

A molecular basis for the self-incompatibility system operating in *Brassica* sp.

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Abstract

Molecules contained in the sporophytically-derived coating of the pollen grain and in the superficial pellicle of the stigmatic papillae control the self-incompatibility response of the breeding system of *Brassica*. The stigmatic pellicle consists of a lipidic matrix in which float a mosaic of proteins many of which can rapidly be renewed from pools in the papillar cytoplasm. A fraction of these proteins are involved in facilitating the passage of water to the pollen whilst another, possibly a glycoprotein, suppresses this activity in an incompatible mating. The pollen coating must also contain two sets of active molecules, one for identifying the stigmatic recognition molecules, and another for effecting the changes that take place to the coat itself on compatible pollination. In essence, the self-incompatibility mechanism appears to operate through the control of water flow from the papilla to the grain. Even when incompatible grains manage to germinate by obtaining atmospheric water, their proteins will often stimulate a reaction in the stigmatic papilla once the cuticle has been penetrated.

INTRODUCTION

The modes of action of the self-incompatibility (SI) mechanisms found in the breeding systems of angiosperms are of considerable academic as well as commercial interest. The acquisition of SI systems has been held to be responsible for the rapid ascendancy of the land plants (Whitehouse, 1950) while, at a cellular level, SI mechanisms provide one of the best-known examples of intercellular communication in plants. In *Brassica* sp., development of self or otherwise incompatible pollen is inhibited immediately on pollination, and the pollen tube, if it grows at all, fails to gain entry into the papillae of the stigma (Stout, 1931; Tatebe, 1939). With the advent of the electron microscope, these short incompatible pollen types were reported to assume an "appresolia-like" aspect (Ockendon, 1972) and, on occasion, to engender some form of cytoplasmic 'reaction' in the stigmatic papillae (Kanno, Hinata, 1969).

Plate I

Except where stated, figures are electron micrographs taken of material prepared according to the methods set out in Dickinson and Lewis (1973a, b).

Fig. 1. Tapetum prior to disintegration showing vacuoles (V) containing protein, and elaioplasts (E) rich in lipids. $\times 1490$

Fig. 2. As Fig. 1, but showing the relatively 'clear' exine (arrows) of the pollen grain. This material is *Raphanus* rather than *Brassica*, but the cells are identical at this stage. $\times 4210$

Fig. 3. Tapetal protoplast prior to disintegration. $\times 6540$

Fig. 4. The beginning of the movement of the sporophytic tapetal cytoplasm onto the exine of the pollen grain. Lipidic (L) and proteinaceous (P) components of the tapetum are clearly distinguishable. Again this material is *Raphanus* rather than *Brassica*. Differences between these plants are described in the text. $\times 6800$

Fig. 5. Elements of the sporophytic tapetum (T) held in the exine (arrows) of the gametophyte. $\times 19900$

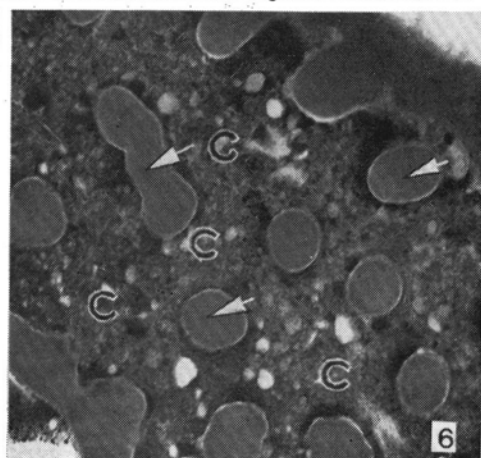
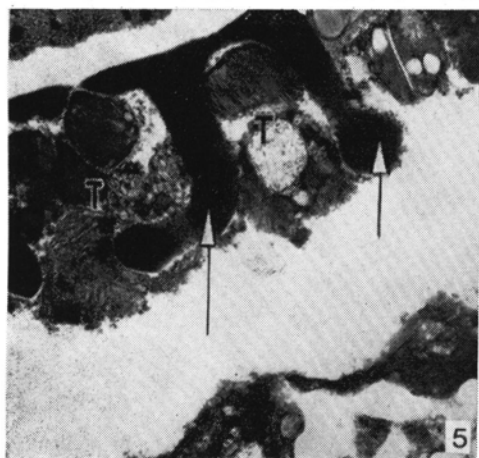
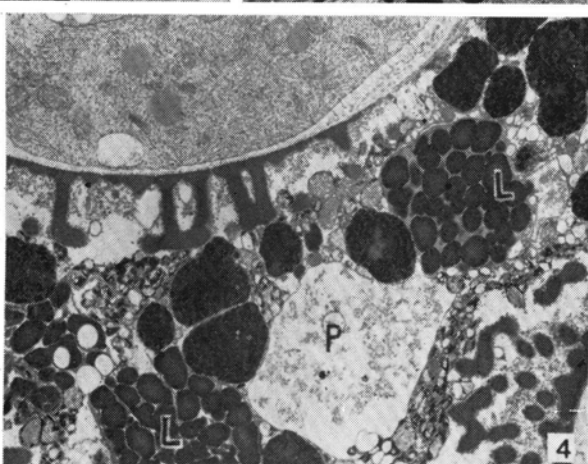
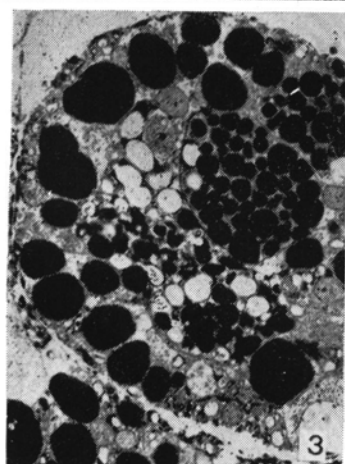
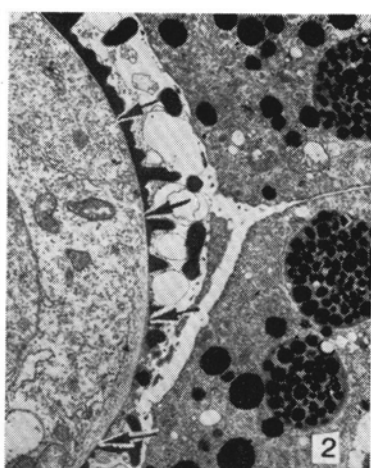
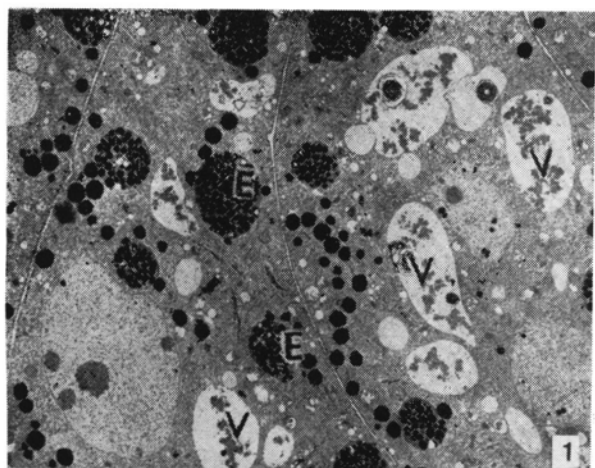
Fig. 6. Tangential section of *Brassica* pollen grain wall just prior to anthesis. The electron-opaque pollen coating (C) can be seen encasing the exine (arrows). $\times 20300$

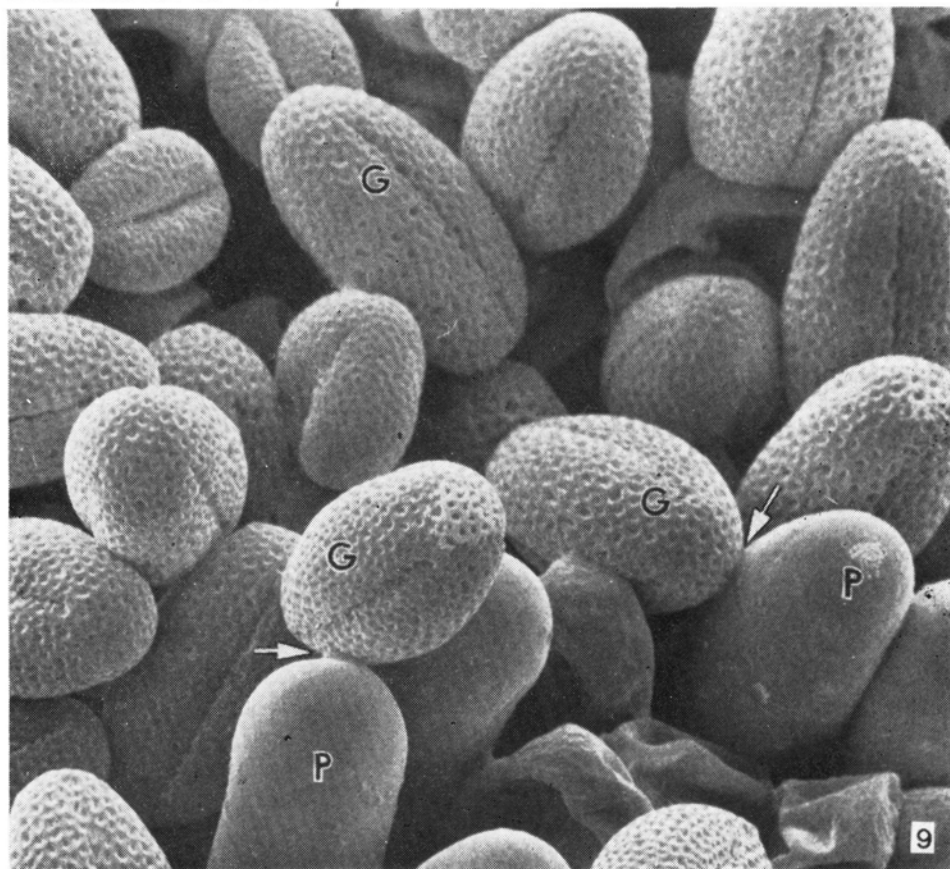
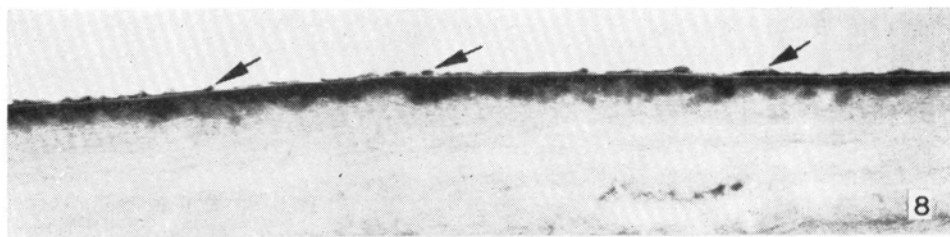
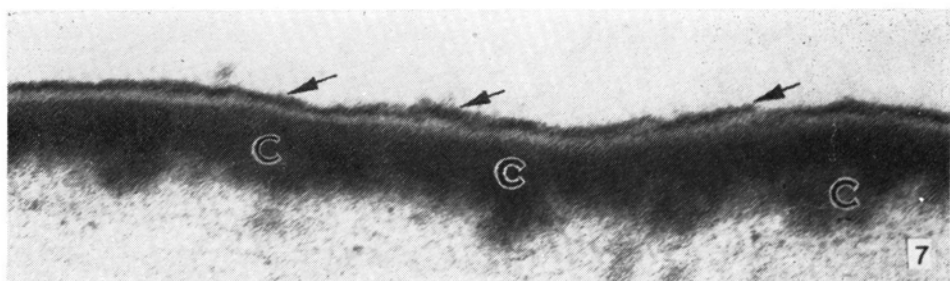
Interestingly, in *Brassica*, in common with many other plants, pollen compatibility with respect to the stigma is sporophytically controlled (Bateman, 1955). This information, especially when considered with the apparently superficial interaction between pollen and stigma, strongly indicates that the initial communication takes place between the surface layers of the pollen and that of the stigmatic papillae. A series of investigations (see Heslop-Harrison, 1975, for reviews) using light and electron microscopic histochemistry, have conclusively shown this to be so. In this paper we record details of the development and properties of both pollen and stigmatic surfaces, and describe how these layers interact to produce the responses observed following compatible or incompatible intra-specific pollinations.

THE DEVELOPMENT AND NATURE OF THE POLLEN GRAIN COATING

In 1968, Heslop-Harrison was able to show that while the main body of the pollen grain was derived directly from the archesporial tissue, elements of its coating were supplied by the nutritive tapetal cells investing it. The extent to which the tapetum physically contributes to the pollen varies considerably, in species such as *Cosmos bipinnatus* in which the tapetum is plasmodial (Dickinson, Potter, 1976) living, organised protoplasm is included into the cavea of the grain exine, while species with a strictly parietal tapetum, such as *Pinus banksiana* (Dickinson, Bell, 1976), display no such transfer. This process has been examined in detail in *Raphanus* (Dickinson, Lewis, 1973a, b) where events appear to be very similar to *Brassica*. Here, prior to pollen mitosis the tapetum becomes rich in electron-opaque

PLATE I





elaioplasts, and elements of the endoplasmic reticulum secrete fibrillar protein into vacuoles within the tapetal protoplasts (Figs 1-3). Shortly following mitosis, the fibrillar protein, followed by the main bulk of the lipid-rich tapetal cytoplasm move on to the surface of the maturing pollen grain exine (Figs 4, 5). As the grain matures this coating becomes progressively more electron-opaque and homogenous, the grain on discharge being invested in a coat containing apparently small vesicles, crystals soluble in preparation media and some small pieces of membrane, all embedded in a lipidic matrix (Fig. 6). The only way in which *Brassica* differs from *Raphanus* during these events is that the transfer of sporophytic materials takes place earlier in *Brassica*, and that larger fragments of tapetal protoplasm are applied to the grain surface than in *Raphanus*. The mature pollen coatings of both plants appear identical under the light and electron microscopes.

The chemical character of the mature coating has proved most resistant to investigation. Using gas-liquid chromatography (GLC) it has been possible to study the lipid component (Roberts et al., 1979) which appears to be composed of a normal range of fatty acids. Similarly, examination of extracted coatings with nuclear magnetic resonance analysis (NMR) has shown the coat to contain very much the same lipids and carotenes as were contained in the tapetal cells. Recent tests with the non-ionic detergent Triton-X have revealed the coating to contain a range of proteins, albeit all in small quantities. Few, if any, of these proteins are glycoproteins. In view of the highly covalent composition of the coating it is surprisingly hydrophilic. Simply placing pollen on a slide in an atmosphere of high humidity will cause the coat to flow from the grain. Similarly, in incompatible crosses where the coat appears not to undergo changes (see elsewhere), the coat frequently flows from the grain onto the stigmatic papillae. Electron micrographs suggest that one of the first steps in hydration is the expansion of the many small vesicles contained in the electron opaque matrix of the coat.

THE NATURE OF THE SURFACE OF THE STIGMATIC PAPILLAE

The stigmatic papillae of *Brassica* arise by the rapid organised growth of the topmost epidermal cells of the young pistil. During this extension, which occurs in buds barely 1 mm long, the cuticle that lies on the surface of these cells becomes highly attenuated and sometimes, in regions near the tips of the papillae, considerably dissected. At this point the cytoplasm commences to synthesise materials which, like waxes and other compounds found on the surfaces of plants, pass through the cuticle, and form a layer investing the cell (Fig. 7).

Plate II

Explanation as in Plate I.

Fig. 7. Mature 'pellicle' (arrows) of *Brassica* stigmatic papilla. Note the irregular cuticle (C) subjacent to it. $\times 189670$

Fig. 8. Young 'pellicle' (arrows) of *Brassica* prior to opening of the bud. Although this layer is hardly discernable, cytochemical preparations reveal the presence of esterase in it. $\times 33910$

Fig. 9. Scanning electron micrograph of pollinated *Brassica* stigma, prepared as described in Roberts et al. (1979). Pollen grains (G) and stigmatic papillae (P) are visible. A "meniscus" is visible (arrow) at each point of contact. $\times 2056$

Mattsson et al. (1974) who first recorded the presence of this layer, named it the 'pellicle', and demonstrated that it not only contained protein, but also that some of this protein possessed esterase activity. Formation of the pellicle is not completed until after the bud opens, but the esterase is one of the first proteins to be included, becoming evident immediately the pellicle becomes detectable (Fig. 8). Heslop Harrison (1975) was also able to show that the pellicle was a dynamic system, and that individual components would, if removed, be replaced within hours.

The proteins of the pellicle are clearly embedded in an electron-opaque matrix, which cytochemistry indicates to be composed, in the main, of saturated lipids. This lipid confers a strikingly hydrophobic character to the stigmatic papillae, and also makes extraction of the proteins from the pellicle rather difficult using mild detergents. However, using the non-ionic detergent Triton-X, these proteins may be extracted and electrofocussing indicates there to be some 20 present in the mature pellicle of *Brassica*. Since these proteins are present in such small quantities, their character is more easily investigated by examining the proteins of extracted whole papillae, and investigating the effects on them of compounds known to affect the pellicle.

Shivanna et al. (1978) proposed that since mature incompatible pollen will successfully develop on immature stigmas in the bud, compounds important to the SI system must be synthesised once the bud is open. In a recent series of experiments, Roberts et al. (1979) have revealed a glycoprotein functional in the SI system, with an isoelectric point of pH 5.6 to be included into the pellicle on opening of the flower. Further, they were able to show that if this glycoprotein was removed from the pellicle, it would be replaced to original levels within 2 hours (Stead et al., 1980). How "dynamic" the system is *in vivo* is unknown; certainly elements of the protein mosaic floating in the lipid matrix may be replaced on removal, but details of any regular turnover of proteins, or lipids, have yet to be recorded. The cuticle subjacent to the pellicle remains very thin and dissected, being composed of small

PLATE III

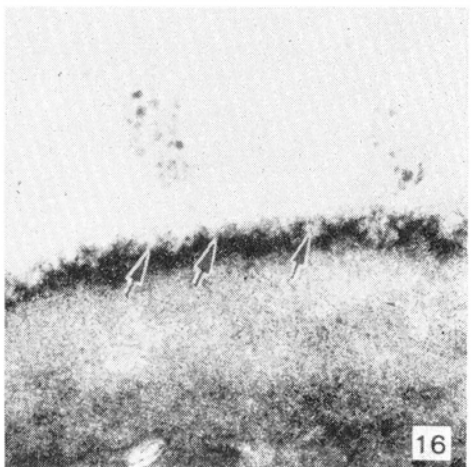
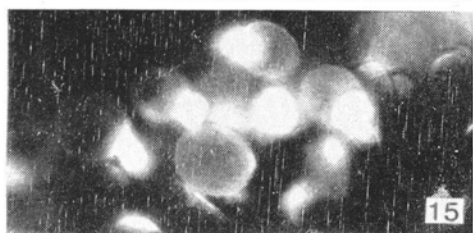
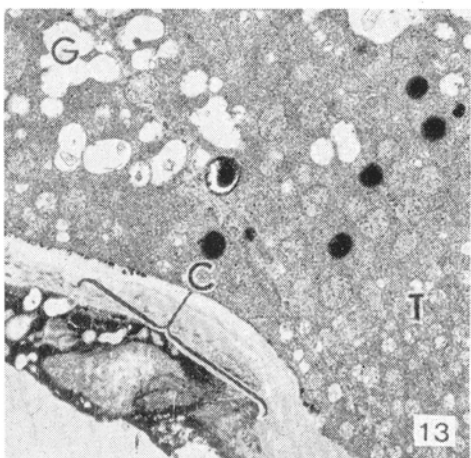
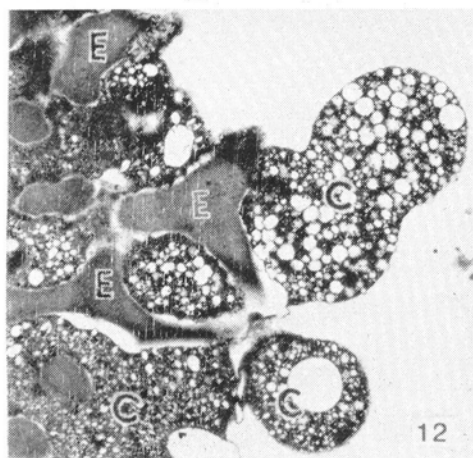
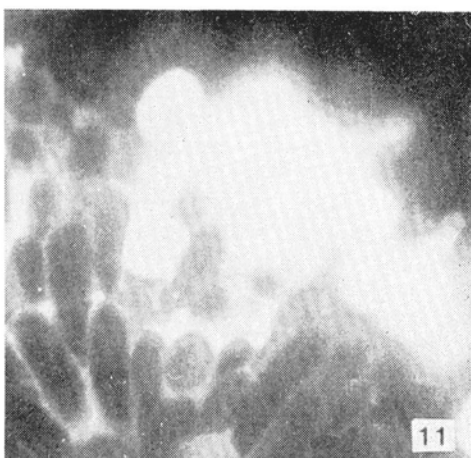
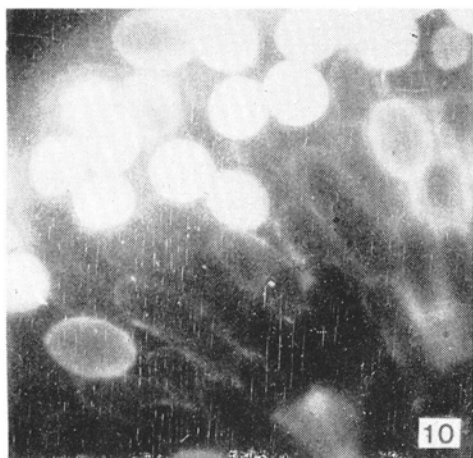


Plate III

Explanation as in Plate I.

Fig. 10. Compatible pollen 10 hours after pollination, stained with 1-ANS (Stead et al., 1979) to show the pollen coat. Note how the coat has moved little subsequent to pollination. $\times 297$

Fig. 11. As Fig. 10, but after an incompatible pollination. Note how the coat has flowed down between the papillae. $\times 250$

Fig. 12. Surface of the pollen grain some 5 hours after an incompatible pollination. Note how the coating (C) has expanded and is apparently 'emerging' from the pollen grain exine (E). $\times 17850$

Fig. 13. Junction between pollen grain (G) and tube (T) 10 hours after a compatible pollination. Note how the coating (C) has expanded and become vesiculate at the point where the tube exits the grain. $\times 7980$

Figs. 14 and 15. Compatible (14) and incompatible (15) pollinations of *Brassica* stained to show the presence of callose (see Roberts, et al., 1979, for details). $\times 250$

Fig. 16. Cuticle (arrows) below interface between pollen coating and pellicle, stained in osmic acid vapour which renders both coating and pellicle electron-lucent. Note the irregular and dissected nature of the cuticle. $\times 90210$

aggregates of electron opaque material embedded in the outer face of the pectocellulosic wall of the papilla.

The lack of copious aqueous secretion from the stigmatic papillae has led Heslop Harrison and Shivanna (1977) to classify the stigma of *Brassica*, in common with many species possessing sporophytically controlled SI systems, as "dry". While this is obviously the case, particularly when compared with plants such as *Lilium*, it should be recognised that the surface of this stigma is not simply a dry cuticle, but a lipid film containing a mosaic of functional proteins, many of them capable of rapid renewal.

EVENTS FOLLOWING COMPATIBLE AND INCOMPATIBLE POLLINATIONS

Differences between compatible and incompatible matings may be detected within minutes of pollination (Fig. 9), for compatible pollen adheres to the stigma far more strongly than incompatible (Roggen, 1972). While this phenomenon may be shown very effectively by an *in vitro* method (Stead et al., 1979) recent tests using carbon fibres attached to pollen grains have shown differences to be apparent within 15 minutes (Roberts et al., 1980). Examination of the pollen grain coat with the fluorescent protein probe 1-ANS shows this adhesion to be reflected in the character of the coating, in that compatible grains maintain a rigid coat, whilst the coat flows readily from the surface of incompatible pollen (Stead et al., 1979) (Figs 10, 11). Following adhesion, the compatible grains hydrate rapidly. Incompatible grains do

hydrate, but more slowly and erratically, and their hydration is much promoted by a humid environment.

Proteins of the stigmatic pellicle are closely involved in the control of adhesion of pollen grains, for pretreatment of the papillae with protease and other agents (Stead et al., 1980) prevents adhesion of compatible grains. However, the process cannot be a simple one, for both in self-pollinations in self-compatible varieties of *Brassica*, and in self bud-pollinations, little or no 'gelling' of the coat occurs, but still the pollen develops in a compatible fashion. It is therefore more probable that the adhesiveness of compatible pollen is a property that has evolved with the SI system, and by promoting the release of self pollen has proved successful in favouring outbreeding in the field. The study of adhesion and hydration of pollen has revealed more concerning the pellicle proteins that control the SI system. For example, a glycoprotein with an isoelectric point of pH 5.6 has been shown to appear in the stigmatic papillae concomitant with the acquisition of the SI system (Roberts et al., 1979) and, since incompatible pollen will develop on the immature stigma, this protein must clearly be involved in the suppression of the other molecules involved in the promotion of grain development.

Following hydration (Fig. 12), the compatible grain develops a tube (Fig. 13) which swiftly enters the stigma through the cuticle of the papilla (Fig. 14). The tube then grows down the papilla, travelling between the pectocellulosic wall and the cuticle. Many incompatible grains do not germinate; if they do, tubes of variable lengths are produced (Stout, 1931; Tatebe, 1939). These tubes may grow for extensive distances over the papillae and then die, others however may penetrate the stigmatic cuticle. When this happens, a reaction is often stimulated in the cytoplasm of the papilla (Kanno, Hinata, 1969) resulting in the formation of a lenticle of the carbohydrate callose, after which the pollen tube invariably dies (Fig. 15). It is also particularly striking that the incompatible pollen tube itself is also far richer in this carbohydrate than its compatible counterpart, a feature of self-pollen tubes in many plants (Heslop Harrison, 1975).

From the preceding data it is not easy to propose a straightforward model for the operation of the SI system in *Brassica*. An efficient germination medium has now been devised for pollen of *Brassica* (Dawes et al., 1980) and during the development of this medium, it became evident that pollen would often germinate well in a humid atmosphere if the correct osmotic and physical environment was provided. It is thus tempting to propose that in compatible crosses cooperation between molecules of the pellicle and pollen coat permit the correct flow of water into the grain. In incompatible matings, such

co-operation is prevented, perhaps in part by the stigmatic glycoprotein, and water passes slowly, or not at all, from the stigma to the grain. Germination would thus only occur could the pollen obtain water from another source, such as a humid atmosphere. This hypothesis is obviously simplistic in the extreme, and there is evidence that far more complex interactions occur, including the synthesis of special proteins by the pollen (Ferrari, Wallace, 1977).

When considering these interactions, it is of paramount importance to dissociate recognition, which must be S-gene specific, from response, which may be under control of any genome. Thus, while it seems likely that recognition occurs between elements of the pellicle (some of which have been shown to be S-gene specific; Nishio, Hinata, 1978) and the sporophytic pollen coating, the response, be it facilitating the passage of water or not, may occur at any point in the system. There appear to be four sites at which the response might take place, the surface of the stigmatic papillae, within the coating of the pollen grain, at the pollen plasma-membrane, or within the pollen protoplast. At the moment no data which point preferentially to any of locations are available. The stigmatic surface, with its dissected cuticle (Fig. 16), and active pellicle would seem particularly suited to this activity, but then the rapid change of the coat during adhesion (admittedly not in self-compatible varieties) would signify changes in property which might involve the flow of germination-promoting substances to the pollen. In all, it is thus probably only fair to say that at present the least likely sites of the response are the pollen plasma membrane, and within the protoplast itself.

Still to be explained is the reaction of the stigmatic papilla to the penetration of incompatible tubes. This reaction is always centred on the point at which the tube has penetrated the cuticle, and this must be a response to substances that pass through this discontinuity. It remains to be determined whether this response is to the sporophytic proteins of the tube coating, or the gametophytic proteins of the pollen tube, both which could make contact with the papillar plasma membrane.

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