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Recognition and rejection phenomena during pollen-pistil interaction

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Abstract. In flowering plants, the pollen grain—the male gametophyte—interacts with the diploid sporophytic tissue of the pistil before liberating the male gametes in the vicinity of the female gamete. During this gametophyte-sporophyte interaction the male gametes are effectively screened by the pistil ensuring the entry of only the appropriate pollen tubes into the embryo sac. This review summarises recent investigations on pollen recognition and rejection, explains the current understanding, and projects future lines of study.

Keywords. Interspecific incompatibility; intraspecific incompatibility; pistil; pollen; recognition; rejection; self-incompatibility; stigma.

1. Introduction

One of the essential features of sexual reproduction is the ability of an organism to recognise and select suitable gametes for fertilization. Unsuitable gametes are effectively prevented from fertilization. This ability of recognition and rejection of gametes enables the species to fulfil two primary functions of sexual reproduction—maintaining stability of the species and permitting a reasonable degree of genetic variability within the species. Unlike animals, which are generally unisexual, the majority of plants are bisexual and the male and female gametes produced by the same individual are likely to come together. For an effective exploitation of sexual reproduction, therefore, it is advantageous for the plants to have mechanisms to discriminate against gametes of the same individual, in addition to those of other species.

Different groups of plants show variations in the operation of recognition and rejection phenomena. In the cryptogams, the male gametes are released freely into the aqueous medium and have direct access to the female gamete. The acceptance or rejection of the male partner, therefore, is at the discretion of the female gamete. No other cell or tissue has any role in this process. Female gametes of many species produce hormones which attract male gametes (see van den Ende 1976).

In the gymnosperms the female gametophyte is enclosed within the nucellus, which is a sporophytic tissue. The pollen grain which is deposited in the pollen chamber has to send out a short tube before liberating the male gametes in the vicinity of the egg. The interaction of the haploid pollen tube and the diploid nucellar tissue is limited, and is confined only to a few cell layers.

In the evolution of the flowering plants, however, the ovule has become encased within the pistil and this has made it obligatory for the male gametophyte to interact with the massive sporophytic tissue before the male gametes and the female gamete can come together. An important outcome of this elaboration of gametophyte-sporophyte interaction has been the transfer of the function of recognition and rejection (of the male gamete) from the egg to the sporophytic tissue of the pistil. Thus, the pistil has developed mechanisms to screen the male gametes, and permit only the appropriate ones to enter the embryo sac. This adaptation has led to the successful establishment of intraspecific incompatibility (self-incompatibility) controlled by multiple alleles at one or many loci in which all the male gametes originating from the same plant, or any other plant having the same genotype, are prevented from gaining entry into the embryo sac. These unique features have made angiosperms the most efficient outbreeding systems. Such effective systems cannot operate in other groups of plants in which the interaction is largely at the gametophyte-gametophyte level as the egg could reject only half the male gametes originating from the heterozygous sporophyte.

The absence of reports of intraspecific incompatibility in gymnosperms (Hagman 1975) supports the concept that an extensive gametophyte-sporophyte interaction is a prerequisite for the establishment of an effective outbreeding mechanism. The transfer of function of recognition and rejection from the female gamete to the sporophytic tissue is probably the most important cause for the dramatic success of angiosperms over other groups of plants. According to Whitehouse (1950), the evolutionary significance of the closed carpel in angiosperms is for preventing the ovules from self-fertilisation rather than for protecting them from desiccation or ravages by predators.

Pollen-pistil interaction is of cardinal importance in the biology of sexual reproduction and of seed formation. Studies on pollen-pistil interaction have direct relevance to plant breeding programme. The plant breeder is continually striving to bring together desirable characters, present in different taxa, through hybridization. Although the technique of somatic hybridization, through isolated protoplasts, has been speculated as an important tool in plant improvement programme, the plant breeder has to depend on the conventional methods of hybridization for many more decades to come. A better understanding of the biology of pollen-pistil interaction would enable the plant breeder to manipulate the screening processes in the pistil more effectively.

The basic information relating to pollen-pistil interaction and fertilization was provided by the investigations of Amici, Schleiden, Strasburger and Nawaschin towards the end of the last century. Subsequent investigators confined their attention largely to gametogenesis, fertilization, and embryo and seed development. Pollen germination and growth of pollen tube through the pistil were largely taken for granted. Following the elucidation of intraspecific incompatibility, the details of pollen-pistil interaction attracted many investigators. During the past three decades extensive literature has accumulated on the genetics, physiology and

biochemistry of intraspecific incompatibility (Lewis 1954; Arasu 1968; Nettancourt 1972, 1977). Nonetheless, integrated approaches to correlate the structure of pollen grain and pistil, to understand the factors involved in pollen recognition and rejection and pollen tube inhibition were lacking. Lately a beginning has been made on these lines and this review attempts to discuss these recent investigations.

2. Receptive surface of the pistil

As the stigma is the recipient of the pollen grain, the initial interaction takes place between these two structures. It is important to understand the structure of the stigma for understanding the nature of the pollen-pistil interaction. The stigmas are traditionally divided into wet and dry types, depending on the presence or absence of exudate at the time of pollination. Recently many investigators have studied the variations found in these two major types of stigma and have divided them into further categories (Heslop-Harrison 1976; Heslop–Harrison *et al* 1975). So far the most comprehensive account of the characteristic features of the receptive surface of the stigma is that given by Heslop-Harrison and Shivanna (1977). They have studied and classified stigmas of about 1000 species covering 900 genera belonging to over 250 families (table 1).

The most important outcome of the work on the structure of the stigma in recent years has been the demonstration of the presence of stigma-surface proteins both in wet and dry types (Mattsson *et al* 1974; Heslop–Harrison *et al* 1975; Shivanna and Sastri 1976; Heslop–Harrison and Shivanna 1977). In the wet type proteins are present on the stigma as a component of the exudate; in the dry type however, proteins occur in the form of a hydrated layer — the pellicle. The pellicle

Table 1. General classification of angiosperm stigma types based on the morphology of the receptive surface, and the amount of secretion present during receptive period (after Heslop-Harrison 1976; Heslop-Harrison and Shivanna 1977). Some examples for each group are given in parenthesis.

Dry stigmas (without apparent fluid secretions)

Group I—Plumose, with receptive cells dispersed on multiseriate branches (Gramineae)

Group II—Receptive cells concentrated in distinct ridges, zones or heads

A—Surface non-papillate (Acanthaceae)

B—Surface distinctly papillate

1. Papillae unicellular (Cruciferae, Compositae)
11. Papillae multicellular
    (a) Papillae uniseriate (Amaranthaceae)
    (b) Papillae multiseriate (Bromeliaceae, Oxalidaceae)

Wet stigmas (surface secretions present during receptive period)

Group III—Receptive surface with low to medium papillae; secretion fluid flooding interstices (some Rosaceae, some Liliaceae)

Group IV—Receptive surface non-papillate; cells often necrotic at maturity; usually with more surface fluid than Group III (Umbelliferae)

is easily demonstrated (histochemically) by its intense non-specific esterase activity (figure 1).

The stigma-surface proteins are present from a very early stage of bud. In taxa with a wet stigma, the stigma-surface proteins are present even before the secretion of exudate. In some taxa, such as *Petunia*, no additional proteins are incorporated along with the secretion of the exudate (Shivanna and Sastri 1976). In others such as *Amaryllis* and *Pancratium*, which are characterised by hollow style, in addition to the proteins present in the pellicle, proteins are also secreted along with the exudate (Sastri and Shivanna, unpublished).

3. Post-pollination events

The major morphological events that occur in the pistil during pollen-pistil interaction are given in figure 2. Pollen grains land on the stigma through various agencies. Following compatible pollination, pollen grains become hydrated followed by the release of pollen-wall proteins on to the stigmatic surface. It is now clearly demonstrated that pollen carries a load of extracellular proteins in both the wall layers—the exine and the intine. The intine proteins are products of pollen cytoplasm, and, hence, of gametophytic origin, whereas the exine proteins are products of the surrounding tapetum, i.e., sporophytic in origin (figure 3; see Heslop–Harrison 1975a, b; Shivanna 1978). The recognition of pollen seems to be established in numerous taxa, as a result of interaction between pollen-wall proteins and stigma-surface proteins (discussed later). Following successful recognition, the pollen grain soon sends out a tube which enters the stigma, and grows through the style. The pistil provides nutrients to the growing pollen tube (see Vasil 1975; Labarca and Loewus 1973; Linskens and Pfahler 1977). The tube, eventually, reaches the ovary and then the embryo sac. When once in the embryo sac, it appears that the male gametes are not exposed to any inhibitory processes before fertilization.

Barring those situations which are strictly self-pollinated, pollen grains of often distantly related plants, land on the stigma during pollination. The pistil, parti-

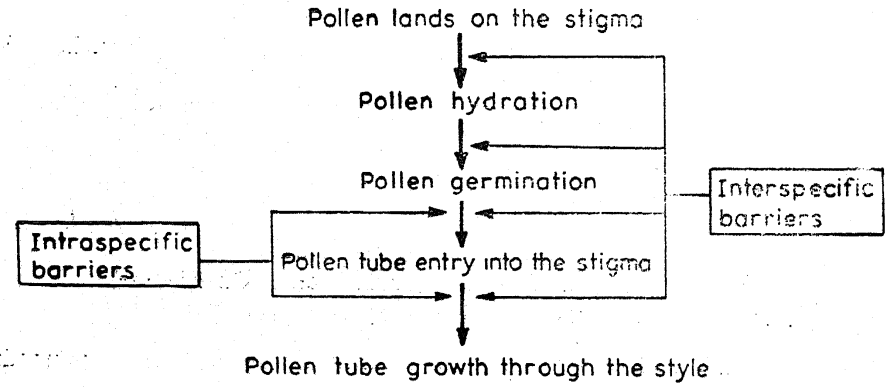

Figure 2. Major post-pollination events involving gametophyte-sporophyte interaction. Intraspecific barriers operate only in the last two stages; interspecific barriers operate at any stage.

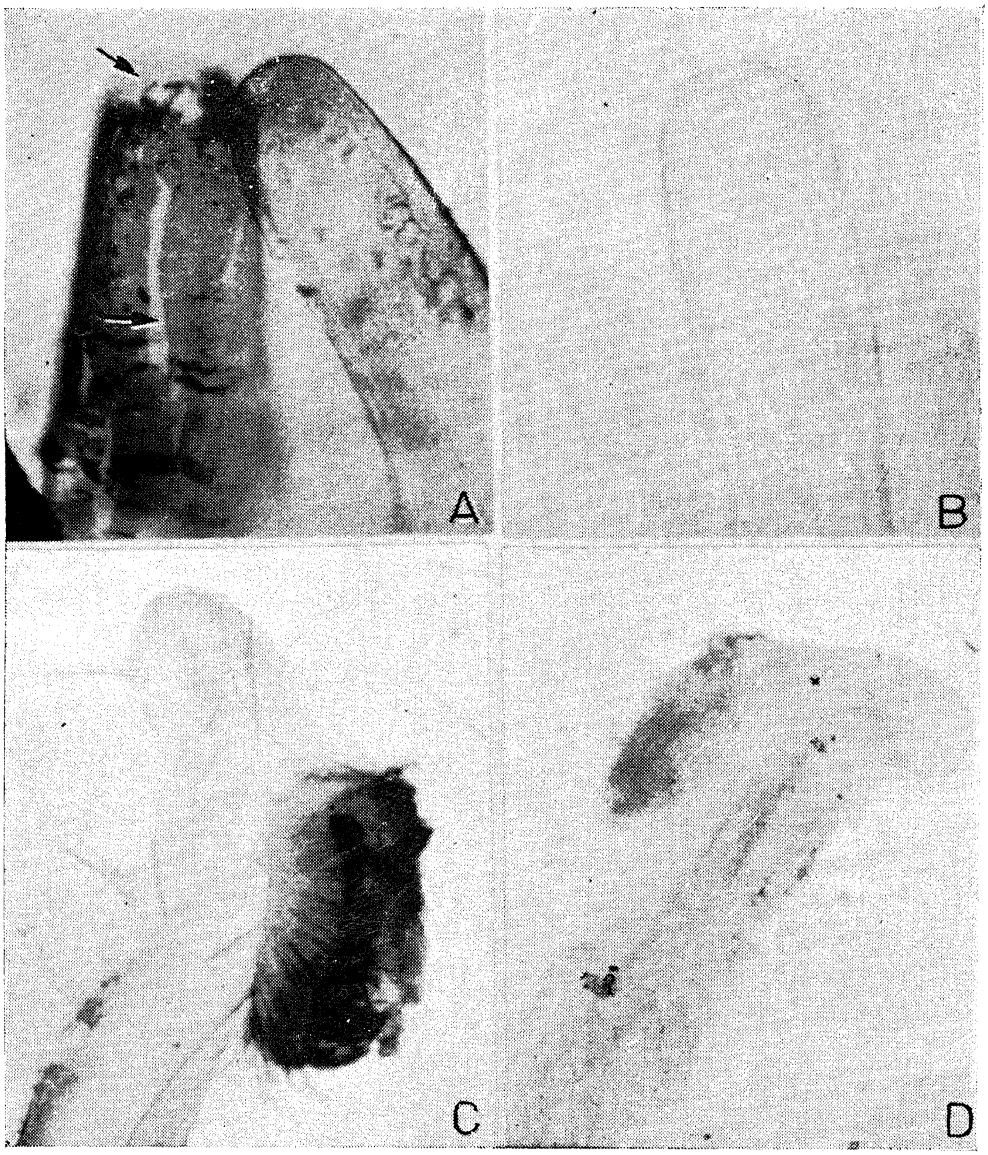

Figure 1. Histochemical localizations of surface-esterases using α-naphthylacetate as a substrate in a coupling reaction with fast blue B salt. A and B, stigmatic papillae of *Acidanthera bicolor* (dry stigma, group II B). A. with substrate. The pellicle is seen as a dark sheath investing the cuticle but is torn at places (arrows) and reveals the underlying cuticle. B. Control for A (without the substrate). C and D. Stigma of *Vigna unguiculata* (wet stigma, group III) Esterase activity is clearly seen on the exudate. D. Control for C (without the substrate).

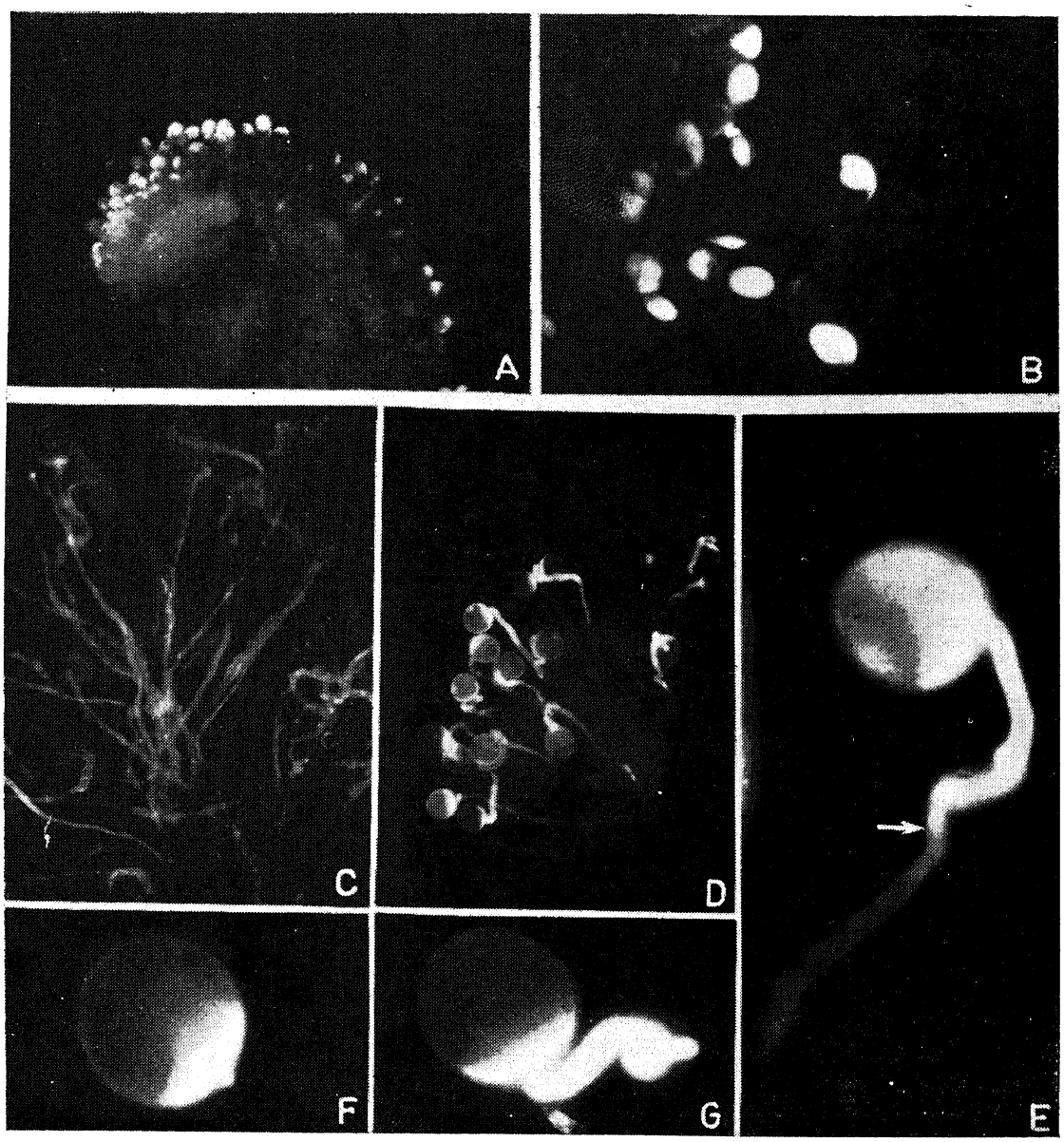

Figure 5. Fluorescence micrographs of stigma 6 hr after pollination following staining with decolorized aniline blue (to localize callose). A and B. *Brassica campestris*. A. Portion of a stigma 6 hr after self-pollination. Observe characteristic rejection reaction in the form of callose plugs at the tip of papillae. Pollen grains get washed off during preparation as the pollen tubes do not enter the stigma. B. Some of the papillae showing callose deposition, at higher magnification. C–G. *Saccharum benghalensis*. C. Part of the stigma following cross-pollination. Observe profuse growth of pollen tubes. Pollen grains are not clearly seen as they hardly show any callose deposition. D. Part of the stigma following incompatible pollination. Pollen tubes are inhibited after growing a short distance in the stigma. E. One of the incompatible pollen at higher magnification. Arrow points to the region of pollen tube entry into the stigmatic papilla. Note pronounced callose deposition in the grain and the tube. F and G. Incompatible pollen grains. Stigmatic papillae are not visible. F. The germpore is blocked with callose inhibiting germination. G. Pollen tube is arrested before it enters the papilla.

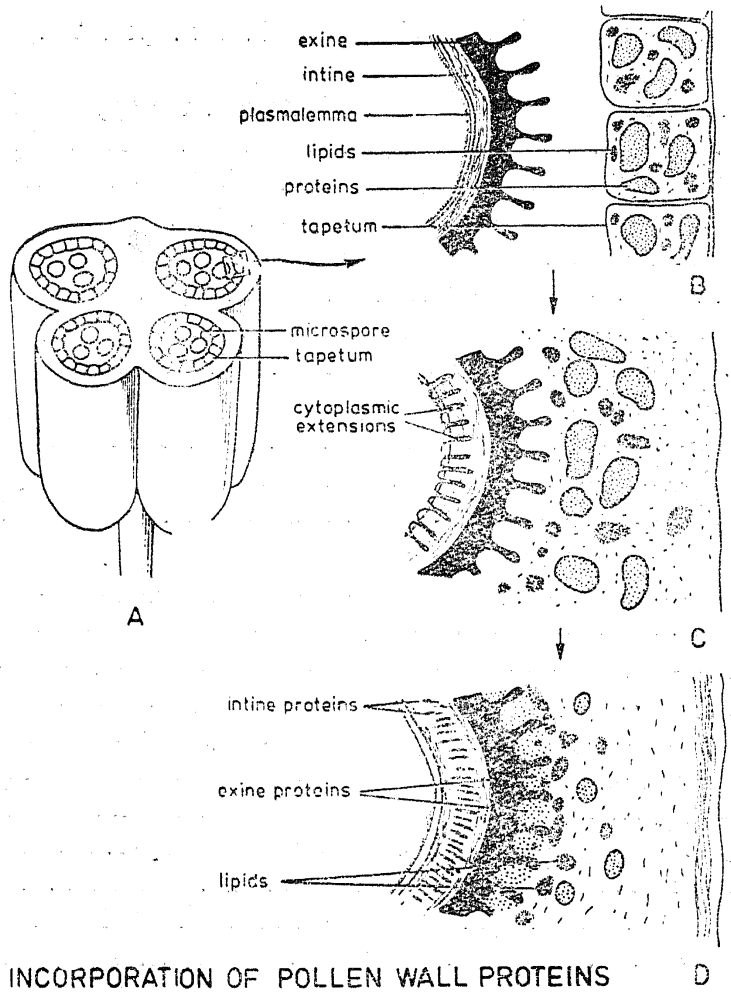

Figure 3. Diagrammatic representation of the origin of pollen-wall proteins. The intine proteins are products of pollen cytoplasm (gametophytic) and the exine proteins are products of the tapetum (sporophytic) (after Shivanna 1978).

cularly the stigma, therefore, should have mechanisms to recognise and reject the pollen grains of alien species. When the pollen is of the wrong type, fertilisation is effectively prevented by arresting post-pollination events at different level (figure 2). Cytological details of rejection following interspecific pollinations have been largely confined to those crosses which exhibit unilateral incompatibility (Lewis and Crowe 1958; see also Nettancourt 1977). In most of the plant breeding programmes, however, the crosses have been classified as compatible or incompatible on the basis of seed set. From the limited information available, it is apparent that the rejection reaction may occur at any level depending on the extent of reproductive isolation of the male partner. Closely related species would accomplish more of the post-pollination events than distantly related species. For example, when the stigma of *Gladiolus* (Knox *et al* 1976) is pollinated with pollen grains of *Crocosmia* belonging to the same family (Iridaceae), pollen hydration and pollen germination are normal, but pollen tubes fail to enter stigmatic papillae. This is because of the failure of tubes to penetrate the cuticle. When the stigma of *Gladiolus* is pollinated with pollen of

Gloriosa (belonging to a different family, Liliaceae), pollen hydration itself is inhibited.

There are no conclusive evidences to indicate that the egg exercises any discrimination against the gametes of other species. Success obtained in the fertilization of crosses involving unrelated taxa, such as those of Solanaceae and Caryophyllaceae (*Melandrium* × *Datura*), by culturing ovules and pollen grains together on a nutrient medium (Zenkteler 1970; Zenkteler *et al* 1975; see Rangaswamy 1977), reemphasises the role of the pistillate tissue in interspecific discrimination. However, interspecific incompatibility may operate after fertilization, resulting in eventual breakdown of the embryo. Post-fertilization barriers are not discussed in this paper.

In self-incompatible taxa, the pistil should also have a mechanism to recognise its own pollen and reject it. The details of inhibition of selfed pollen tubes have been investigated in a large number of taxa. There is a correlation between the genetic control of self-incompatibility, and the zone of pollen tube inhibition (Brewbaker 1957; Lewis 1956). In taxa having sporophytic type of incompatibility (in which incompatibility in the pollen is controlled by the genome of the parent sporophyte) pollen tubes are inhibited from entering the stigma. In taxa with gametophytic incompatibility (in which incompatibility is controlled by the haploid genome of the pollen) the growth of the pollen tube is inhibited in the style. Also, sporophytic systems are generally associated with the taxa with dry stigma, whereas, gametophytic systems are associated with both wet and dry types of stigma (Heslop-Harrison *et al* 1975; Heslop-Harrison and Shivanna 1977). In a few instances pollen tubes may be inhibited even in the ovary. Intraspecific incompatibility is invariably a pre-fertilization barrier and is due to active inhibition of pollen tubes, presumably by the production of an inhibitor. In *Theobroma cacao* incompatible pollen tubes are reported to enter the embryo sac, and also discharge the male gametes (Cope 1962). This is the only report in which the function of recognition and rejection, following incompatible intraspecific pollination, is retained by the gametophyte. However the report of Cope (1962) needs to be confirmed.

4. Role of stigma-surface proteins

Many investigations have implicated stigma-surface proteins in pollen recognition and rejection. Most of these investigations have been confined to the taxa with dry type of stigma. In members of the Caryophyllaceae (Heslop-Harrison and Heslop-Harrison 1975) and *Gladiolus* (Knox *et al* 1976) digestion of pellicle-proteins by pronase does not affect pollen germination but totally inhibits the entry of pollen tubes into the stigmatic papillae. Obviously, some factors in the pellicle are required for the effective operation of the cutinase system in the pollen. In *Raphanus sativus* enzymatic digesion of the pellicle reduces pollen germination, and totally inhibits entry of even the compatible tubes into the stigmatic papillae (Shivanna *et al* 1978).

More direct evidences for the involvement of stigma-surface proteins have come from the work of Knox *et al* (1976) on *Gladiolus*. Concanavalin A (con A) was demonstrated to bind specifically to the pellicle. Stigmas of very young buds,

free from pellicle, did not bind to con A. Con A binding on the stigmatic surface did not inhibit pollen germination, but prevented the entry of tube. Thus, the components involved in con A binding were necessary for pollen tube entry, and not for pollen germination. Washing of stigmas with sodium deoxycholate removed the ability of the stigma to support pollen germination as well as its ability to bind to con A. Based on these studies Knox *et al* suggested that stigma-surface receptors are composed of many components, some of them being involved in pollen germination and others in entry of tubes.

In interspecific crosses involving *Populus deltoides* × *P. alba*, pollen tubes are inhibited on the stigma. The treatment of the stigma with organic solvents such as anhydrous hexane and ethyl acetate, before pollination, was remarkably effective in overcoming incompatibility (Willing and Pryor 1976). Presumably, the components involved in incompatibility are removed or denatured as a result of the treatment.

Investigations on the details of intraspecific incompatibility have also implicated stigma-surface proteins in pollen recognition and rejection. Nasralla and Wallace (1967) have demonstrated, through immunodiffusion technique, the presence of S-allele specific proteins in the diffusates of intact stigmas. Also, water-soluble substances released from the undamaged stigmas have been reported to inhibit, selectively, in vitro germination of self-pollen but not of cross-pollen (Ferrari and Wallace 1976). Apparently, these proteins emanate from the pellicle.

Studies carried out on bud receptivity also implicate stigma-surface proteins in pollen-stigma interaction. In *Petunia* (Shivanna and Sastri 1976) although younger buds are free from the exudate, they support pollen germination and pollen tube entry into the stigma. Receptive stigmas of buds are invariably covered with a layer of pellicle.

The details of bud receptivity have also been worked out in many taxa belonging to Cruciferae (Shivanna *et al* 1978). Buds of all stages in *Raphanus sativus* and *Cheiranthus cheiri* showed a layer of pellicle on the stigmatic papillae; and all of them permitted satisfactory pollen germination. Entry of incompatible pollen was confined only to a few younger stages (figure 4). The number of papillae that showed callose deposition following incompatible pollination (characteristic rejection reaction, see figures 5A, B) also decreased in younger buds. Many of the papillae showed diffuse callose deposition, extending to the lower region of the papillae, an indication of inefficient operation of rejection reaction. The variety of *Sinapis alba* tested did not allow the entry of self-pollen in buds of any stage. Shivanna *et al* (1978) explained that in *R. sativus* and *C. cheiri*, the factors responsible for pollen germination and pollen tube entry are present in the pellicle from a very early stage; the factors responsible for incompatibility accumulate in sufficient quantities in the pellicle only in older stages of buds. In *S. alba*, on the other hand, factors involved in pollen germination, pollen tube entry, and incompatibility are secreted almost simultaneoulsy, with the result there is no receptive stage of buds free from incompatibility factors.

5. Role of pollen-wall proteins

Direct evidences for implicating pollen-wall proteins in recognition function are largely based on studies on intraspecific incompatibility. In members of the

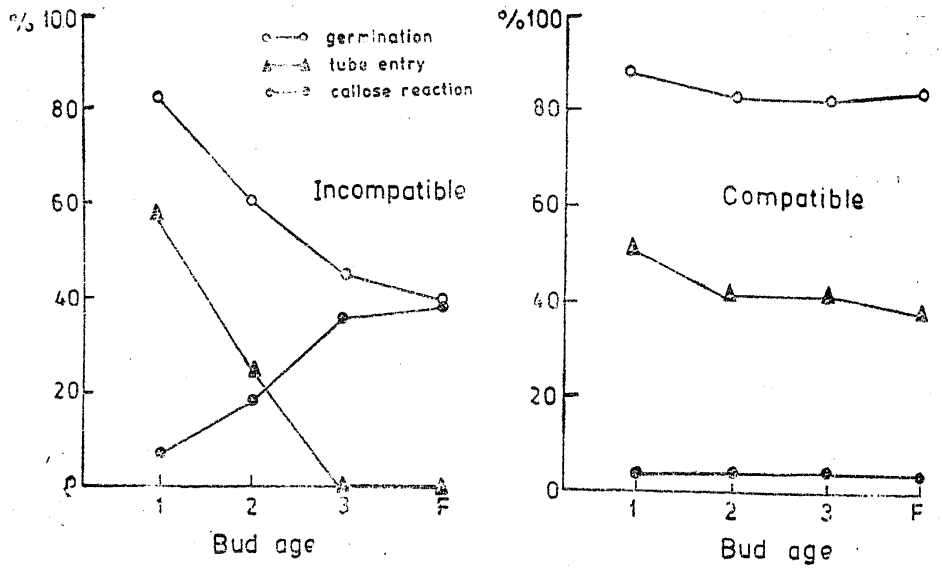

Figure 4. Responses of developing buds of *Cheiranthus cheiri* to compatible and incompatible pollinations. Bud age 1 represents the youngest stage studied; 2 and 3 are successively older stages. F is freshly opened flower (after Shivanna *et al* 1978).

Cruciferae and Compositae, incompatible pollen induces a characteristic rejection reaction in the adjoining stigmatic papillae in the form of deposition of a lenticular plug of callose between the cell wall and plasmalemma (figure 5A, B; Dickinson and Lewis 1973; Heslop-Harrison *et al* 1974; Howlett *et al* 1975; Shivanna *et al* 1978). This has been used as a bioassay to analyse pollen factors involved in incompatibility. Deposition of an agarose film in *Iberis* into which exine proteins from incompatible pollen were allowed to diffuse, was effective in inducing rejection reaction in the stigmatic papillae (Heslop-Harrison *et al* 1974). The fragments of the tapetum (before releasing the proteins into anther locule) could also induce rejection reaction. No rejection reaction was induced when exine proteins from compatible pollen, or tapetal fragments from the compatible anther, were deposited. In *Raphanus* also Dickinson and Lewis (1973) have demonstrated the efficacy of pollen-kitt material, isolated from incompatible pollen through high speed centrifugation, in inducing rejection reaction. These studies have clearly shown, that in taxa characterized by the sporophytic type of intraspecific incompatibility, exine proteins, the products of the sporophytic tissue (tapetum), are the pollen factors involved in incompatibility. Recognition is presumably established as a result of interaction between exine and stigma-surface proteins; the message would then move into the papilla and pollen cytoplasm, initiating the rejection reaction.

The knowledge of the pollen factors involved in incompatibility has been effectively utilized to manipulate incompatibility responses. In *Cosmos bipinnatus* (Howlett *et al* 1975), application of pollen-wall proteins, obtained from compatible pollen, on to the stigma, before pollinating with self-pollen, was effective in overcoming incompatibility. Obviously, some factors present in the compatible wall proteins confuse the recognition mechanism of the stigma, with the result that the stigma becomes incapable of inhibiting all the selfed pollen tubes.

In a majority of taxa having gametophytic type of incompatibility, the pollen tubes are not inhibited on the stigma but in the style. It is, therefore, difficult to carry out experiments comparable to those on members of the Cruciferae and Compositae. In grasses (which show gametophytic incompatibility), however, pollen tubes are inhibited on the stigma. The role of exine and intine proteins has been studied in many gramineaceous taxa (Heslop–Harrison 1975c, 1976; Shivanna *et al* 1976). The stigmatic papillae do not produce callose following incompatible pollination. However, callose deposition is very conspicuous in pollen tubes (figure 5 C–G). In many of the taxa, different individuals show variation in the intensity of incompatibility. In moderately incompatible individuals, pollen tubes are inhibited only after they have grown to various distances in the papillae (figure 5 D, E). In plants showing strong incompatibility, the intensity of pollen inhibition depends on the orientation of the germpore on the papilla. When the germpore is away from the papilla, pollen tube is initiated soon after pollen hydration, grows down on the pollen grain, and comes in contact with the stigma. Soon after it touches the papilla, the pollen tube is plugged with callose, and is effectively inhibited (figure 5G). When the germpore lies in direct contact with the papilla, incompatible reactions are initiated soon after pollen hydration; the germpore itself is plugged with callose (figure 5F) inhibiting germination.

The differences in the inhibition reaction in relation to the orientation of the germpore can be rationally explained on the basis of the differences in the time of the release of intine proteins. When the germpore is away from the papilla, exine proteins are released soon after hydration. As the initiation of pollen tube is followed soon after pollen hydration, pollen tube emerges along with its intine-load of proteins before they are released. Intine proteins are released on to the pellicle only when the pollen tube touches the papilla. This triggers off rejection, and results in plugging the pollen tube tip with callose. When the germpore is in contact with the papilla, intine proteins are also released along with or soon after the exine proteins, thus initiating rejection reaction immediately before the emergence of pollen tube. It appears, therefore, that release of only the exine proteins on the stigma does not initiate rejection reaction. In those instances in which pollen tubes are inhibited after entering the papilla, it is possible that proteins synthesised in the pollen tubes are involved in incompatibility. Nonetheless, involvement of exine proteins is ruled out. It is obvious that in grasses which are characterised by the gametophytic type of incompatibility, intine proteins (products of the gametophyte) are involved in intraspecific incompatibility. In other taxa in which pollen tubes are inhibited in the style, such as *Petunia* and *Nicotiana* it is logical to expect the involvement of intine proteins or pollen tube proteins in rejection reaction.

Pollen-wall proteins seem to be involved in interspecific incompatibility also, although the evidences are less direct. Knox *et al* (1972a, b) have successfully overcome interspecific incompatibility in *Populus* (*P. deltoides* × *P. alba*) by pollinating the stigma with a mixture of killed compatible pollen termed recognition pollen, with live incompatible pollen. These authors explained that the killed compatible pollen provide recognition substances to the incompatible pollen, making them compatible. However, location of these recognition factors in pollen wall is yet to be conclusively established. The technique of using recognition pollen

has also been effective in overcoming incompatibility, on the stigma, in the cross involving *Sesamum indicum* and *S. mulayanum* (Sastri and Shivanna 1976).

6. Sites of recognition and rejection in intraspecific incompatibility

In taxa characterised by the inhibition of incompatible tubes on the stigma, recognition of incompatible pollen as well as rejection are completed on the stigma. Obviously, pellicle proteins are involved in both these processes. In taxa characterized by the inhibition of pollen tubes in the style, the sites of recognition and the involvement of stigma-surface proteins in recognition and rejection are not clear.

Van der Donk (1975a, b) studied the synthesis of RNA, DNA, and the size of the free nucleotide pool at 3 hr intervals in the styles of *Petunia hybrida* following self- and cross-pollinations. He noted distinct differences in self- and cross-pollinated pistils in all the three metabolic activities within the first 3 hr period. These differences were explained on the basis of recognition taking place much earlier, on the stigma itself, soon after pollination (Van der Donk 1975a, b; Linskens 1975). In *Prunus avium* (Raff and Knox 1977), another taxon in which the incompatible tubes are inhibited in the style, the presence of stigma is necessary for pollen tube inhibition. When the pollen grains were deposited on an artificial stigma made on the cut stump of the style (after removing the stigma), both compatible and incompatible tubes grew through the style and reached the ovary (figure 6A). Therefore, in *Petunia*, *Pyrus*, and probably other genera with solid

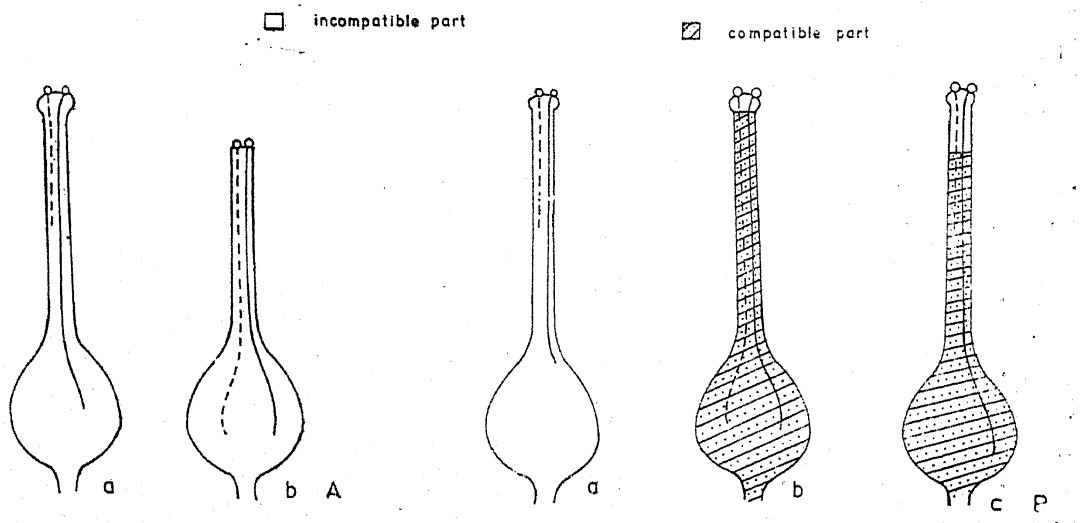

Figure 6. Diagrammatic representation of the responses of self (broken line) and cross (solid line) pollen to various treatments in *Prunus avium* (A) and *Lilium henryi* (B). In both taxa, incompatible pollen tubes are inhibited in the style. In *P. avium* removal of stigma was effective in overcoming pollen tube inhibition indicating that incompatible message is received in the stigma. In *L. henryi* grafting of incompatible stigma on compatible style was not effective in inhibiting incompatible pollen (b). However, when incompatible stigma together with upper quarter of the styles was grafted on to compatible style, incompatible pollen tubes were promptly inhibited (c). These studies indicate that the stigma has no role in recognition of incompatible pollen. The recognition is established by the time pollen tube has grown through a quarter of the style [modified from Lawson and Dickinson 1975 (B), and Raff and Knox 1977 (A)].

style, recognition of the incompatible pollen takes place in the stigma, soon after pollination, whereas inhibition of pollen tubes is completed in the style about 24 hr later. Stigma-surface proteins are likely to be involved in this recognition.

In *Lilium* which is characterised by hollow style, the stigma does not seem to play any role in pollen recognition and rejection. By a series of grafting experiments, Lawson and Dickinson (1975) have shown that in *Lilium henryi* the incompatible message is not received in the stigma, but is registered only after the pollen tubes have grown through a quarter of the style (figure 6 B). Similar findings have been recorded in *L. longiflorum* (Gladding and Paxton 1975). Additional evidence is provided from experiments involving hot water treatment of the pistil (Fett *et al* 1976). In *L. longiflorum* treating the pistil with hot water at 50° C for 6 min, prior to pollination, is effective in overcoming self-incompatibility. Application of hot water to only the stigma did not alter incompatibility responses. Hot water treatment of only the lower part of the style was adequate to overcome incompatibility. This indicates that both recognition and rejection are confined to the style. This may be the situation in other hollow-styled taxa. It is interesting that in *Lilium* the removal of loosely bound wall materials (proteins and carbohydrates) does not affect pollen germination and pollen tube growth (both in vivo and in vitro), as well as self-incompatibility reaction (Fett *et al* 1976).

7. Sequence of recognition and rejection

Based on the investigations discussed in the preceding pages, a probable sequence of recognition and rejection that occurs during pollen-pistil interaction is presented in figure 7. This is largely based on the site of recognition and rejection, and the factors involved in recognition. The details of biochemical events involved in these processes are not considered, as our present understanding on these aspects is very meagre.

It is apparent that the data on interspecific incompatibility is fragmentary. Unlike the situation in intraspecific incompatibility, there is no uniform pattern in the operation of interspecific incompatibility. Pollen-wall and pellicle components are probably involved in the first three stages of inhibition, i.e., pollen hydration, pollen germination, and cutinase activation. No information is available on the factors involved in pollen tube inhibition in the style. Although pollen hydration appears to be a physical phenomenon, there are many instances in which pollen hydration is prevented following interspecific pollinations. The mechanism involved in such inhibition is not clear. It is likely that non-proteinaceous factors such as phenolic compounds and carbohydrates also play a role, particularly during pollen hydration and pollen germination. Recently, phenolic compounds have been shown to selectively promote or inhibit pollen germination (Martin 1970, 1972; Martin and Ruberte 1972; Tara and Namboodiri 1974; Sedgley 1975). In *Impatiens sultani* the mutants orange, crimson and pink, are partially or fully male sterile but produce normal embryo sacs (Tara and Namboodiri 1974). Pollination of orange and crimson varieties with viable pollen resulted in seed set, but not of pink. This was caused by the failure of the stigma of pink variety to support germination of viable pollen. Ultraviolet

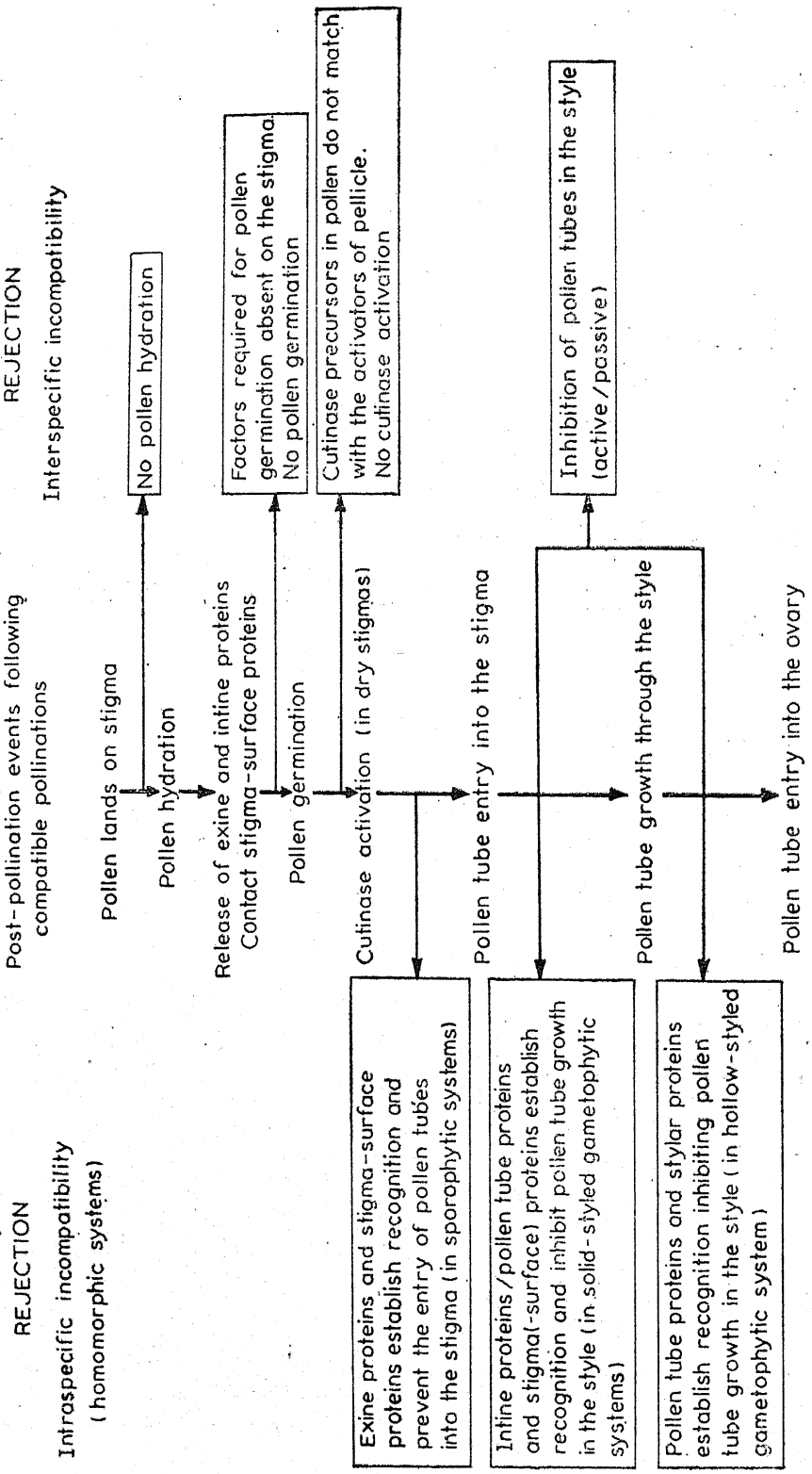

Figure 7. Probable sequence of recognition and rejection during pollen-pistil interactions.

absorption profiles of the stigmatic fluid showed that the crimson and orange varieties showed an absorption peak at 261 nm, which was lacking in the stigmatic fluid of the pink variety. Chromatographic studies showed that two phenolic spots, common to orange and crimson varieties, were absent in the pink variety (Tara and Namboodiri 1976). The authors suggested that absence of these phenolic compounds in the stigma of pink variety is responsible for its failure to support pollen germination. Although such a mechanism cannot operate in intraspecific incompatibility, it may be widespread in controlling interspecific incompatibility in distantly related species. It represents passive rejection in which there is no need for pollen recognition.

Another method of passive rejection would be the inability of pollen tubes to penetrate the cuticle of the stigma as has been shown in the cross involving *Gladiolus* (♀) and *Crocosmia* (♂) (Knox *et al* 1976). This happens when the cutinase precursors in the pollen do not match with the activators of the pellicle. These passive rejections are more like a lock and key mechanism in which incompatibility is due to the absence of a suitable key (with one of the partners for the lock present with the other partner). Closely related species would have the right key at the stigma level, but may be unable to grow through the style. Inability of the pollen tube to utilize stylar nutrients, which may be due to the lack of positive recognition, may often be the reason. In very closely related species, particularly in instances of unilateral incompatibility between self-incompatible (♀) and self-compatible (♂) individuals, it may be due to active inhibition.

Although the data on intraspecific incompatibility are more sound, generalisations have been based on a few systems, on circumstantial evidences rather than by direct demonstrations. Investigations are yet to be extended to other systems. Nonetheless the scheme, apart from presenting the available data in a proper perspective, emphasises more prominently the areas which require critical investigations for a better understanding of the pollen-pistil interactions. As more data become available, the scheme, will have to be altered or modified.

8. Mechanism of inhibition

8.1. *Intraspecific incompatibility*

Although significant progress has been made in understanding the factors involved in intraspecific incompatibility, not much progress has been made in understanding the mechanism of inhibition of incompatible pollen tubes. Many models have been developed based on circumstantial evidences (Linskens 1965; Lewis 1965; Ascher 1966; see Nettancourt 1972). Basically these models envisage the production of an S-allele-specific polypeptide identical in pollen and style. When the polypeptide of the pollen comes in contact with the identical polypeptide in the style (upon self-pollination), it dimerises on the surface of the pollen tube to form a repressor (figure 8) which inibits the growth of the pollen tube.

Pandey (1975) modified the concept of Lewis (1960) concerning S-gene determination, based on the tripartite nature of S-gene (figure 9). S-allele-specific protein coded by specificity part is identical in pollen and style. But this protein unites with the tissue-specific complementary protein in the style and pollen. Thus,

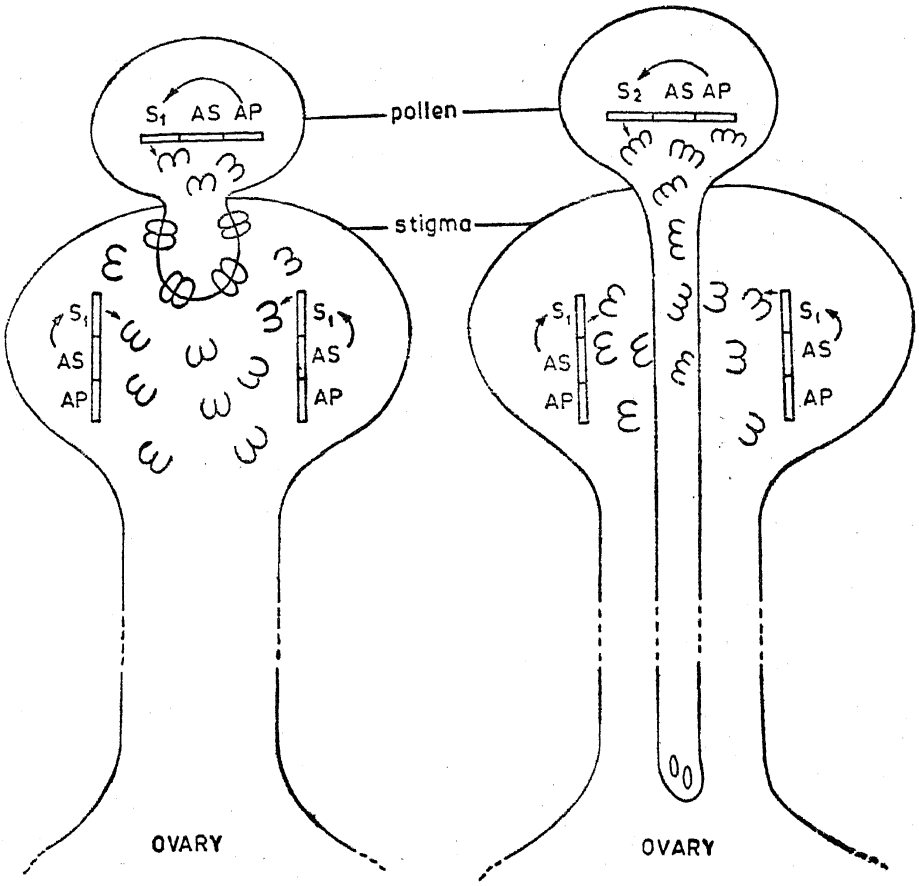

Figure 8. A generalised model to explain the function of S-allele. This is based on the tripartite nature of the S-locus (Lewis 1960) having a specificity part (S) common to both pollen and style, and two activity parts controlling the reaction in pollen (AP) and style (AS). Each S-allele produces a specific polypeptide as a result of activation by AP (pollen) or AS (style). When polypeptides of the pollen and the style are identical, they dimerize on the surface of the pollen tube to form a repressor inhibiting pollen tube growth. When polypeptides of the pollen are different from those of the style, no repressor is formed and hence the pollen tube is not inhibited (modified from Nettancourt 1972).

the S-allele proteins in the pollen and style have an identical specificity-protein, and tissue-specific adaptive protein (figure 9). This model explains the failure of the stylar, or the pollen proteins to react amongst themselves, but allow for the interaction between them to produce a repressor of the genes involved in pollen tube growth.

The mechanism by which the repressor inhibits the growth of the pollen tube is not clear. According to Lewis (1965), the repressor acts as a genic regulator to induce the synthesis of an inhibitor, or to repress the synthesis of an auxin responsible for the growth of pollen tubes. A few investigators have implicated the involvement of peroxidases (Pandey 1967; Bredemeijer 1974; Bredemeijer and Blass 1975) and esterases (Pandey 1973) in pollen tube inhibition, which may probably be under the control of the repressor. These findings are largely circumstantial and no conclusive proof for the involvement of any specific enzyme is available.

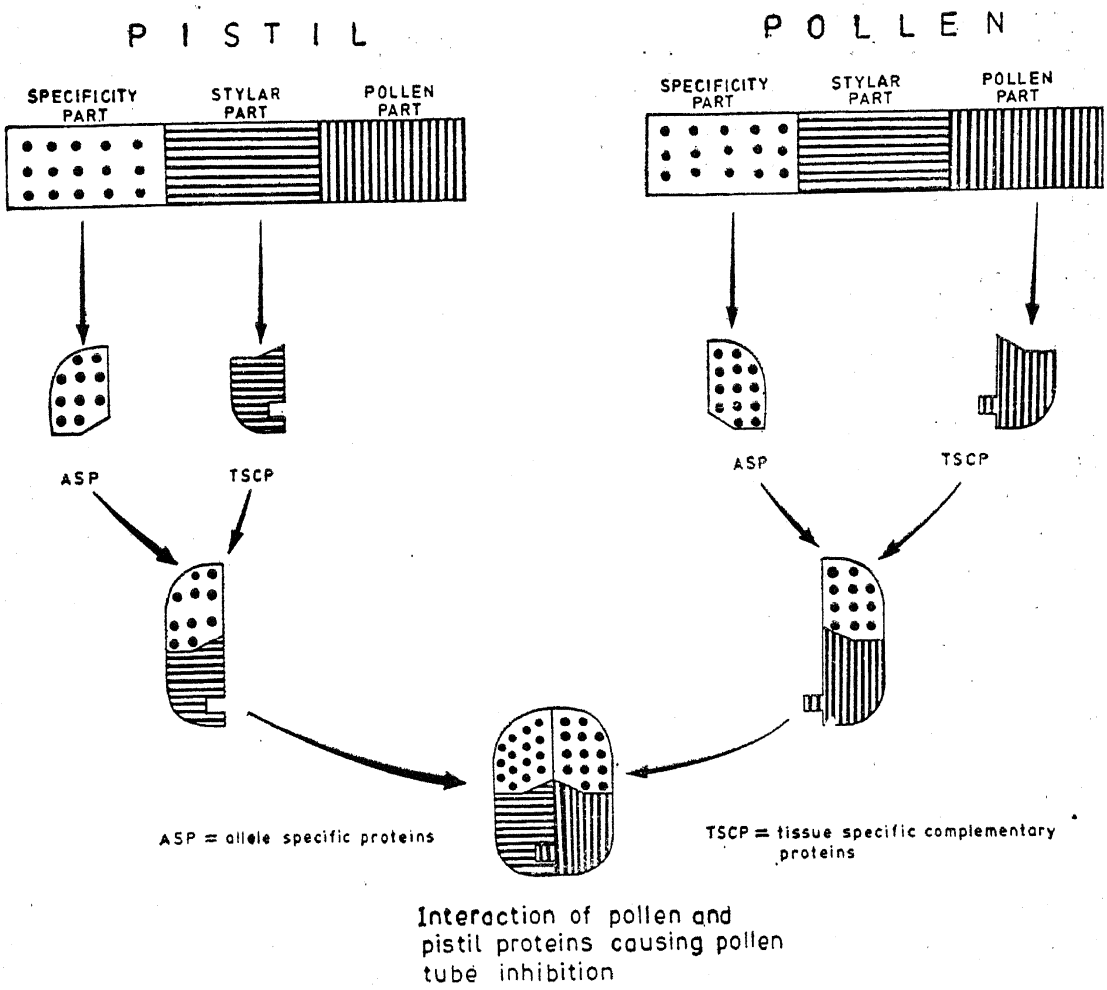

Figure 9. Diagrammatic representation of the hypothesis concerning S-gene action (modified from Pandey 1975). This explains the formation of mutually reactive S-allele-specific proteins in the stigma and the style.

According to Ascher's model (1966) the S-allele acts as a regulator gene governing two sets of operons in the pollen tube which control pollen tube metabolism. One set (the low velocity operon) controls the metabolism utilizing the reserves available in the pollen itself. Activation of this operon results in the limited growth characteristic of incompatible pollen tubes. The other (high velocity operon) controls a different metabolic pathway which enables the pollen tube to utilize stylar nutrients. Activation of this operon results in the continued growth typical of compatible tubes. When the S-allele specific pollen regulator product is the same as the stylar regulator product (upon self-pollination), the two molecules unite to form a dimer repressor which attaches itself to the high velocity operon. Thus, the high velocity operon remains repressed; and hence, the pollen tubes are unable to utilize stylar nutrients and cease to grow after pollen reserves are depleted. When the pollen regulator products are different from the stylar regulator products, no functional repressor is formed. The high velocity operon is activated and enables the pollen tubes to utilize the stylar nutrients for continued

growth. Although ultrastructural studies of the pollen tube tip in compatible and incompatible pistils of *Lilium* (Rosen and Gawlik 1966) are in agreement with this hypothesis, biochemical evidences have not indicated differential utilization of metabolites by compatible and incompatible tubes (Labarca and Loewus 1973).

One of the characteristic ultrastructural features of incompatible pollen tubes in the pistil of *Lycopersicum* is the alteration in endoplasmic reticulum into whorls of concentric layers and its subsequent degradation (Nettancourt *et al* 1974). Based on these evidences Nettancourt *et al* (1974, 1975) suggested that one of the early manifestations of intraspecific incompatibility is the general cessation of protein synthesis in pollen tubes (see also Cresti *et al* 1977). Biochemical investigations are necessary to substantiate this hypothesis.

It must be emphasized that these models explaining the mechanism of pollen tube inhibition, although elegant, are not based on direct evidences. The only direct evidence has been the demonstration by immunological methods of the formation of S-allele-specific proteins in pollen grains of *Oenothera* (Lewis 1952; Lewis *et al* 1967) and *Petunia* (Linskens 1960), and in pistils of *Petunia* (Linskens 1960) and *Brassica* (Nasrallah and Wallace 1967). Of these, the most comprehensive studies have been on *Brassica* by Nasrallah and his associates who supplemented immunodiffusion studies with electrophoretic techniques and genetic analysis (Nasrallah and Wallace 1967; Nasrallah *et al* 1970, 1972; Nasrallah 1974). They demonstrated that each S-allele-specific protein had a different electrophoretic mobility and, hence, each of them could be localised to a specific band on the gel. These proteins are heritable as shown by the presence, in the heterozygous plants, of both the S-allele specific bands of the parents (Nasrallah *et al* 1970).

Nasrallah *et al* (1972) also studied the inheritance of S-allele-specific proteins in 3 homozygous self-incompatible genotypes of *Brassica oleracea*, and their F_1 and F_2 progenies. By the use of both immunodiffusion and disc electrophoresis independently, to test the presence or absence of self-incompatible proteins, they demonstrated that S-allele specific proteins present in the stigma of F_1 and F_2 plants were exactly correlated with the segregation of S-alleles as determined by genetical analysis (phenotypic expression of self-incompatibility). Recent studies of Nasrallah (1974) on a self-incompatible and a self-compatible line of *B. oleracea* have provided further evidences to S-allele-protein-phenotype relationship.

Although these studies conclusively demonstrated the formation of S-allele-specific proteins in the pollen and the style, their similarity has so far been demonstrated only in *Petunia*. Attempts to demonstrate S-allele-specific proteins in the pollen grains of *Brassica* have not been successful (Nasrallah and Wallace 1967). Heslop-Harrison (1975b) has pointed out the possibility that the immunological methods which use the rabbit system may not be sensitive enough to detect the differences in the pollen-proteins of different S-genotypes of *Brassica*. This is supported by the report that different rabbits show variation in the response to produce antisera against S-allele-specific proteins of the stigma extract of *Brassica* (Sedgley 1973).

One of the significant demonstrations of intraspecific incompatibility is the report of Van der Donk (1975c) that the RNA, extracted from the pollinated styles of *Petunia*, when injected into the oocyte of *Xenopus levis* could synthesize the proteins characteristic of the injected RNA. Further, when *Petunia* stigmas

were treated with protein extracts from *Xenopus* egg following injection of RNA from selfed style, compatible pollen tubes were inhibited to the same extent as selfed tubes. This observation is rather unexpected, as it is well established that the presence of selfed pollen tubes in the pistil does not affect the growth of compatible tubes. Nonetheless, these experiments should pave the way for a fuller understanding of the inhibitory processes.

Recently, Kroes (1973) put forward an entirely different hypothesis to explain the mechanism of self-incompatibility. According to this theory, S-allele has two functions: in the pollen it inhibits the production of a specific enzyme, and in the style it produces a specific complex in which an essential nutrient required for pollen tube growth is bound to a macromolecule. If the S-allele in the pollen and style are the same, the pollen tube cannot grow through the style, because it lacks a specific enzyme to release the stylar nutrient; but it can grow through the styles having other S-alleles, as it will have enzymes for breaking stylar components bound to any other S-allele. Thus, pollen grains of different S-alleles differ not in the substance they produce, but in the substance they lack. This theory interprets self-incompatibility reaction more as a passive phenomenon, rather than as active inhibition. It is not consistent with our present knowledge on the physiology and biochemistry of self-incompatibility. Many of the incompatibility phenomena such as high respiratory rate following selfing, success of bud pollination and delayed pollination, ability of self-incompatible pollen tube to grow through the style in many interspecific and often intergeneric crosses, and the requirement of RNA synthesis for self-incompatibility, are difficult to explain on the basis of this theory (see also Nettancourt *et al* 1974).

It is generally thought that the mechanism of inhibition in the gametophytic system is basically different from that in the sporophytic system. Many of the hypotheses discussed above were formulated to explain, largely, the gametophytic system. Based on the light microscopic studies, which indicated the cuticle of the stigmatic papillae as the barrier for the entry of incompatible tubes, Christ (1959) put forward cutinase hypothesis to explain incompatibility phenomenon in crucifers. He suggested that either (*a*) cutinases present in the pollen are inactivated on an incompatible stigma, or (*b*) the cutinase precursors of the pollen require specific activators found only on compatible stigmas. Subsequent demonstration of the presence of cutinases in germinating pollen of Cruciferae by Linskens and Heinen (1962) supported cutinase hypothesis. Experiments of Kroh (1966) on self-incompatible *Arabis arenosa* and *Brassica nigra* involving transfer of pollen grains from one stigma to the other supported the second alternative of cutinase hypothesis.

However, recent electron microscopic studies on cruciferous stigma (Kanno and Hinata 1969; Dickinson and Lewis 1973) have clearly shown that the cuticle is eroded following self-pollination also. Cuticular erosion occurs even when the pollen coat substances are isolated and deposited on the stigma. Cutinase hypothesis is, therefore, no more tenable to explain sporophytic incompatibility. As the incompatible tubes in the sporophytic systems are also inhibited in the pectocellulosic wall of the papillae (as it happens in many gametophytic systems), the zone of inhibition appears to be of secondary importance in distinguishing the two types of incompatibility. Analysis of details of inhibition in other systems makes this more apparent.

Although in most of the gametophytic systems incompatible pollen tubes are inhibited after growing through a considerable distance in the style, in members of Gramineae, and in *Oenothera organensis*, inhibition occurs on the stigma itself or in the region immediately subjecent to the stigma (Dickinson and Lawson 1975; Heslop-Harrison 1976). In some species of *Gaudinia* and *Saccharum* individual plants belonging to the same species show variation in the zone of inhibition. It is noticed either on the surface of the stigma, or after the pollen tube has grown to various lengths through the stigmatic papillae (Shivanna *et al* 1976). In yet another grass, *Cynodon dactylon*, inhibition has been recorded in the main axis of the style (Thomas and Murray 1976). In taxa characterised by hollow style, pollen tubes are inhibited at any distance between half-way down the style and the ovary. Thus, there is much variation in the zone of inhibition amongst the taxa having the same genetic basis of incompatibility. The zone of inhibition may be based more on the cytomorphology of the stigma and style, and the intensity of incompatibility reaction, rather than on the differences in the mechanism of inhibition.

In some taxa both recognition and rejection are completed on the stigma itself. In others recognition is established on the stigma, but rejection is delayed until pollen tubes reach the style. In yet others, both recognition and rejection take place only in the style. It appears that there are no basic physiological differences in the mechanism of inhibition between the sporophytic and gametophytic systems. The models of incompatibility put forward to explain the gametophytic systems may as well apply to the sporophytic systems.

8.2. *Interspecific incompatibility*

Our knowledge on the details of inhibition following interspecific pollinations is meagre. As pointed out earlier, the rejection at the initial stages of pollen-pistil interaction (i.e. pollen hydration, pollen germination, and pollen tube entry into the stigma) does not appear to involve active inhibition. The mechanism involved in pollen tube inhibition in the style seems to vary greatly. It may be passive inhibition caused by absence of factors in the pistil required for pollen tube growth. In many instances the crosses involve parents which differ in the length of the style. For example, in species of *Nicotiana* and *Datura* the stylar length ranges from 1 to 10 cm. When a species having a shorter style is used as the male parent to cross with a species having a longer style, pollen tubes generally fail to reach the ovary, while the reciprocal cross results in seed set (see Maheshwari and Rangaswamy 1965). Thus, the maximum length attained by the pollen tube in the style of a given species is generally equal to the length of its style. In such crosses involving species with different stylar lengths, the failure of the pollen tube to reach the ovary may be due to its intrinsic inability to grow beyond a certain length, rather than to an active inhibition.

In crosses involving closely related species, particularly in instances of unilateral incompatibility, pollen tube inhibition is an active process. According to some investigators (Lewis and Crowe 1958; Pandey 1968, 1969, 1970) the S-allele has dual function and is involved in both intra- and interspecific incompatibility. Other investigators consider interspecific incompatibility to be controlled by genes

not belonging to the S-locus (see Abdalla 1974). Recently, Takahashi (1974) carried out detailed analysis of intra- and interspecific incompatibility in *Nicotiana alata* and *N. langsdorffii* (both self-incompatible taxa). Fully self-compatible clone (L_4 having S_5 S_f alleles) of *N. langsdorffii* showed stronger incompatibility when crossed with pollen of *N. alata*, than clone L_5 (having S_6 S_7 alleles) which is fully self-incompatible. Thus, the S_f gene did not weaken interspecific incompatibility. Also, when self-incompatible clone of *N. alata* was crossed with pollen of L_4 clone of *N. langsdorffii*, both S_5 and S_f pollen were inhibited. Based on these studies Takahashi concluded that the genes controlling interspecific incompatibility are different from those controlling intraspecific incompatibility.

Hogenboom (1975) put forward the concept of 'incongruity' to explain interpopulation incompatibility. According to him, there are two distinct mechanisms for the non-functioning of pollen-pistil relationship: incompatibility due to the identity of S-alleles, and incongruity due to incompleteness of the relationship. Incongruity results from lack of genetic information in one partner about some relevant character of the other, and hence varies from system to system. Incongruity is a by-product of evolutionary divergence. Amongst the freely interbreeding population, a sub-population may differentiate as a result of changed environment. This may bring in an extra barrier in the pistil of one of the populations with the pollen of the other. This results in incongruity between populations, being slight at first but increasing as the divergence proceeds. Thus, incongruity is more complex, and involves many genes, depending on the extent of divergence. Incongruity may also be concerned with the post-fertilization barrier.

Physiological studies on interspecific incompatibility are limited. Roggen and Linskens (1967) studied pollen tube growth and respiration pattern following intergeneric crosses between *Petunia hybrida* and *Salpiglossis sinuata*. The morphological abnormalities of incompatible pollen tubes (branching, swelling of tube-tip, increased callose deposition, etc.), and the respiratory pattern following intergeneric crosses were similar to those following selfing. They suggested that the mechanism of inhibition of pollen tubes is similar in both self- and intergeneric incompatibility. However, in *Lilium*, self-incompatibility can be overcome by hot water treatment of the pistil whereas interspecific incompatibility cannot be overcome (Ascher and Peloquin 1968), indicating that the mechanism of action in both types of incompatibility is different.

Further physiological and biochemical studies on interspecific incompatibility are necessary for a clearer understanding of the mechanism of inhibition. It is apparent that unlike intraspecific incompatibility, there is no uniform method of inhibition of pollen tubes following interspecific pollination. The mechanism varies greatly between crosses, depending on the extent of reproductive isolation of the two species.

9. Concluding remarks

From the foregoing account it is obvious that the progress in our knowledge on the biology of pollen-pistil interaction is impressive, but far from complete. Detailed investigations, whether structural, or physiological, or biochemical, have

been confined only to a few selected taxa. In addition to the need for intensification of studies on the established taxa, it is important to study other systems.

More attention needs to be paid to investigate the physiology of the pollen and stigma, as their interaction during the initial stages of pollination determines subsequent events in the pistil. Localisation of pollen-wall proteins and stigma-surface proteins and their implication in pollen-pistil interaction have been some of the important achievements in recent years. Although the details of the origin of pollen-wall proteins and their incorporation have been followed in a few systems, the details of the stigma-surface proteins, particularly in dry types, are yet to be investigated. Attempts must also be made to characterize the heterogeneous components of the stigmatic surface and pollen wall, and in identifying the role of individual components in pollen physiology, stigma receptivity, pollen recognition, and eventual acceptance, or rejection of the pollen tube. In this direction the investigation of Knox *et al* (1976) on *Gladiolus* is significant.

There are no direct evidences for the involvement of the sporophytic tissue in the function of recognition in the gymnosperms. Recently, however, extracellular glycoproteins of the sporophytic origin in the ovule of gymnosperms have been implicated in recognition phenomenon (Pettitt 1977a, b). In *Ginkgo biloba* and many cycads, the megaspore wall which encloses the megagametophyte has been shown to be similar (both structurally and chemically) to the pollen wall. The chambered exine of the megaspore becomes covered with the degeneration products of the surrounding nucellar cells—the sporophytic tissue (Pettitt 1977a). These products show strong esterase activity but no detectable acid phosphatase activity, similar to exine components of angiosperm pollen. Also, a glycoprotein moiety which is capable of binding to con A is also present in the wall components (Pettitt 1977b). In cycads although the megaspore wall above the archegonium breaks down before the male gametes are released, the liquid present in the archegonial chamber would have these nucellar components. These glycoprotein fractions have been suggested to be involved in the function of recognition (Pettitt 1977b). Further studies on these primitive gymnosperms and extension of these studies to other gymnosperms, would no doubt yield valuable information on the recognition phenomenon in gymnosperms. Also, in many gymnosperms the female gametophyte develops only after pollination. It is to be established whether the development of the female gametophyte is a response to the recognition (of the right pollen) established in the pollen chamber, or whether it results from a general activation in response to non-specific substance(s) released from the pollen. Experimental investigations are required to clarify these points.

Whereas studies on interspecific incompatibility have remained fragmentary, recent investigations on various aspects of self-incompatibility have been rewarding. The evidences for implicating exine-proteins in controlling sporophytic incompatibility are more direct and conclusive, but the role of intine proteins in gametophytic incompatibility needs more convincing proof. There is urgent need to extend these studies to other taxa. The ultimate aim of these investigations should be to isolate and characterize the proteins involved in pollen recognition and rejection.

The progress in the understanding of the mechanism of incompatibility has been limited. Many hypotheses have been put forward to explain the gametophytic incompatibility, on the basis of interaction of identical pollen and pistil-

proteins. However, the similarity of S-specific proteins from the pollen and pistil has been immunologically shown only in *Petunia*. Extensive immunological and electrophoretic investigations, similar to those carried out on *Brassica* by Nasrallah and his associates, need to be conducted on other systems.

Since the classical work of Lewis on the mechanism of heteromorphic incompatibility in *Linum grandiflorum* (see Lewis 1954) there have been hardly any physiological or biochemical studies on heteromorphic systems. In the light of recent progress on the role of pollen-wall proteins and stigma-surface proteins and on the mechanism of incompatibility in homomorphic systems, it would be worthwhile to extend the studies to heteromorphic taxa also.

Although self-incompatibility is widespread in flowering plants, experimental investigations have so far been confined to a few selected systems, largely to members of the Cruciferae, Compositae, Liliaceae, and Solanaceae. Even with limited experimentation, variations in the physiology and biochemistry associated with pollen tube inhibition have become significant. It is likely that each group of plants has a unique mechanism of inhibition. Self-incompatibility inhibition may be more complex than presently considered. The linkage of self-incompatibility with other characters in heteromorphic systems, the involvement of more than one S-gene in self-incompatibility in many taxa (Lundqvist 1975; Verma *et al* 1977; Lewis 1977; Larsen 1977), the genetic analysis indicating polygenic control, the effects of genetic background, and the appearance of new specificities due to inbreeding (Nettancourt *et al* 1975) are some of the complexities. It is hoped that the problems outlined above would engage the attention of investigators in the coming years.

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