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Stigma-pollen recognition: a new look

C. DUMAS, T. GAUDE

Université Cl. Bernard-Lyon 1, Biologie végétale, A.T.P. "Biologie des Membranes", 43, Bd du 11 Novembre 1918, 69622 Villeurbanne, France

Abstract

During the last two decades, there have been several conceptual developments in our understanding of pollen-stigma recognition and molecular mechanisms involved. The main models proposed are compared. Based on additional data a hypothesis to complete these models especially for pollen hydration and adhesion is proposed. After attachment of the pollen to the stigma surface a close interaction exists involving lipoproteic membrane-like compounds (pollenkitt and stigma pellicle) and pollen agglutinating ability.

INTRODUCTION

No evidence for the presence of an immunological system producing antibodies or a histoincompatibility system encoded by genes has been found in plants (East, 1931; cited in Lewis, 1976). The main particularity for the flowering plant recognition and reproduction system is the recognition and discrimination against self. This mechanism gives a selective advantage in fertilization. In contrast, the immune system of vertebrate animals is programmed to recognize and respond to non-self rather than against self. But. Katz and Skidmore (1978) consider that the immune system is not an exception to the general rule. Some analogies can be seen between the immune system of higher vertebrates, mechanisms of self-recognition in lower colonial marine organisms (Burnet, 1971; Theodor, 1970) and in the mechanisms preventing self-fertilization in higher plants.

Lewis (1963) has proposed 2 hypotheses some years ago to explain mechanisms of self-recognition in flowering plants. A complementary stimulus (or an oppositional inhibition) to pollen tube germination and growth by a complementary (or an oppositional) interaction of two unlike (or like) S gene products in pollen and pistil. This would positively stimulate (or inhibit) pollen tube germination or development for compatible (or incompatible) combinations (Lewis, 1979). In plants it has not been

possible to decide definitively between interaction of 2 identical proteins (or polypeptide determinants) or 2 sterically complementary patterns. However, Clarke et al. (1977) provided some evidences in favor of the hypothesis that interactions occur between identical proteins. But Nasrallah (1979) from immunodiffusion results says that pollen-stigma coordination "is achieved by the expression of a specific glycoprotein antigen in the stigma, and of a complementary but different receptor molecule in the pollen", and therefore that different genetic units are responsible for the pollen reaction.

During the last two decades, there have been several conceptual developments in our understanding of pollen-stigma recognition and molecular mechanisms involved. It is interesting to compare the main models proposed.

I. SOME DIFFERENT MODELS PROPOSED TO EXPLAIN PISTIL-POLLEN RECOGNITION

We successively envisage different models starting from the model of Christ (1959) to the biochemical model of Ferrari and Wallace (1977).

1. Model of Christ (1959) or cutinase hypothesis

Christ suggested that either cutinases present in the pollen are inactived on an incompatible stigma or the cutinase precursors of the pollen require specific activators found only on compatible stigmas. The presence of cutinases (glycoprotein-like compounds) in germinating pollen of *Cruciferae*, supported this hypothesis (Linskens, Heinen 1962) but recent observations have shown that the cuticle is slightly modified after self-pollination also (Dickinson, Lewis, 1973a).

2. Model of Heslop-Harrison (1975)

It seems that the self-incompatibility (in *Brassica*, for example) may be considered to consist of five or six stages (see Fig. 1, explanation in Heslop-Harrison, 1975) with:
a - an initial recognition between stigma pellicle and pollen grain wall (proteins); b - an inhibition phase; c - the rejection of the incompatible pollen. A preponderant stigma control into pollen rejection is proposed in this model. It has been assumed that the mechanism is oppositional rather than complementary (De Nettancourt, 1977).

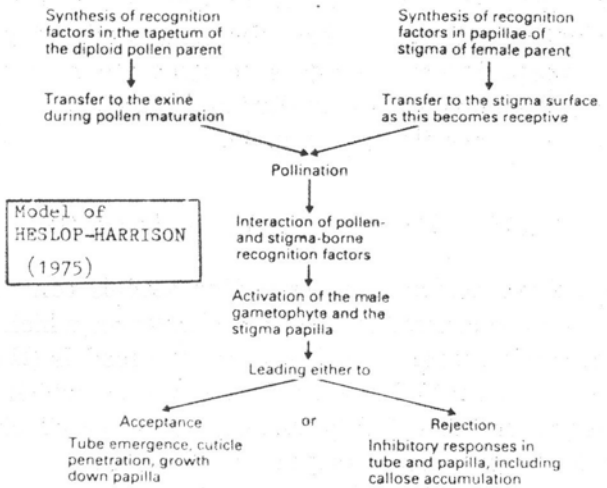

Fig. 1. Scheme for the interaction of pollen and stigma in species with the sporophytic self-incompatibility system

3. Model of van der Donk (1975)

This author believes that the recognition reaction in the gametophytic system is not based upon the interaction of identical S-polypeptides (Lewis' hypothesis, 1963), but on the matching of different substances in pollen and styles respectively produced by the pollen part

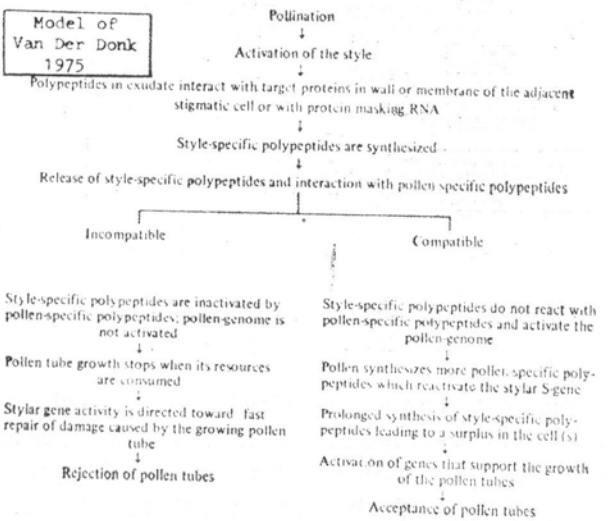

Fig. 2. Sequences of events postulated to occur by van der Donk (1975) after compatible and incompatible pollinations in species with gametophytic monofactorial self-incompatibility

and the stylar part of the S-locus. Following De Nettancourt (1977), this biochemical model has the great advantage to explain "the apparent incapacity of mutagens to generate new specificities at the S-locus and the apparent contradiction between self-incompatibility and interspecific incompatibility" (Fig. 2).

4. Model of Ferrari and Wallace (1977)

The major difference from the preceding models concerns the hypothetical events for the control of the tube elongation which occurs in the pollen grain after self-recognition. The preceding models (Heslop-Harrison, van der Donk) both emphasize events which occur in the stigma (sporophytic incompatibility, *Brassica* type) or which fail to occur in the stigma (gametophytic incompatibility, *Petunia* type) as the cause of inhibition of the tube elongation.

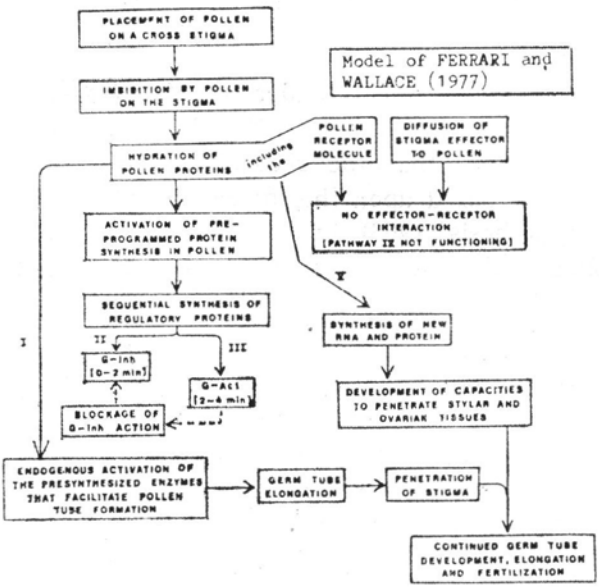

Fig. 3. *In vivo* pathways functioning in *Brassica* to control germ tube emergence following a cross pollination

Ferrari and Wallace recognized at least eight stages (Fig. 3): "1 - recognition event; 2 - interactions of substances released by pollen with other products from stigmatic tissues; 3 - subsequent development of the regulatory-system which controls enzymes involved with germ tube formation; 4 - germ tube formation as mediated by a pre-synthesized enzyme system; 5 - development of separate capacities to penetrate tissues of the papillae; 6 - stigma; 7 - stylar conducting tissue; 8 - ovary".

These authors conclude that "for either sporophytic or gametophytic control new protein synthesis is required for expression of self-incompatibility, but not for initial germ tube elongation" and "the regulation of incompatibility for sporophytic and gametophytic control systems is basically the same".

5. Model of Shivanna (1979)

She has not presented the details of biochemical events involved in the sequence of recognition and rejection of pollen but this model is based largely on the site of recognition and rejection (Fig. 4).

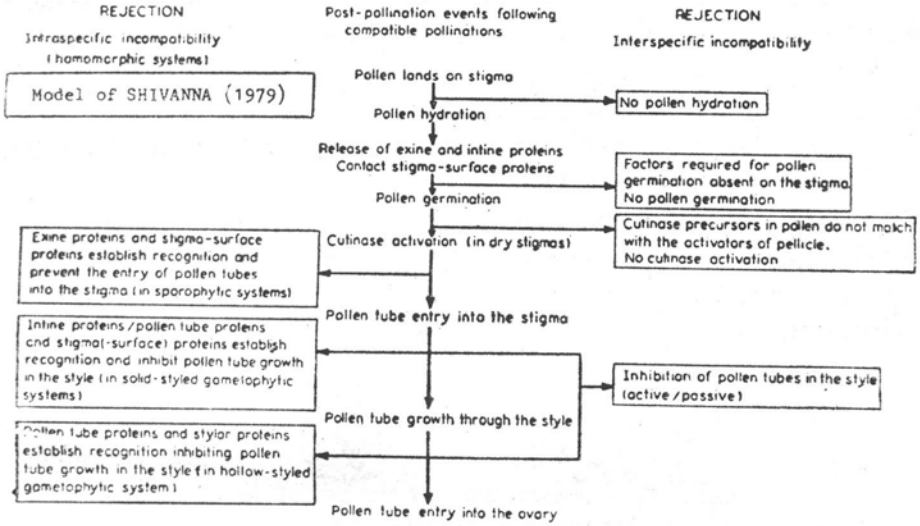

Fig. 4. Probable sequence of recognition and rejection during pollen-pistil interactions

These 5 models cannot explain the preliminary steps of pollination and the events involved in hydration. We propose some hypotheses to complete these two late models.

II. ADHESION

Cell adhesiveness is a fundamental cell property. It probably plays an important role during developmental processes such as pollen hydration and the first steps of pollen germination or rejection.

In *Brassica*, for example, two different approaches to studying the problem of pollen adhesion may be characterized: the adhesion of pollen to each other, and the adhesion of pollen grains to extracellular

substrata: stigma cells or artificial medium (Fig. 5). Pollen adhesion is a dynamic process where the rate of pollen adhesion depends on the rate of initial contact of pollen grains with the substratum (biological or physical) or with each other and the subsequent formation of attachment bonds. According to Grinnell (1978), separation is independent of contact interaction, whereas it is precisely the contact interaction that is rate-limiting for adhesion. Differences in the adhesion of self- or cross-pollen to the stigma may occur a few minutes after pollination since 10-15 minutes after pollination, self-pollen is easier to remove than cross-pollen (Roggen, 1975). When pollen adhesion is observed with a light or scanning electron microscope, many events are seen to occur, but overall there is an important shape change after hydration (Heslop-Harrison, 1975; Stead et al., 1979) (Fig. 6). To understand this adhesion, three phenomena may be involved:

a) which cell surface components are involved adhesion?
b) what constitutes the bond of adhesion?
c) which "pollen envelopes" and perhaps cytoplasmic forces are responsible for the shape change?

It becomes clear that pollen adhesion occurs in a series of steps named pollination, contact, attachment, hydration. These successive steps probably correspond to a variety of physical, mechanical and physiological mechanisms.

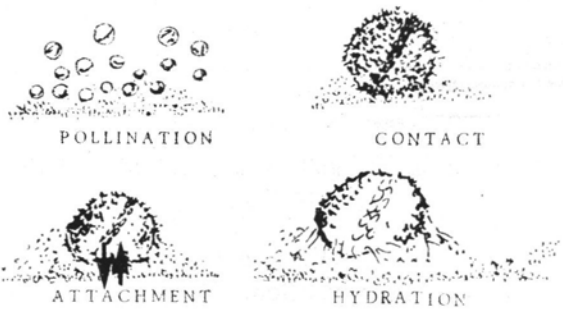

Fig. 5. Multistep of pollen adhesion

1. Pollination

Pollination mechanisms are the means by which transference of functional pollen is achieved, or avoided, from the pollen-bearing structure to the compatible and receptive surface of the pistil. The success of pollination requires a fine degree of reciprocity and coadaptation between the pollen wall (pollen envelopes) and the stigma cell surface (review in Frankel, Galun, 1977).

2. Pollen contact

Pollen contact with the substratum (biological or physical) may be dependent on the presence of an electrostatic barrier between the two surfaces. No data do exist to day on this particular point.

3. Pollen attachment

Following contact of the pollen grains with the substratum, bonds of attachment form. Only bonds between pollen grains and protein-coated substrata appear to be mediated by specific ligand-receptor-like interactions: contact between the stigma pellicle and the pollen proteins of exine, for example. Many papers on the ultrastructure of the stigma in recent years have been published (Heslop-Harrison, 1975; Dumas, 1975; Heslop-Harrison, Shivanna, 1977). The most important outcome of the latest work has been the demonstration of the presence of stigma-surface proteins both in wet and dry types. In the dry type, proteins occur in the form of a thin pellicle with an intense nonspecific esterase activity.

The bonds between "pollen envelopes" and protein-free substrata are probably non-specific and a specific pollen grain adhesion may require the redistribution of cell surface adhesion receptors. Where are these receptors localized in "pollen envelopes"? The mature pollen grain is enclosed in a double wall structure; the inner one called the intine, the outer — the exine. Two sorts of compounds are accumulated into the exine crypts and on the outside of the mature pollen grain. The "pollen-kitt" is an oily layer found on the outside; it appears to be principally hydrophobic lipids and species-specific carotenoids. The tryphine appears to be a complex mixture of hydrophilic substances (Echlin, 1971; Dickinson, Lewis, 1973b) .

4. Pollen hydration

After initial contact and adhesion of the pollen grain, there is a general modification observed *in vitro* and associated with hydration. *In vivo*, some pollen grains do not become hydrated (Shivanna, 1979). Close related species would accomplish more of the post-pollination events than distant related species (Knox et al., 1976). When the stigma of *Gladiolus* (*Iridaceae*) is pollinated with pollen belonging to a different family (*Liliaceae*, for example) the pollen hydration step locked. Compatible pollen grains probably require a specific adhesion to the stigma cell in order to grow and primary recognition probably exists between contact and attachment. A Petri dish containing hydrated me-

dium does not correspond to a specific substratum but we always observe pollen hydration. An early observation on animal cells indicating the importance of substratum chemistry in adhesion was the dependence of adhesion on the type of glass substratum used. A critical number of negative charges on the glass was thought to be required; it contains at least 5 negatively charged groups per 10 nm (Maroudas, 1977).

Many times, high relative humidity (RH) was reported to overcome the self-incompatible response (Carter, McNeilly 1976; Ockendon, 1978). After cross-pollination, Stead et al. (1979) have demonstrated that the adhesion between the pollen grains and the stigma surface was better than after self-pollination and the mobility of proteinaceous component of the pollen grain coating was greater after self-pollination. For Stead et al. (l.c.), the significance of pollen grain adhesion into the self-incompatibility system in *Brassica* is not clear. These authors propose that the differences in adhesion result from physical or chemical changes taking place in the components that bind the pollen to the stigma. A change in the "pollen envelope" characters and properties would certainly be accompanied by a change in its affinity to water.

III. POLLEN ENVELOPE CANDIDATES FOR RECOGNITION

Direct histochemical and biochemical evidences for the presence of proteins and lipids in the pollen wall have been given (Stanley, Linskens, 1974; Dickinson, Lewis, 1975; Heslop-Harrison et al., 1975) and pollen wall has been considered as a living structure.

A successful approach to indentify morphological pollen-substratum attachments must be effective in looking for *in vitro* correlates of *in vivo* structures. We have observed a tripartite membrane-like structure for the pollenkitt in *Cruciferae* and *Caryophyllaceae* species (Dumas, Gaude, unpublished data). This membrane-like structure is PTA positive. Is it a candidate for pollen grain attachment? Other experiments are actually in progress on this topic. Some compounds involved in "pollen print" deposits (Heslop-Harrison et al., 1975) easily stained with Coomassie blue are removed with the Folch' mixture and are probably lipoprotein-like compounds. The same results may be obtained with stigma prints (Dumas, Gaude, unpublished data). For Roggen (1974), the "pollen-coat" in *Brassica* "is similar to what is called "recognition substances" (see model of Heslop-Harrison, Fig. 2) and probably involved lipoproteic compounds". Roberts et al. (1979) have not observed fundamental changes in lipids of the pollen grain coating of *Brassica* following self- or cross-pollination. Neutral lipids appear not to be involved in the incompatibility system in *For-*

sythia (Dumas, 1977). The constant presence of lipids in the stigma-pollen interface remains to be explained. In addition, it is almost certain that proteins are involved in all biological reactions of lipids. The elucidation of lipid-protein interactions is therefore one of the most important problems of biological chemistry to day (Morrisett et al., 1975; Wickner, 1979).

In Fig. 6, we have envisaged that active cell adhesion is highly specific. The idea of highly specific adhesion receptors is well known in biology (Greaves, 1976) and cell surface ligand-receptor interactions are thought to mediate cell-cell recognition and adhesion. The model of cell adhesion in Fig. 6 is based on specific pollen grain surface receptors binding to the substratum surface. Roth (1973) proposed that surface glycosyltransferases on one cell may react with appropriate receptors on a second cell surface to form a stable enzyme-substrate complex which would constitute the bond of attachment. To day, no glycosyltransferases on the pollen grain nor on the stigma pellicle have yet been reported.

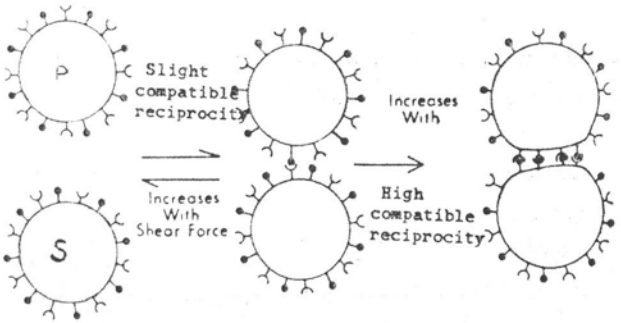

Fig. 6. A simple model for stigma pollen-adhesion
P — pollen, S — stigma

The presence of lectin-like sugar binding molecules on one side and appropriate sugar sequences or glycoproteins bearing the appropriate sugars on the other have been involved in the specificity of attachment. The opposite arrangement can be envisaged, with diffusible lectine-like proteins and heterosaccharides forming part of cell-bound receptors. Concanavalin A (ConA) can bind to the stigmatic surfaces of certain species (Heslop-Harrison, 1976; Pettit, 1980) demonstrating that, at least, lectin-binding sites are present in the region where pollen is deposited. It has been shown that the stigma surface of a number of species is able to bind ConA and, in at least one species, *Gladiolus*, pollen tube penetration is inhibited if the receptor sites for ConA are occupied (Knox et al., 1976; Knox, Clarke, 1978). The specific binding with ConA has never been proved (Pettit, 1980).

Recently, Golynskaya et al. (1976) have proposed that aqueous extracts of *Primula* pistils exhibited agglutinating ability in relation to red blood cells. These phytohemagglutinins of the pistil have been involved as possible proteins of generative incompatibility. Although lectins have a definite recognition function in their specific binding to carbohydrate receptors, their role is yet not clear (Sequeira, 1978). Clarke and Knox (1978) postulated a specific role for the proteoglycans distinct from the extensin. Arabinogalactan proteins associated with the stigma surface of *Gladiolus* (Knox, Clarke, 1977) can form "non covalent associations with other macromolecules such as cell-surface receptors and hence have the potential for both specific and non-specific interactions" (Clarke, Knox, 1978).

A pollen agglutinating property has been observed in *Cruciferae*, but none of the sugars tested was effective to inhibit the agglutination. The nature of this compound must be elucidated (Dumas, Gaude, unpublished data).

CONCLUSION AND PERSPECTIVE

How does the pollen substratum recognition system function from pollination until the pollen tube germination step?

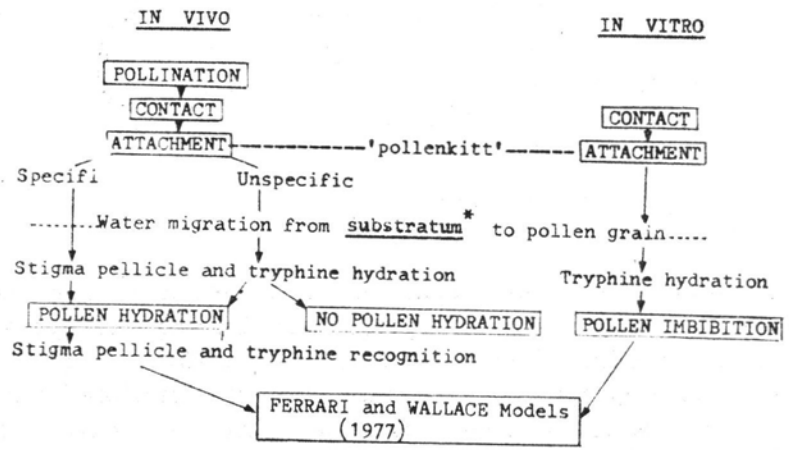

Fig. 7. Post pollination events before pollen germination *in vivo* and *in vitro* aspects
*stigma cells or culture medium

Our model is speculative and it may be tested during research designed to determine the molecular and physiological bases of the preliminary events occurring during pollen-substratum recognition. The sequences from pollination to pollen germination (Figs 7 and 8) mark increasingly close interactions of progressively greater specificity.

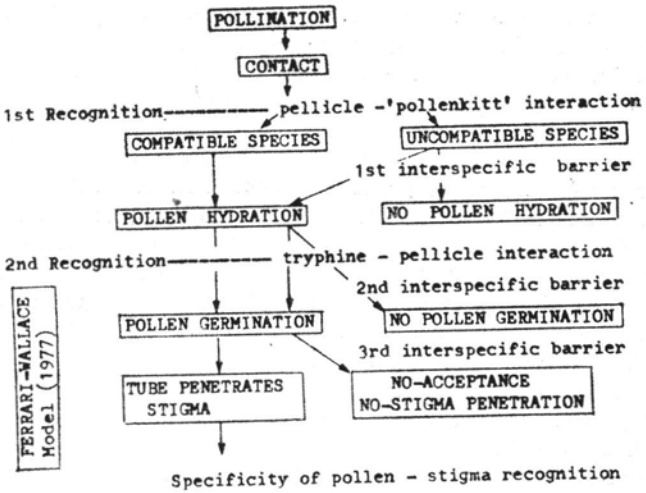

Fig. 8. Specificity of the pollen-stigma recognition

REFERENCES

Burnet F. M., 1971. "Self-recognition" in colonial marine forms and flowering plants in relation to the evolution of immunity. Nature 232: 230-238.

Carter A. L., McNeilly T., 1976. Increased atmospheric humidity post pollination: a possible aid to the production of inbred line seed from mature flowers in the Brussels sprout (*Brassica oleracea* var. *gemmifera*). Euphytica 25: 531-536.

Christ B., 1959. Entwicklungsgeschichtliche und physiologische Untersuchungen über die Selbsterilität von *Cardamine pratensis* L. Z. Bot. 47: 88-112.

Clarke A. E., Knox R. B., Harrison S., Raff J., Marchalonis J. 1977. Common antigens and male-female recognition in plants. Nature 265. 161-163.

Clarke A. E., Knox R. B., 1978. Cell recognition in flowering plants. Quart. Rev. Biol. 53: 3-28.

Dickinson H. G., Lewis D., 1973a. Cytochemical and ultrastructural differences between intraspecific compatible and incompatible pollinations in *Raphanus*. Proc. R. Soc. Lond. (B), 183: 21-38.

Dickinson H. G., Lewis D., 1973b. The formation of tryphine coating the pollen grains of *Raphanus*, and its properties relating to the self-incompatibility system. Proc. R. Soc. Lond. (B), 184: 149-165.

Dickinson H. G., Lewis D., 1975. Interaction between the pollen grain coating and the stigmatic surface during compatible and incompatible intraspecific pollinations in *Raphanus*. In: "The biology of the male gamete", J. G. Duckett and P. A. Racey ed. Publ. Linn. Soc. Lond., Academic Press, pp. 165-175.

Donk J. A. W. M. van der, 1975. Recognition and gene expression during the incompatibility reaction in *Petunia hybrida* L. Molec. Gen. Genet. 141: 305-316.

Dumas C., 1975. Le stigmate et la sécrétion stigmatique. Thèse Lyon (2 vol.).

Dumas C., 1977. Lipochemistry of the progamic stage of a self-incompatible species. Neutral lipids and fatty acids of the secretory stigma during its

glandular activity, and of the solid style, the ovary and the anther in *Forsythia intermedia* Zab. (heterostylic species). Planta 137: 177-184.

Echlin P., 1971. The role of the tapetum during microsporogenesis of Angiosperms. In: "Pollen, development and physiology" J. Heslop-Harrison ed., Butterworths, London, pp. 41-61.

Ferrari T. E., Wallace T. H., 1977. A model for self-recognition and regulation of the incompatibility response of pollen. T. A. G. 50: 211-225.

Frankel R., Galun E., 1977. Pollination mechanisms, reproduction and plant breeding. Monographs Theor. App. Gen., 2, Springer-Verlag, Berlin-New York.

Golynskaya E. L., Bashkirova N. V., Tomchuk N. N., 1976. Phytohemagglutinins of the pistil in *Primula* as possible proteins of generative incompatibility. Transl. Fiziol. Rast. 23: 88-97.

Greaves M. F., 1976. Cell surface receptors: a biological perspective. In: "Receptors and recognition" P. Cuatrecasas and M. F. Greaves, eds., pp. 1-42.

Grinnell F., 1978. Cellular adhesiveness and extracellular substrata. Internat. Rev. Cytol. 27: 65-144.

Heslop-Harrison J., 1975. Incompatibility and the pollen-stigma interaction. Ann. Rev .Plant Physiol. 26: 403-425.

Heslop-Harrison J., Knox R. B., Heslop-Harrison Y., Mattson O., 1975. Pollen-wall proteins; emission and role in incompatibility responses. In: "The biology of the male gamete" J. G. Duckett and P. A. Racey, Publ. Linn. Soc. Lond., Academic Press, pp. 189-204.

Heslop-Harrison Y., 1976. Localization of concanavalin A binding sites on the stigma surface of a grass species. Micron 7: 33—36.

Heslop-Harrison Y., Shivanna K. R., 1977. The receptive surface of the Angiosperm stigma. Ann. Bot. 41: 1233-1258.

Katz D. H., Skidmore B. J., 1978. Self-recognition as a means of cell communication in biologic systems; the immune system is not an exception to the general rule. In: "The molecular basis of cell-cell interaction" R. A. Lerner, D. Bergsma ed., Al. R. Liss, New York, pp. 327-342.

Knox R. B., Clarke A. E., Harrison S., Smith P., Marchalonis J. J., 1976. Cell recognition in plants: Determinants of the stigma surface and their pollen interactions. Proc. Nat. Acad. Sci. (U.S.A.) 73: 2788-2792.

Knox R. B., Clarke A. E., 1977. Localization of AGP's in plant tissues. In: "Arabinogalactan Protein News" A. E. Clarke ed. Univ. Melbourne, pp. 42-45.

Knox R. B., Clarke A. E., 1978. Localization of proteins and glycoproteins by binding to labelled antibodies and lectins. In: "Electron Microscopy and cytochemistry of plant cells" J. L. Hall ed., Elsevier North-Holland, pp. 149-185.

Lewis D., 1963. A protein dimer hypothesis on incompatibility. In: "Genetics Today" S. J. Geerts ed., Internat. Congress Gen., 1963 vol. 3, 1965, pp. 657-675.

Lewis D., 1976. Incompatibility in flowering plants. In: "Receptors and recognitions" ser. A, vol. 2, P. Cuatrecasas and M. F. Greaves Chapman and Hall London, pp. 165-198.

Lewis D., 1979. Sexual incompatibility in plants. Studies in Biology 110: 1-59.

Linskens H. F., Heinen W., 1962. Cutinase-Nachweis in Pollen. Z. Bot. 50: 338-347.

Maroudas N. G., 1977. Cited by Katz D. H., Skidmore B. J. 1978.

Morrisett J. D., Jackson R. L., Gotto A. M., 1975. Lipoproteins: structure and function. Ann. Rev. Biochem. 44: 183-206.

Nasrallah M. E., 1979. Self-incompatibility antigens and S-gene expression in *Brassica*. Heredity 43: 259-263.

Nettancourt D. de, 1977. Incompatibility in Angiosperms. Monographs Theor. Appl. Gen. 3, Springer-Verlag, Berlin-New York.

Ockendon D. J., 1978. Effect of hexane and humidity on self-incompatibility in *Brassica oleracea*. T. A. G. 52: 113-117.

Pettit J. M., 1980. Reproduction in sea grasses: nature of the pollen and receptive surface of the stigma in the *Hydrocharitaceae*. Ann. Bot. 45: 257-271.

Roberts I., Stead A. D., Dickinson H. G., 1979. No fundamental changes in lipids of the pollen grain coating of *Brassica oleracea* following either self- or cross-pollinations. Incompatibility Newsletter 11: 77-79.

Roggen H., 1974. Pollen washing influences (in) compatibility in *Brassica oleracea* varieties. In: "Fertilization in higher plants", H. F. Linskens ed., North-Holland Publ. Co., Amsterdam, Oxford, pp. 273-278.

Roggen H., 1975. Stigma application of an extract from rape pollen (*Brassica napus* L.) effects seelf-incompatibility in Brussels sprouts (*Brassica oleracea* L. var. *gemmifera* Dc.). Incompatibility Newsletter 6: 80-86.

Roth S., 1973. A molecular model for cell interactions. Quart. Rev. Biol. 48: 541-563.

Sequeira L., 1978. Lectins and their role in host-pathogen specificity. Ann. Rev. Phytopathol. 16: 453-481.

Shivanna K. R., 1979. Recognition and rejection phenomena during pollen-pistil interaction. Proc. India Acad. Sci. 88: 115-141.

Stanley R. G., Linskens H. F., 1974. Pollen. Biology, Biochemistry, Management. Springer-Verlag, Heidelberg-New York.

Stead A. D., Roberts I. N., Dickinson H. G., 1979. Pollen-pistil interaction in *Brassica*. Planta 146: 211-216.

Theodor J. L., 1970. Distinction between "self" and "non self" in lower invertebrates. Nature 227: 690-702.

Wickner W., 1979. The assembly of proteins into biological membranes: the membrane trigger hypothesis. Ann. Rev. Biochem. 48: 23-45.