEM; ×72 500.

Fig. 11. Tangential section showing the interaction between membranous vesicles and the cell surface. The discharging vesicles (arrows) seem to merge with the intine (i) of the pollen wall. TEM; ×77 000.

Fig. 12. A stabilized area of the cell surface of a hydrating pollen grain. Although there are suggestions of a plasma membrane, the major part of the cell surface is taken up by vesicles (arrows). The ER cisternae (c) in the peripheral layer are well shown. TEM; ×47 900.

Fig. 13. Vapour-fixed hydrating pollen grain showing cisternae of endoplasmic reticulum (arrows) traversing the fibrillar layer (f). The protoplast surface (s) is at the top right of the micrograph. TEM; ×51 000.

Fig. 14. Material as shown in Fig. 2, but showing details of the spherical inclusions (s) bounded by elements of the endoplasmic reticulum (arrows). The whole system is encrusted with ribosomes. Note the mitochondrion (m). TEM; ×32 000.
pellicle. However, this may well be its sole function since fusion is observed in both compatible and incompatible matings.

While clearly not a membrane in the conventional sense, the CSL both binds and excludes lanthanum, a property of true biological membranes, and its reaction with Alcian Blue and phosphotungstic acid indicates the presence of a mucopolysaccharide. The CSL does not correspond with the Exinic Outer Layer, described by Gaude & Dumas (1984) and Dumas, Knox & Gaude (1984). These authors describe a 'membrane-like' boundary investing the sexine of the sporopollenin pollen wall, not the pollen wall coating. While the function of the Exinic Outer Layer has yet to be conclusively proven, there is little doubt that it plays an important part in the binding of the coating to the exine surface (Gaude & Dumas, 1984; Dickinson & Roberts, 1985).

Changes in the pollen coat during hydration on the stigma

Both compatible and incompatible contacts between the stigma and the pollen grain result in a dramatic conversion of the pollen coat. Close examination with the electron microscope enables us now to distinguish two forms assumed by this layer: (1) the electron-translucent granular form of dry pollen, anther-held grains or after hydration in a moist atmosphere; (2) the converted coat, which stains very heavily with uranyl acetate and lead citrate and appears to consist of many membranous inclusions.

The granular form of the coat seems to be the state in which the coat is at its most mobile. The coat flows very readily on contact with a stigma, or even another pollen grain, and it is probably after this movement that coat conversion first occurs. Surprisingly, Alcian Blue not only induced some coat conversion but also caused quite extensive reorganization, with the coat flowing around the bacula. These observations suggest that coat conversion is the consequence of contact between the CSL and another surface or substance with which it can interact. The flowing of the coat that precedes conversion is a mechanism that presumably results in an increase in the area of contact between grain and stigma. The significance of coat conversion is not immediately clear; while it is likely that it indicates the passage of water through the coat, it is not known whether it is the cause or the result of this passage. The fact

![Fig. 15. Fully hydrated pollen cytoplasm, vapour-fixed. The region shown is that where the tube emerges from the grain, and part of the intine (i) is visible. Note the lack of any form of stratification in this cytoplasm. TEM; \( \times 34000 \).](image1)

![Fig. 16. A. Vapour-fixed dry pollen coat showing a thin superficial layer (csl) and small electron-opaque bodies (arrows) within the coating, e, exine. TEM; \( \times 95000 \). B. Material as depicted in A, but showing the coating superficial layer (csl) to advantage. e, exine. TEM; \( \times 108000 \). C. Material as depicted in A, but showing the coating superficial layer (csl) being compressed into folds (arrows) around the bacula of the exine (e). TEM; \( \times 90000 \). D. Material as depicted in A, but showing detachment of the coating superficial layer (csl) around the colpal region (arrows) of the grain. TEM; \( \times 15000 \).](image2)

![Fig. 17. Dry pollen fixed 'conventionally' using glutaric dialdehyde and osmic acid. Note that the pollen coating (c) has become electron-opaque and vesicular. This layer is clearly mobile during this treatment, for it appears to have moved out from the exine (arrows). TEM; \( \times 19000 \).](image3)
that pollen grains hydrated in moist atmosphere show no coat conversion suggests that when water enters through the colpal regions no conversion takes place. The binding of lanthanum or Alcian Blue to the CSL significantly increases the degree of coat conversion occurring in pollen fixed while hydrating on a liquid medium, reinforcing the view that conversion on the stigma surface occurs as a response to the interaction between the pellicle and CSL.

**Cytoplasmic transformation during hydration**

In *B. oleracea* both compatible and incompatible matings result in some hydration of the pollen grains. Pollen on self or cross stigmas proceeds from the characteristic dry state, containing many spherical bodies at the periphery of the protoplast, through the stratified-fibrillar condition characterizing partial hydration, to the fully hydrated grain. If, as seems to be the case, water from the stigma is equally available to compatible and incompatible grains alike, we must again consider the role of inhibitors in controlling incompatibility. The 'recognition event', wherever that may occur, must provide a signal for the synthesis or mobilization of an inhibitor either from the stigma (Hodgkin & Lyon, 1984), or possibly in the grain itself. This ultrastructural study of the hydrating cytoplasm has, however, provided no firm clues as to the point at which the incompatible grain ceases development.

**The protoplast membrane**

In a comprehensive examination of pollen plasma membranes of many species Dumas & Gaude (1981) quote *B. oleracea* as possessing an intact plasma membrane at all times. For this reason we anticipated that vapour fixation would reveal the presence of this membrane in pollen at all stages of dehydration, and rehydration. Even taking into account the fact that osmium tetroxide often fails to stain membranes positively, the organization of the protoplast periphery of dry or rehydrating pollen presents a confusing picture. There is certainly some evidence of a plasma membrane even in the driest pollen, but it is discontinuous and appears to possess...
fine connections with the intine of the pollen wall. Closer examination of this layer suggests that whilst many of these strands are extensions of the fibrillar intine, others have a unit-membrane-like profile. Thus, in some regions the 'plasma membrane' may be derived from the innermost face of a palisade of small vesicles situated at the proplast surface. Indeed, in favourable sections these vesicles may be seen. As hydration continues the plasma membrane is seen rapidly to assume full continuity, although the stranded connections with the intine remain present. When the pollen achieves full hydration, a more conventional organization is observed at the proplast surface, and a clear plasma membrane may easily be discerned.

The consequence of these observations is not easily evaluated. It appears that the proplast is bounded by membranes at all times, albeit not always in the form of a monolayer. Indeed, during the earliest stages of hydration the surface of the proplast membrane appears to be composed of a mosaic comprising an organized plasma membrane in some regions, active vesicular cytoplasm in others, and yet other areas occupied by layers of small vesicles. It is not easy to predict how such a cell surface would function physiologically, although it would certainly possess some of the features of a selectively permeable plasma membrane. This type of surface organization is also present in the pollen grains in the anther during the final stages of the hydration; indeed, the cytoplasmic organization of these anther-held pollen grains is very reminiscent of the stratified stage of rehydrating grains (Dickinson & Elleman, unpublished). This similarity in cytoplasmic organization suggests that essentially similar processes are taking place both before and after anther dehiscence, and that they are merely arrested by the final desiccation of the grains. The principal events taking place in these cells appear to be the discharge of vesicles derived from both the dictyosomes and the cisternae of the endoplasmic reticulum and, under normal circumstances, these would fuse with an intact plasma membrane. As, however, water is progressively withdrawn from the pollen proplast the simple bimolecular leaflet of the plasma membrane appears to be unable to reorganize to form a complete layer and, instead, the vesicles are left to discharge directly into the intine. In the final stages of dehydration even the organization of the membranes investing these vesicles seems to be lost – for the driest pollen contains very little membranous material at all. During rehydration of the grain, either in vitro or on the stigma, the reverse of these events takes place with the cell surface first being composed of a layer of active vesicles, and then bounded by an organized plasma membrane. It seems therefore that in B. oleracea a condition exists that is intermediate between that seen in the Gramineae (Heslop-Harrison, 1979), where lipidic micelles are formed at the cell surface, and that of plants with wet stigmas such as Lilium (Dickinson, Moriarty & Lawson, 1982), where pollen possesses a plasma membrane at all times.

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