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A Model for Self-Recognition and Regulation of the Incompatibility Response of Pollen*

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Summary. Recent biochemical studies with *Brassica* indicate that the pollen grain has a primary role in the control of self incompatibility. Combining this new evidence with that from prior genetic, biochemical, and ultrastructural studies, a working model is hypothesized for the molecular events which occur during self recognition and the subsequent control of pollen germination. Self recognition is postulated to involve the interaction of a presynthesized, genotype-specific recognition molecule (effector) produced by the stigma with a presynthesized receptor molecule produced by and located in or on the pollen grain. The consequence of self recognition is a selective inhibition of pollen protein synthesis within about 2-4 minutes after imbibition. We deduced that protein synthesis is programmed to occur in pollen - unless interrupted as a consequence of self-recognition - and leads to the sequential production of opposing regulators: first a germination inhibitor (G-Inh), then a germination activator (G-Act). These regulators in turn control the activities of presynthesized, and probably sequestered enzymes required for germ tube formation. Sequential appearances of the G-Inh and G-Act occur unless synthesis of the G-Act is blocked as a result of self recognition. Thus, following a self pollination, recognition occurs in sufficient time to block production of the G-Act but not of the G-Inh, and inhibition of germination (incompatibility) results. For a cross pollination, there is no self recognition and production of the G-Act is unimpeded; it then nullifies the effect of the G-Inh and pollen germination (compatibility) results. The model and evidence for its support are discussed in detail.

Key words: Incompatibility - Control - Pollen - Recognition - Style

Introduction

Sexual or self incompatibility of plants is a genetically-directed inhibition of pollen function. If incompatibility results only when identical (homogenic) incompatibility alleles (S-alleles) are present in both pollen and pistillar tissues, then the system is termed "gametophytic". The system is termed "sporophytic" when the genotype of the sporophyte rather than of the male gamete determines the active S allele of pollen (Townsend 1971). Incompatibility occurs with selfing and with crossing between plants carrying the same S-allele, even though the male and female gametes are functional. Much information exists about the genetics, ultrastructure and physiology of S-allele-controlled sexual incompatibility (see reviews of Sears 1937; Lewis 1954; Arasu 1968; Rosen 1968; Lundquist 1969; Townsend 1971; de Nettancourt 1972; Vasil 1974; Brewbaker 1957). Several hypotheses exist concerning the control mechanisms preventing pollen tube growth after an incompatible pollination (Lewis 1949, 1965; Nasrallah et al. 1970; Pandey 1970; Ascher 1966; Kroes 1973; Heslop-Harrison et al. 1975; van der Donk 1975). However, no hypothesis has gained univeral acceptance.

Two recently hypothesized models conclude that "... the whole information base from which the discrimination between different pollen genotypes is made must be available in the papillae" for species with sporophytic control of the pollen reaction (Heslop-Harrison et al. 1975), or in the style for species with gametophytic control (van der Donk 1975). Both these tissues are female parts of the flower pistil. Heslop-Harrison et al. (1975) assign a major aspect of pollen rejection to events occurring in or on the stigma of *Brassica*. Van der Donk (1975) concludes that the act of recognition between pollen and pistil and the rejection of incompatible pollen is mediated primarily by events occurring in the stylar tissues. With the stated emphasis on activities in the pistil,

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both Heslop-Harrison's and van der Donk's models come near to suggesting that pollen only supplies an S-allele specific recognition molecule. However, much evidence summarized by Pandey (1970) and more recent results with *Brassica* indicate the reverse - an active role for pollen in recognition and rejection while the stigma supplies only an S-allele specific recognition molecule. Using the new evidence, we hypothesize for *Brassica* a model describing the active events occurring in pollen during S-allele-mediated self recognition and the subsequent molecular control of germination. Results accumulated over several decades by investigators in several laboratories were used to eliminate or accept concepts and alternatives considered in deriving the model. A number of observations inconsistent with past hypotheses are explained. The model is speculative in part because evidence is incomplete concerning some of the events postulated to occur during recognition and subsequent manifestation of incompatibility. The model is presented so that it may be tested during research designed to determine the molecular mechanisms through which incompatibility is expressed.

Components of the Model

We hypothesize that expression of self-incompatibility in *Brassica* is the consequence of a multiple component system including:

A) An effector molecule - a presynthesized S-allele specific recognition factor, produced by and located on stigmatic papillae.

B) A receptor molecule - a presynthesized S-allele specific recognition factor, located in or on pollen.

C) A complete set of presynthesized enzymes which mediate at least initial germ tube formation.

D) A pollen germination inhibitor (G-Inh).

E) A pollen germination activator (G-Act) - The G-Inh and G-Act are regulatory proteins synthesized by pollen; by opposing actions they control the activity of the presynthesized enzymes.

Components A and B: S-allele specific recognition molecules. Serologically- and electrophoretically-detected S-allele specific proteins, generally accepted as recognition molecules functioning in self-incompatibility (Townsend 1971; Heslop-Harrison 1975; Ascher 1966), have been extracted from *Brassica* stigmas (component A, effector molecule) (Nasrallah and Wallace 1967; Nasrallah et al. 1970; Sedgley 1974a, 1974b). In F_2 progenies, these stigma proteins segregated in absolute correlation with the expressed self- and cross-incompatibilities of each segregating plant (Nasrallah et al. 1972). Low quantitites were present in stigmas of immature flower buds which accept self pollen; and, as the quantity of the protein in stigmas of developing flowers increased, expression of incompatibility increased (Nasrallah and Wallace 1967; Nasrallah 1974). In addition, *Brassica* stigmas release a substance (probably the stigma effector molecule) capable of inhibiting in vitro germination of self pollen, with extracts from cross stigmas having much less effect (Ferrari and Wallace 1976). Serologically detected S-allele specific proteins were also found in *Petunia* styles (Linskens 1960), and from pollen (component B, receptor molecule) of *Oenothera* (Lewis 1952) and *Petunia* (Linskens 1960; Mäkinen and Lewis 1962). Using the callose deposition response of stigmatic papillae to self-pollen extracts as a biological test of activity, active substances were extracted from pollen of *Brassica*, *Iberis* and *Raphanus* (review of Heslop-Harrison 1975).

Component C: sequestered, presynthesized enzymes required for pollen tube elongation. Mature pollen, from plants with both gametophytic or sporophytic control of incompatibility, contains a large number of presynthesized enzymes prior to germination, many of which are concerned with metabolism of wall precursors (reviews by Mascarenhas 1975; Mäkinen and Macdonald 1968; and Brewbaker 1957). Based primarily on data obtained using inhibitors of protein and nucleic acid synthesis (reviewed by Mascarenhas 1971, 1975), it is evident that new enzyme syntheses are not required for initial (at least) tube elongation.

Therefore, a key question concerning manifestation of incompatibility is what keeps the enzymes that facilitate germ tube formation from functioning in pollen on self stigmas? First, we consider the possibility that these enzymes are free in the cytoplasm and that a hydrolase-like inactivation of their

function is responsible for the inhibition of incompatible pollen germination. We rejected this possibility because the mechanism controlling enzyme activity in *Brassica* must be reversible. Reversibility is indicated in that pollen transferred from an incompatible to a compatible stigma retained its ability to germinate and penetrate papilla cells (Kroh 1967). It is unlikely that a hydrolase-like inactivation would be reversible. We also exclude a second possibility that the stigma provides a preformed regulator that directly controls one or more pollen enzyme activities, or a third that the stigma must provide a required germination-limiting enzyme as hypothesized by Kroes (1973). Functioning of a stigmatic substance with direct control of pollen enzyme activity is unlikely because pollen from many species can germinate in vitro in the absence of stigma factors, and tube lengths about 100 times the grain diameter have been observed (Ferrari and Wallace 1975). This innate ability of pollen to germinate and elongate in chemically defined media indicates that the pollen grain has all the enzymes required for at least initial tube elongation.

Because there must be many enzymes operating in tube formation, we retain the concept that a common control mechanism maintains all or most of them in an inactive state following an incompatible pollination. After excluding the three alternatives discussed, we favor the possibility that the enzymes required for germination are sequestered in the pollen. Membrane bound bodies detectable in electron micrographs of pollen tubes indicate compartmentation as a possible alternative for maintaining enzymes in an inactive state. Some potential compartments are the "enzyme bodies" detected in pollen tubes of *Oenothera* by Dickinson and Lawson (1975), the lipoid bodies surrounded by rough endoplasmic reticulum in *Impatiens* pollen (van Went 1974) and the bipartite particles observed in arrested germ tubes of *Esculentum* by de Nettancourt et al. (1973, 1974). These and other cellular inclusions with unknown functions were prevalent in micrographs of tubes from incompatible pollen. Proof of this compartmentalization concept requires identification of "compartments", their "associated enzymes" and any developmental changes they undergo during pollen tube development.

Because pollens from numerous species are capable of germinating in vitro, the mechanism for releasing enzymes from the hypothesized compartment must operate endogenously. How then is the control of enzyme release mediated following an incompatible pollination? We suggest that a "germination inhibitor" (component D) in pollen prevents endogenous enzyme release following an incompatible pollination.

Component D: The germination inhibitor. The following evidence is supportive of the existence of a germination inhibitor. High concentrations (2×10^{-4}M) of cycloheximide or cordycepin (10^{-3}M) (inhibitors of protein synthesis and of RNA processing, respectivley) strongly inhibited *Brassica* pollen germination in vitro when added to the germination media 1 to 2 minutes after the pollen (Ferrari and Wallace 1976). However, these same inhibitors at the same high concentrations did not reduce germination when present in the medium at the time pollen was added, i.e. when present at time zero and throughout the subsequent germination period. As a working hypothesis to explain these findings, we deduce that an inhibitor of germination (the G-Inh) is synthesized by pollen during the first 1 to 2 minutes after imbibition; thus, the presence of cycloheximide or cordycepin at the onset of imbibition can prevent synthesis of the G-Inh and thereby allow germination to occur.

Hypothesized kinetics for production of the G-Inh are shown in Fig. 1A. The relationship of this hypothe-

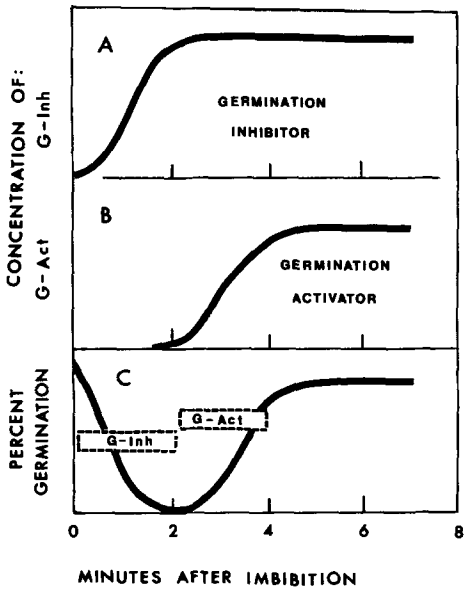

Fig. 1. Kinetics for sequential appearances of the hypothesized (A) germination inhibitor (G-Inh) and (B) germination activator (G-Act), and (C) their effect on pollen germination

tical pollen germination inhibitor to the physiology of self incompatibility became apparent when the time- and concentration-dependent effects observed with cycloheximide and cordycepin were also obtained with extracts from self- but not cross-stigmas (Ferrari and Wallace 1976).

Two predictions arise if these hypothesized kinetics for synthesis by pollen of a G-Inh are correct. First, germination should be inhibited at lower rather than higher concentrations of the exogenous inhibitors of protein synthesis and RNA processing, because inhibitor uptake might then be slow enough to permit biosynthesis of the G-Inh. Second, pollen pretreated with high concentrations of cycloheximide or self-stigma extract [previously shown to rapidly block self-pollen protein synthesis but not tube elongation (Ferrari and Wallace 1976)] should overcome self incompatibility in situ. Both predictions were fulfilled with *Brassica* pollen. Germination in vitro was inhibited when pollen was exposed to lower concentrations (2×10^{-4}M vs. 10^{-3}M) of cordycepin and to lower levels of self-stigma extract (Ferrari and Wallace 1976). More significantly, cycloheximide pretreated pollen germinated, adhered to papillae, and penetrated into self stigmas (Ferrari and Wallace 1977). Controls indicated that these responses were not due to inhibitor leakage onto the stigma from the treated pollen. In addition, preliminary results indicate that pollen which was pretreated with crude extracts from self stigmas also germinated on self stigmas in situ; however, this response differed from pollen pretreated with cycloheximide in that the germ tubes which formed lacked orientation: the tubes grew in all directions and did not penetrate into stigmatic tissues. Actinomycin-D-pretreated pollen from *Lycopersicum* also grew through self styles (Sarfatti et al. 1974), suggesting that RNA synthesis might be required for control of incompatibility for plants with the gametophytic system.

Inhibition of germ tube elongation in vitro at low concentrations by some vitamins and hormones, and lack of inhibition or sometimes strong stimulation at higher concentrations (compared to controls with no treatment) was reported for pollen from *Areca* (Raghaven and Baruah 1959), *Cucumis* (Vasil 1960), *Ceratozamia* and *Pinus* (Anhaeusser 1953). An inhibitor of RNA synthesis, 6-methylpurine and several of its analogs, at increasingly higher concentrations, strongly enhanced *Petunia* and *Nicotiana* germ tube elongation in vitro (Tupy et al. 1965). This is consistent with the interpretation that, for pollen from some species, synthesis of RNA is involved with production of an inhibitor of tube elongation. That high concentrations of maleic hydrazide, transcinnamic acid, iodoacetate, abscisic acid, auxin and ethylene promoted tube elongation, again in vitro, yet inhibited germination to varying extents, led McLeod (1975) to conclude that an active endogenous inhibitory system is established in pollen soon after germination.

That incompatibilities on *Brassica* stigmas and in *Lycopersicum* styles, respectively, were overcome in situ by pretreating pollen with an inhibitor of protein synthesis (Ferrari and Wallace 1977) and of DNA transcription (Sarfatti et al. 1974) suggests the following: A) The G-Inh is a protein regulator which inhibits the activities of enzymes required for germ tube formation, B) synthesis of this regulator is programmed to occur in the pollen - not the stigma -, and C) appearance of G-Inh activity requires at least new protein synthesis, new RNA processing (data for *Brassica*) or new RNA synthesis (data for *Lycopersicum*).

The above rationale for the presence of a G-Inh requires that it be synthesized rapidly by pollen. Can pollen synthesize protein within minutes after addition to germination medium? With *Tradescantia* and *Petunia* pollen, polyribosomes were assembled within 1 to 2 minutes after pollen imbibition, and protein was synthesized both in vivo (Mascarenhas and Bell 1969) and in vitro (Linskens et al. 1970). Leucine-^{14}C incorporation into *Brassica* pollen proteins was linear, was detected as early as 10 minutes after the start of pollen incubation, and extrapolation of incorporation rates to zero time indicated little or no lag phase (Ferrari and Wallace 1976). Imbibition (water uptake) in vitro is complete seconds after adding *Brassica* pollen to germination medium (Ferrari and Wallace, unpublished data). Hence, uptake by pollen of low molecular weight exogenous inhibitors should occur fast enough to rapidly block protein synthesis.

How does the pollen G-Inh turn off germination? We speculate that it acts by preventing release from

a sequestered compartment of the presynthesized enzymes required for pollen tube elongation.

Component E: The germination activator. Two observations suggest that shortly after programmed biosynthesis of the hypothesized G-Inh, it must either be inactivated, or that its blockage of germination is circumvented by another mechanism. First, pollen from many species germinates readily in vitro, i.e. in defined medium lacking stigma extract or inhibitors of transcription and translation. Second, although germination was strongly inhibited by adding high concentrations of cycloheximide, cordycepin or self stigma extract to germination medium 1 to 2 minutes after pollen imbibition, germination was less and less inhibited as time of addition was delayed beyond 2 minutes (Ferrari and Wallace 1976). Therefore, we suggest the sequential synthesis by pollen of first the G-Inh, and then a germination activator (G-Act) (Figs. 1A and 1B). In the absence of exogenous or stigma-derived inhibitors of protein synthesis, the action of the G-Inh would first increase as it was synthesized, and then decrease as the G-Act overcame the G-Inh-mediated block of germination. The G-Act could act by direct inactivation of the G-Inh, or by indirectly circumventing the G-Inh imposed block on germination. At any time, the effective concentration of G-Inh would be inversely proportional to its effect on germination. This analysis is consistent with observed inhibition kinetics (Ferrari and Wallace 1976). When added about 2 minutes after the start of imbibition by pollen, high concentrations of cycloheximide, cordycepin or stigma substance inhibited germination by apparently allowing time for biosynthesis of the G-Inh (indicated by the bar, Fig. 1C) but not of the G-Act. The G-Inh: G-Act control system is analogous to the sequential appearances in potato tuber disks of first phenylalanine ammonia-lyase and then of a lyase-inactivating protein (Zucker 1968). Hotta and Stern (1963) observed that disappearance of the induced activity of thymidine kinase in lily anthers also required conditions which favored protein synthesis.

Regulatory Regimes of Pollen Germination

The proposed biochemical model for recognition and control of incompatibility includes at least 4 interacting pathways (Figs. 2-4). [A fifth pathway (Fig. 4), discussed in the conclusion section, concerns developmental stages of pollen which occur after the completion of recognition and control of incompatibility.] The various interactions (regulatory regimes) among pathways I, II, III and IV explain the following situations: (i) the contrasting time- and concentration-dependent effects on pollen germination of inhibitors of protein synthesis (Fig. 2); (ii) the consequence of an incompatible or self-pollination (Fig. 3); (iii) the consequence of a compatible (cross) pollination (Fig. 4); and (iv) the in vitro germination of pollen.

Situation (i): Pollen germination in the presence of protein synthesis inhibitors. When pollen is suspended in synthetic germination medium, the grain imbibes water within seconds and proteins are hydrated. If the medium contains an effective inhibitor of protein synthesis, formation of the G-Inh via pathway II and of the G-Act via pathway III (both regulatory proteins) are blocked (Arrow A, Fig. 2). To account for the fact that germination can occur in the presence of such inhibitors, an endogenous mechanism must then release the presynthesized, sequestered enzymes that mediate pollen tube elongation (Fig. 2, pathway I). Thus, one condition of our model is that new protein synthesis is not essential for at least initial germ tube formation to occur.

When the inhibitor of protein synthesis is not added to the medium until about 2 minutes after imbibition, only formation of the G-Act is blocked (Arrow B, Fig. 2): The G-Inh, synthesized in the first 2 minutes via pathway II, can then block the endogenous release of the enzymes involved with tube formation [cf. (ii) and Fig. 3].

In the absence of protein synthesis inhibitors, the control of germination by the regulatory proteins would be as described for a cross pollination [cf. (iii) and Fig. 4].

Situation (ii): Pollen having the same S-allele phenotype as the stigma. After "self" pollination, events [Pathways I, II, and IV functioning (Fig. 3)] would proceed as follows: the S-allele-specific recognition effector molecule on the stigma surface diffuses to the pollen grain, either concomitant with imbibition of water or immediately thereafter. Within minutes, interaction of the stigma effector mole-

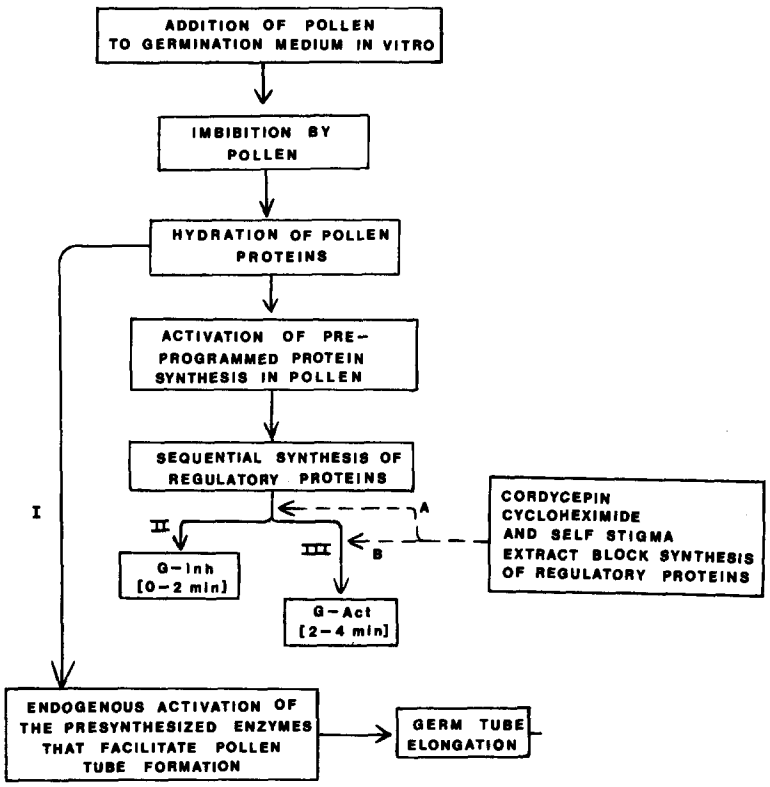

Fig.2. In vitro patheays functioning in *Brassica* to control germ tube emergence after placement of pollen in synthetic germination medium.

High concentrations of cycloheximide or cordycepin have no effect on germination when added to pollen before G-Inh and G-Act synthesis (Arrow A). Release of enzymes required for tube elongation occurs endogenously. When added at 2 min, inhibitors directly block germination by permitting synthesis of the G-Inh and blocking synthesis of G-Act (Arrow B). The G-Inh and G-Act act respectively as described for self (Fig.3) and cross pollinations (Fig.4)

cule with a recognition receptor molecule in the pollen grain or on its surface occurs via Pathway IV. This effector-receptor interaction, i.e. self-recognition, occurs at a rate slow enough to permit biosynthesis of the G-Inh via protein synthesis in the pollen (0-2 min, Pathway II), but fast enough to block by 2-4 minutes, either directly or indirectly, synthesis of the G-Act via Pathway III. With G-Act synthesis blocked, the G-Inh is free to prevent the endogenous release of the sequestered, presynthesized enzymes required for tube elongation.

Situation (iii): Pollen having a different S-allele phenotype than the stigma. After a cross pollination [Pathways I, II, III and V functioning (Fig.4)], stigma effector and pollen receptor molecules would be incapable of interaction; self-recognition via Pathway IV would not occur (Fig.4). Without self-recognition, formation of the G-Act occurs unimpeded and its biosynthesis is completed within 2-4 minutes via Pathway III. We speculate that the G-Act facilitates germination by either inactivating the G-Inh, or by releasing in some manner the enzymes required for tube elongation. Germ tube elongation can then proceed normally, and as yet poorly understood interactions will occur between the germ tube and pistillar tissues: Pathway V relates to these events which occur during tube growth through the style, entrance into the ovary and fertilization. (See the conclusion section for discussion of the many stages of germ tube development.)

Situation (iv): in vitro pollen germination [Pathways I, II and III functioning (Fig.2)]. In the absence of any stigma substances or exogenous growth regulators, in vitro pollen germination occurs via

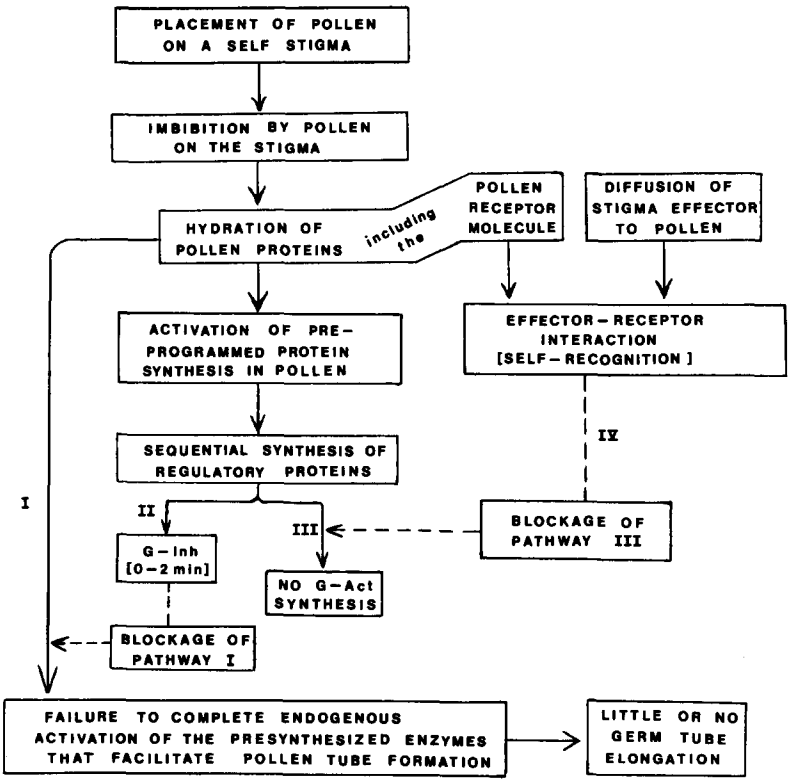

Fig.3. In vivo pathways functioning in *Brassica* to control germ tube emergence following a self pollination

Pathway I. Pathways II and III in the pollen also operate, as described for a cross pollination (situation (iii) and Fig.4]. Because no stigmatic tissues or substances are present, there can be no stigma-pollen interactions. Therefore, Pathway IV cannot function, and if events related to Pathway V do occur they might not be detected in the absence of pistillar tissues.

Timing of Regulatory Events

Our experiments indicated that control of incompatibility can occur in vitro as early as 2 to 4 minutes after pollen imbibition (Ferrari and Wallace 1976). Completion of recognition in situ by as early as 10 minutes was recognized by Heslop-Harrison (1974) and Heslop-Harrison et al. (1975). Results from in situ pollen transfers (Kroh 1967) support our in vitro evidence that recognition of self is completed within about 4 minutes in *Brassica*. Kroh (1967) found that self pollen of *Brassica* germinated and penetrated papillae at rates comparable to cross pollen if it was first placed on a cross stigma for a minimum of 4 minutes. This indicates that the "programmed" events that result from failure of pollen to recognize self are irreversibly completed during the 4-minute "preincubation" of pollen on a cross stigma. In contrast, cross pollen penetrated papillae after a 4-minute "preincubation" on self stigmas, indicating that the inhibition of pollen germination resulting from self recognition can be reversed.

Events occur rapidly in *Brassica* pollen subsequent to self recognition or to lack of self recognition. And, inhibitors of translation and transcription fail to block pollen germination for several plant species (Shivanna et al. 1974a, b; Ferrari and Wallace 1976; Mascarenhas 1971, 1975). These observations indicate that the biochemical processes leading to both recognition and tube elongation are presynthesized or "programmed" to occur in *Brassica* pollen. The evidence indicates that the presynthesized components include stigma effector and pollen recptor molecules, and enzymes responsible for tube elongation. We have no

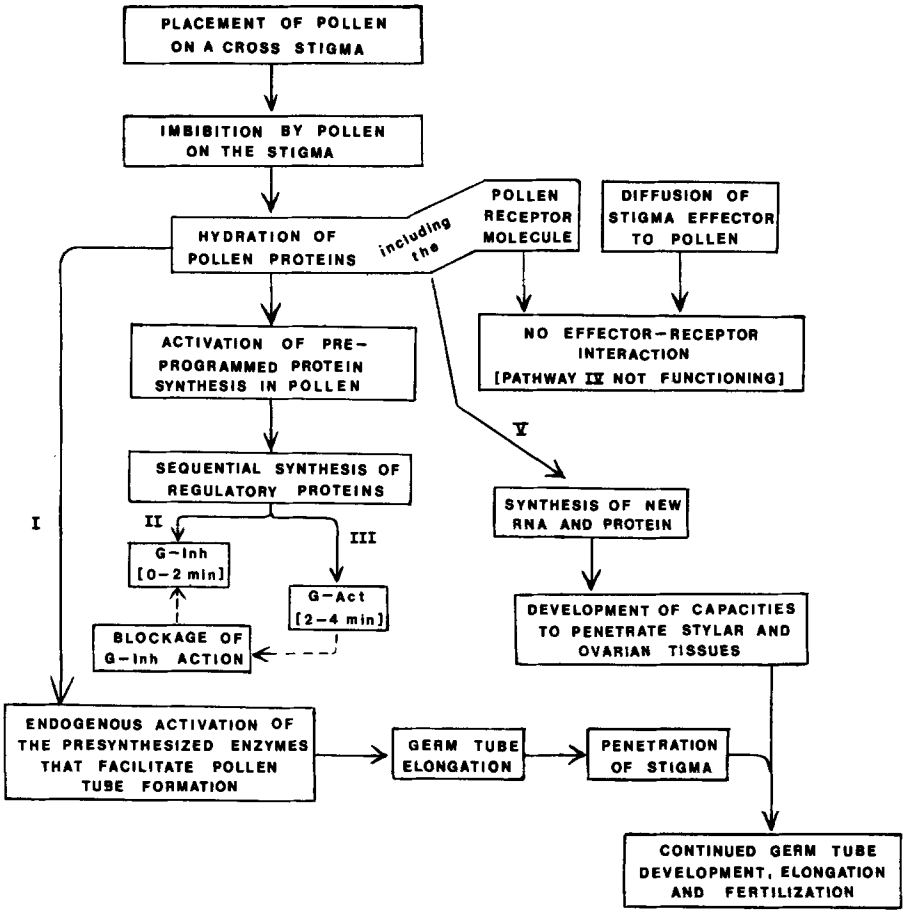

Fig. 4. In vivo pathways functioning in *Brassica* to control germ tube emergence following a cross pollination

direct evidence indicating the exact chemical nature of the "program(s)" coding for the A-Act and G-Inh. However, the speed that their synthesis occurs in *Brassica* pollen, and that cordycepin affects pollen germination in the same manner as self-stigma extract, suggests the possibility that preformed RNA codes for these regulatory components (G-Act and G-Inh). Pollen does contain stable RNA (Tupy 1966; Mascarenhas et al. 1974) and the adenine content of pollen RNA is greater after germination than before (Mascarenhas 1971; Mascarenhas and Bell 1969).

Consistency with Mutation Effects

The proposed model explains why mutation studies with pollen have produced only negative effects on self-incompatibility (review of de Nettancourt 1969): mutations cause self-fertility (S_f) and have not produced new S alleles. Radiation-induced mutations most frequently cause DNA deletions and lead to production of nonfunctional proteins. The model predicts that nonfunctional molecules of (A) stigma effector, (B) pollen receptor or (C) pollen G-Inh would all give an S_f phenotype. Mutations causing secondary structure changes of components (A) stigma effector or (B) pollen receptor molecules would prevent effector-receptor interaction (self-recognition) after pollination between plants with like S-alleles. The consequence of no self-recognition is that G-Act synthesis will occur as for a cross pollination. The G-Act would then either release directly the enzymes responsible for germination, or inactivate the previously synthesized G-Inh. In either case, tube elongation would result (Pathways I, II and III, Fig. 4). (C) A mutation causing a defective G-Inh molecule would prevent it from blocking the endogenous release of enzymes required for germination - even

if effector-receptor interaction occurred. Thus, each of the 3 mutations (A, B, or C) would cause the system to operate as for a cross pollination: each would cause an S_f phenotype. Provided reversion does not occur, as often happens with pollen, S_f would be established in genomes of the progeny (Lewis 1949, 1951; Lewis and Crowe 1953; de Nettancourt 1969).

Mutation at the structural gene coding for the G-Act (or genes regulating its transcription or translation) would result in a "functionally lethal" system. Germination could not occur following a cross-pollination because a defective G-Act would be unable to prevent or circumvent action of the G-Inh. Since fertilization could not occur, the mutation would be "functionally lethal" unless restored by gene complementation as can occur in pollen (Lewis 1958; Pandey 1965, 1967).

The model provides a rationale of why no new S-allele incompatibility genotypes have appeared following mutation experiments with plants (de Nettancourt 1969). To create a new, functional S-allele genotype, the model requires 4 successive, stringent events. First, a mutation would be required that caused a structural modification in the stigma effector molecule (or pollen receptor) which would have to be constructive. Second, the original mutation must be followed by a constructive mutation in the pollen receptor molecule (or stigma effector). Third, the mutation-induced change in secondary structure of the stigma effector and of the pollen receptor molecules must each be such that a new specific effector-receptor interaction (self recognition) arises. Fourth, the two mutations enabling formation of a new effector-receptor heterodimer must not interfere with that dimer's ability to block G-Act formation (Pathway IV, Fig.3). A priori, we feel the probability is too small (less than 10^{-20}, assuming an induced mutation frequency of 10^{-5} for each event) for all 4 criteria to be met in 1, or even a 2-step mutation event.

Lewis (1949) reported that induced mutations at the S-locus of plants with gametophytic incompatibility were not expressed in pollen when irradiation was performed after meiosis of the pollen mother cells. He concluded that factors controlling incompatibility must be laid down in the cytoplasm prior to tetrad formation. This is consistent with our hypothesis (previous section) that a presynthesized RNA "program" in pollen codes for biosynthesis of the regulatory components - G-Act and G-Inh. Radiation would not normally prevent translation of preformed "RNA programs" in mature pollen.

Consistency with Temperature Effects

Generally, enzymatic processes have larger temperature coefficients (Q_{10}) than nonenzymatic processes (e.g. passive diffusion). Therefore, a temperature change will usually cause a greater rate change for an enzymatic reaction (Q_{10} greater than 2) than for a diffusion process (Q_{10} about 1). Our model for self-recognition and control of germination simultaneously involves both protein synthesis (enzymatic reactions) and diffusion (a physical process) of effector from the stigma surface to a receptor site on or in pollen. Because these processes respond differently to temperature, one prediction from the model is that incompatibility might be poorly expressed at relatively high or low temperatures.

For example, at relatively high temperature, enzyme reactions leading to G-Inh and G-Act synthesis could be completed before diffusion-limited events leading to effector-receptor interaction (self-recognition). Self recognition might still occur, but it will be too late to prevent G-Act synthesis and its consequent activation of germination. This prediction is consistent with frequent observations that following self pollination at high temperature incompatibility is weakened or sometimes does not occur (Lewis 1954; Linskins 1975; Townsend 1971; Ascher and Peloquin 1966, 1970). Weakening of incompatibility by increased rate of G-Act synthesis caused by relatively high temperature should be distinguished from high-temperature-induced inactivation of stigma and/or stylar recognition components (Townsend 1971) which can also prevent effector-receptor interaction and lead to loss of incompatibility.

That self-incompatibility is often not expressed at low temperatures is also consistent with the model. Low temperatures would retard protein synthesis more than diffusion, so diffusion of effector to receptor would occur before the G-Inh and G-Act could be synthesized, and the effector-receptor interaction

would then block synthesis of both the G-Inh and G-Act. The enzymes mediating tube elongation would then be released endogenously via Pathway I (Fig. 2), permitting both incompatible and compatible pollen to germinate. Therefore, the diffusion-limited events leading to self recognition and the consequent blockage of protein synthesis would occur before the G-Inh and G-Act could be synthesized by slower biochemical processes. These predictions are valid assuming that phase changes of membranes at low temperatures are not indirectly responsible for altering effector-receptor binding properties at a membrane site. Phase transitions of membranes do not occur in chilling-resistant genera (Lyons 1973) such as *Brassica*, *Lilium*, *Medicago*, etc. In support of the model's prediction that incompatibility will be weakly expressed at low temperature, there are reports that incompatible tubes formed at low temperature frequently attain lengths similar to those of compatible tubes at normal temperatures of 20-30°C where incompatibility is maximally expressed (Lewis 1942; Modlibowski 1945; Odland and Noll 1950; Ascher and Peloquin 1966, 1970). Greater self-seed set has sometimes been measured at low (15-20°C) and high temperatures (30-40°C) (Townsend 1971; Ronald and Ascher 1975; Nasrallah and Wallace 1968).

Comparison with Other Models

Our model for self-recognition and control of incompatibility (Figs. 2, 3 and 4) in sporophytic incompatibility of *Brassica* was derived with data from biochemical experiments. The components of the model correspond to structural components of the S-allele as derived from genetic and mutation experiments with the gametophytic system (Lewis 1954). For example, Lewis hypothesized that the S-allele coded for (A) a pollen part, and (B) a style part, each with (C) a specificity part, and (D) an activity part. Our biochemically-derived model proposes that following an incompatible pollination, (A) a presynthesized stigma component and (B) a presynthesized pollen component (C) interact specifically (specificity part) to (D) inhibit protein synthesis (an activity part). Further, Lewis predicted that interaction of stigma and pollen parts caused either production of a germination inhibitor after an incompatible pollination or production of an antidote to an inhibitor after a compatible pollination. Likewise, our model involves functioning of an inhibitor (the G-Inh) after an incompatible pollination; and of an antidote (the G-Act) to the G-Inh after a compatible pollination. After pollen hydration both compatible and incompatible pollinations include preprogrammed synthesis of the G-Inh and control over the sequential and subsequent synthesis of the G-Act.

Techniques detecting S-allele-specific molecules on *Brassica* stigmas have not identified these same molecules in pollen (Nasrallah and Wallace 1967; Heslop-Harrison 1975). Thus, our model does not require interaction (dimer formation) between identical stigma effector and pollen receptor molecules as Lewis (1965) and Ascher (1966) propose. Dimerization of identical recognition molecules from stigma (or style) and pollen would require some means of preventing two molecules in the pollen or style from spontaneously dimerizing and thereby indirectly preventing pollen germination. It is more reasonable as we, van der Donk (1975), Heslop-Harrison (1975) and Burnet (1971) indicate, to hypothesize that the very different cellular environments of the pollen and stigmas or styles result in transcription, respectively of structurally different male and female S-allele designated molecules. This is in full agreement with observed separation of male and female S allele activities (Lewis 1954; Nasrallah 1974).

Ascher's model for gametophytic incompatibility of *Lilium* (1966) proposes that identical stigma and pollen recognition molecules dimerize following an incompatible pollination. The dimer then represses DNA transcription in pollen of a high velocity operon, thereby preventing post-pollination synthesis of the enzymes required for continued tube elongation. Our model differs from Ascher's in that control of pollen germination is by regulating the activity of preformed enzymes that mediate tube elongation. We reject control via regulation of DNA transcription for the following reasons. Results obtained with transcriptional and translational inhibitors indicate that the enzymes required for tube elongation of pollen of many species, including *Brassica* (Ferrari and Wallace 1976, 1977), are presynthesized prior to pollen-stigma interactions (Sarfatti et al. 1974; Shivanna

et al. 1974a, b; Franke et al. 1972; Mascarenhas 1971, 1975). As required by our model for *Brassica*, lily pollen must also contain in presynthesized form all the enzymes necessary for germination, because germination in vitro (in the absence of any dimer repressors of DNA transcription) was unaffected by cycloheximide (Ascher and Drewlow 1970).

These observations with *Lilium* (gametophytic incompatibility) are consistent with our conclusions for *Brassica* (sporophytic incompatibility) that protein synthesis in pollen is not required for tube elongation but is required for expression of incompatibility. This conclusion is supported by recent findings that prepollination treatment of pollen with cycloheximide (*Brassica*, Ferrari and Wallace 1977) and actinomycin D (*Lycopersicum*, Sarfatti et al. 1974), respectively, overcame inability of incompatible pollen to germinate in situ on intact stigmas and to grow through styles. Gates and Ochendon (1976) report a similar effect of cycloheximide on pollen germination of *Brassica*.

Inhibitor studies indicate that regulation of incompatibility in the gametophytic system of *Lilium*, like the sporophytic system of *Brassica*, requires presynthesized recognition components. For example, when lily styles were pretreated 6 hours before pollination with 6-methylpurine (an RNA synthesis inhibitor), incompatible pollen tube growth increased to lengths observed with compatible tubes receiving no treatment (Ascher 1971). Thus, during this six hour pre-pollination time at least part(s) of the self-recognition system was presynthesized in the style as a result of DNA transcription. Since inhibitor treatment of stigmas just prior to or after pollination did not alter the pollen incompatibility response (Ascher 1971), the effect of 6-methylpurine must have been on presynthesis of the stylar component (a stylar effector?) of the self-recognition system, and not on pollen components.

The model for control of gametophytic incompatibility proposed by van der Donk (1975) for *Petunia*, compares with our model for sporophytic incompatibility of *Brassica* as follows:

1. The *Brassica* model assumes that before pollination the stigma is prepared to facilitate germination and growth of all intraspecies pollen except pollen with an active S-allele corresponding to one in the stigma. In contrast, the *Petunia* model assumes that the style is prepared to reject all pollen; to accept any pollen the style must first be activated through sequential steps most of which are discussed below.

2. Both models involve a pre-synthesized and S-allele-coded recognition monomer in or on the pollen.

3. The *Brassica* model also includes a presynthesized and S-allele-coded recognition monomer located in or on the stigma. In contrast, the *Petunia* model includes only post pollination synthesis of the S-allele-coded female recognition molecule. This monomer is not synthesized in the style until after activation of the style by contact with intraspecies pollen of any or all S-allele genotypes.

4. For both models the pollen and stigma or stylar recognition molecules are assumed to be non identical, even when coded by the same S-allele.

5. Both models assume that the non-identical male and female recognition monomers are complementary when coded by the same S-allele, and that they combine to form a heterodimer molecule.

6. For an incompatible pollination, the *Brassica* model hypothesizes that the heterodimer molecule is active: it directly or indirectly blocks synthesis in pollen of a germination activator, thereby indirectly causing the failure of pollen to germinate. On the contrary, the *Petunia* model hypothesizes that the heterodimer is inactive, but that its formation reduces concentrations of the pollen and stylar S-allele-coded recognition monomers. In the *Petunia* model these monomers are active - they cooperatively facilitate the pollen germination that constitutes compatibility.

7. The *Brassica* model requires no post-pollination synthesis of the S-allele coded recognition monomers of either pollen or stigma. On the contrary, the *Petunia* model hypothesizes that when these two monomers do not heterodimerize (when they are coded by different S alleles and self recognition does not occur), the post-pollination synthesized stylar recognition monomer activates additional synthesis (post-pollination) of the pollen recognition monomer. This additional pollen monomer in turn activates prolonged synthesis of the stylar recognition monomer. A resulting surplus (above a threshold quantity in the stylar cells) of the monomer

then activates in the style the genes that support pollen tube growth in the style.

8. It follows from 7. (above) that in the *Petunia* model both the pollen and stylar recognition molecules act as both effector and receptor molecules, and both also play a direct role in control of incompatibility. In the *Brassica* model, however, the pollen recognition monomer is the receptor, the stigma monomer is the effector and the only direct role of these molecules is differentiation of self vs. non-self S-allele genotypes.

The model of Heslop-Harrison et al. (1975) for sporophytic incompatibility, which is derived from studies with *Brassica* and *Raphanus*, other Cruciferae, and with Compositae, Caryophyllaceae and Malivaceae, is presented with less precisely hypothesized biochemical detail than either our *Brassica* model or van der Donk's *Petunia* model. Our *Brassica* model is consistent with the concept of Heslop-Harrison et al. (1975) that the pollen recognition molecule originates from tapetal (sporophytic) cells. It is also consistent with their conclusion that the stigmatic recognition molecule is located on the surface of the stigmatic papillae in the hydrated proteinaceous pellicle (coating) which they consider to be a partially dried-down secretion. The research of Heslop-Harrison et al. (1975) has emphasized callose deposition in the stigma papillae, rather than a germination inhibitor in pollen, as a major cause of the pollen tube inhibition. They do deduce that self recognition also initiates processes affecting metabolism of pollen (see also Heslop-Harrison 1975). Because of research emphasis on the callose rejection response, this group labels the stigma recognition molecule as the receptor and the pollen molecule as the effector - the reverse of our *Brassica* model.

Dickinson and Lewis (1973a, b; see also Heslop-Harrison 1975) identify 3 stages of expression of self-incompatibility. The first stage operates within the grain and determines whether it will germinate. If a tube is formed for an incompatible grain, the second stage is difficulty in penetrating the cutin layer beneath the pellicle. The third is a migration of products from the pollen tube into the cytoplasm of papillae cells where this causes the callose rejection response which is the last line of defense. If a pollen tube penetrates beyond this callosic barrier, it has bypassed all stages of expression of incompatibility. The first 2 stages, failure to germinate and inability to penetrate the cutin, are in agreement with our *Brassica* model.

The model of Heslop-Harrison et al. (1975) and van der Donk (1975) propose that the pollen-borne recognition factor(s) diffuses to receptor sites in the style (van der Donk 1975) or on sitgmatic papillae (Mattsson et al. 1974; Heslop-Harrison et al. 1975). Recognition then elicits secondary messages which leave the extracellular recognition sites and enter both pollen grain and stigma cells, initiating in each the action appropriate to the combination of genotypes: action leading to acceptance or rejection. If these hypotheses are correct, then the extracellular secondary messages cannot be subject to random diffusion; if they were, a secondary recognition system in pollen or germ tubes would be necessary to interpret them. Otherwise, randomly diffusing secondary messages would cause "confusion" when compatible and incompatible pollen or pollen tubes are adjacent to each other. A number of workers (Emerson 1940; Arasu 1968; Rosen 1968; Mäkinen and Lewis 1962; Tammisola and Ryynanen 1970) have reported that after mixed compatible and incompatible pollinations, pollen grains are not "confused" and incompatibility or compatibility occurs according to specific genotype. No extracellular, secondary messages diffusing between pollen and papillae are required by our model; but secondary "messages" in the form of intracellular pollen regulators (the G-Inh and G-Act) are proposed. Internal production of the G-Inh and G-Act by pollen obviates need for an additional pollen-stigma recognition system to discern them.

Our model proposes that germination is programmed to occur even in the event there is no recognition reaction. Therefore, pollen does not require that a secondary "acceptance signal" or "stimulus" be communicated to it from the stigma following recognition, as proposed by Heslop-Harrison et al. (1975). Pollen of several species can germinate in vitro in the absence of stigma or stylar messages; hence an "acceptance signal" or "stimulus" must not be necessary for germination to proceed.

With our model for *Brassica* and Ascher's (1966) for *Lilium*, the hypothesized events for control of tube elongation occur in the pollen grain after self

recognition. On the contrary, the models of Heslop-Harrison et al. (1975; see also Heslop-Harrison 1975) for *Brassica* and van der Donk (1975) for *Petunia* both emphasize events which occur in the stigma (*Brassica*) or which fail to occur in the stigma (*Petunia*) as the cause of inhibition of tube elongation.

Conclusion

It has become clear, as suggested by Hogenboom (1973), that many developmental stages occur during pollen tube growth through pistillar tissues. Two stages were defined by Pandey (1973) and Heslop-Harrison (1974); three were recognized by Dickinson and Lewis (1973a, b); five by Heslop-Harrison et al. (1975); six by Heslop-Harrison (1975); and the discussion here indicates at least eight stages. They include: (A) a recognition event; (B) subsequent development of the regulatory-system which controls enzymes involved with germ tube formation; (C) germ tube formation as mediated by a presynthesized enzyme system; and development of separate capacities to penetrate tissues of the (D) papillae, (E) stigma, (F) stylar conducting tissue and (G) ovary. Interactions of substances released by pollen with other products from stigmatic tissues cause - for an incompatible pollination - at least one additional developmental stage to occur. This stage results in production of a callose rejection body in papillae. Eight developmental stages are listed (most are included in Fig. 4), though future research on pollen tube development will certainly reveal more. We emphasize the uniqueness of these stages to provide a framework for discussing and furthering research of pollen germination and incompatibility. Using metabolic inhibitors and purified fractions of stigma and stylar extracts, it should be possible to "dissect" the developmental stages of pollen tube growth and study the stages independently.

In the literature there is a tendency to consider the mechanisms for control of sporophytic and gametophytic incompatibility as different. However, the evidence discussed in presenting our model was drawn from researches on both the gametophytic and sporophytic systems. Plant species do exhibit individuality in expression of incompatibility; but, they posses striking similarities in recognition, control of germination, and response to environmental factors. Considerable evidence suggests that the control of incompatibility may be the same, or at least similar for both systems, but that it occurs at different stages of the developing pollen tube (as suggested by Pandey 1970). For example, for plants with gametophytic control of incompatibility, inhibition of pollen tube development occurs on the stigma for *Papavar rhoeas* (Lawrence 1975); in the stigma for *Oenothera organensis* (Dickinson and Lawson 1975); in the upper, mid or lower styles for many other species; and at the ovary for still others (see Pandey 1970).

Evidence cited earlier indicates that for either sporophytic or gametophytic control new protein synthesis is required for expression of self-incompatibility, but not for initial germ tube elongation. The temperature induced breakdown of incompatibility for species with both systems also argues for a common control mechanism. Considering these and other similarities, we presume that the regulation of incompatibility for sporophytic and gametophytic control systems is basically the same, with the tissue and time of expression varying with species. Additional research is required to determine if the recognition mechanism and subsequent control of pollen germination for the sporophytic system differs extensively from that of the gametophytic system. Continued research will identify those aspects of each of the discussed models that can be accepted or modified, or that must be rejected, and will lead to a more unified concept of the control of self incompatibility.

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