Pollen-Stigma Interaction and Cross-Incompatibility in the Grasses

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Summary. The failure of interspecific and intergeneric crosses in the grasses is commonly due to zygotic inviability, embryo abortion, or endosperm abnormalities, all of which occur after fertilization. Often, however, there is difficulty in obtaining fertilization because the pollen tube fails to reach the embryo sac. This article concerns recent research on pollen-stigma interaction in the grasses and the light it is beginning to throw on prefertilization self- and cross-incompatibilities.

The quest for greater yields, disease resistance, and better agronomic qualities in our major crop plants demands the continual enlargement of the gene pools available for breeding. Since the beginning of the 19th century, hybridization has been the principal way to achieve this; and while we may expect that a significant contribution will eventually come from methods of attaining genetic recombination which totally bypass the sexual system, much remains to be done in manipulating the sexual process itself to extend the range of hybridity. Invariably, the limitation in practice is the existence of bars to crossing, notably at specific and generic levels. Among crop plants and their wild relatives, zygotic failure and partial or complete F_1 inviability or sterility commonly form the principal blocks to achieving gene transfer. But often there is difficulty in getting even as far as the first generation because of prefertilization barriers operating between the receipt of pollen and the entry of the tube into the embryo sac.

This is the situation in the most important agronomic family, the Gramineae (grasses). Attempts to hybridize different species can be frustrated by the difficulty of obtaining the first, critical fertilizations. Research on the pollen-stigma interaction in the grasses is beginning to reveal some of the controls governing pollen tube behavior and to suggest how they may operate in various cases of self- and cross-incompatibility.

Stigma Features and the Pollen Tube Transmitting Tract

In most grass genera, the so-called stigma is composed of two stylodia. These are clothed, at least in the upper part, by secondary stigma branches made of up to five ranks of cells, the papillate tips of which bear the pollen-receiving surfaces. The stigma lacks free-flowing surface fluid (Fig. 1A) but, as in all other stigmas of this type, the receptive surfaces themselves carry a thin secretion (*1*).

The stratigraphy of the wall of a receptive papilla of rye is shown in Fig. 1C. The superficial proteinaceous pellicle can be stripped with mild detergent treatment under conditions that do not disrupt the underlying cells. The protein composition of the eluate, as revealed by gel electrophoresis and isoelectric focusing, is highly heterogeneous (*2*). An example of fractionation on a gradient gel of the surface eluate from stigmas of *Pennisetum glaucum* is illustrated in Fig. 2. Some 30 protein bands are present, with three zones of esterase activity. Four bands are identifiable as glycoproteins on this gel; the principal one, D, has a molecular weight of about 170,000.

The surface proteinaceous and pectic-gel layers clothe a discontinuous cuticle. The stratum underlying this has a thinly dispersed microfibrillar content, and the staining responses indicate a considerable pectic component. This material is continuous with that of the layer separating the cells and occupying the enlarged intercellular spaces in the core of the secondary stigma branch (Fig. 1B); morphologically, therefore, it corresponds to the middle lamella. The inner wall layer of the papillate cells is cellulosic. It is probably important for the peculiar osmotic properties of these cells that as pollen receptivity is attained the tonoplast undergoes dissolution.

Each secondary stigma branch forms, in effect, a cuticle-invested pollen tube transmitting tract, since passage for the tube is offered both beneath the cuticle and through the intercellular spaces. At the junction with the stylodium, this tract links with an ill-defined zone of transmitting tissue in the core of the stylodium. This connects in turn with the transmitting tract of the upper ovary wall, which terminates at the thinly cuticularized roof of the ovary cavity. After penetrating into the cavity, the tube grows over the outer integument toward the micropyle (Table 1).

Pollen Characteristics

Grass pollens show an unusual degree of structural and functional specialization, reflecting the advanced status of the family among the monocotyledons (*3*). At the time of dispersal the pollen is tricellular, with a vegetative cell and two preformed gametes. The wall has one operculate aperture, the operculum being formed by a boss of sporopollenin subtended by a tenuous membrane of the same wall polymer (*4, 5*). The exine is tectate, but the voids of the sexine have no protein content comparable to that of many other tectate exines (*5, 6*). Both layers of the exine are perforated by micropores. The pectocellulosic intine contains a ramifying system of protein-containing channels concentrated in a thickened zone underlying the aperture (*7*). In his early ultrastructural studies of grass pollens, Rowley (*8*) showed that the intine at the aperture site is overlaid by a lens-shaped body, to which he applied the name of zwischenkörper. This structure, seemingly composed of pectic polysaccharides (*9*), is intimately concerned with the early events of germination.

The desiccation associated with final ripening in the anther reduces the water content of the pollen to 25 to 35 percent, as estimated by loss on drying, although the value at anthesis is evidently much affected by the water status of the parent plant (*10*). In the partially dehydrated, freshly shed grain, the membranes of the cytoplasm and organelles are ill defined, and even in electron micrographs prepared from material fixed in aqueous fixatives—that is, under conditions allowing some rehydration—a continuous plasmalemma cannot be resolved (*11*).

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Pollen Germination and Tube Entry

In the grasses the processes associated with germination and passage of the pollen tube into the stigma are accomplished with astonishing rapidity. In rye, for example, the interval between pollen capture and tube entry can be as short as 90 seconds. The initial interaction involves pollen attachment, hydration, and lifting of the operculum at the germination aperture and emergence of the tube tip. These events have been investigated in detail in no more than a handful of species, but the relative uniformity of pollen and stigma structure throughout the family suggests that the findings can be generalized with reasonable confidence.

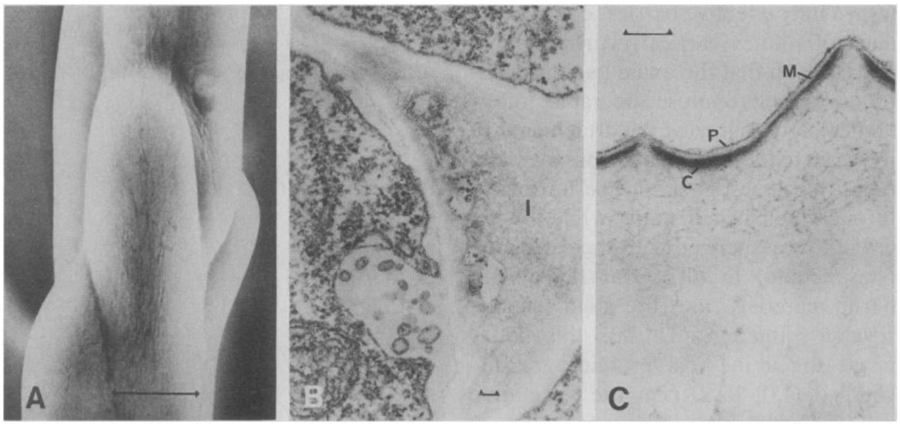

Fig. 1. (A) Scanning electron micrograph of the receptive surface of the stigma of *Pennisetum glaucum* R. Br. The thin surface secretion is molded to the underlying fissured cuticle. Scale bar, 15 μm. (B) Transmission electron micrograph of the transmitting tract of the stigma of *Secale cereale* L. (rye) in transverse section, showing the interstitial material (*I*). This material, which has pectic and protein components, is derived by secretion from the adjacent cells; the secretion may involve paramural bodies, one of which is seen in the cell to the left, adjacent to protein-rich vesicles in the intercellular space. Scale bar, 1 μm. (C) Transmission electron micrograph of the wall of the receptive zone of a stigma papilla of rye. The surface bears a thin proteinaceous pellicle (*P*), which extends over a mucilaginous layer (*M*) some 100 nanometers thick. The cuticle (*C*) is thin and fissured, and it overlies an outer zone of the wall which is rich in pectins and has a thinly dispersed microfibrillar component. This layer is continuous with the interstitial material seen in (B), and the pollen tube may pass through either. Scale bar, 1 μm.

The capture of pollen depends on the "stickiness" of the receptive zones of the stigmatic papillae. It is uncertain whether the capture is in any sense selective, although pollen attaches much less readily to stigmas from which the surface proteins have been removed enzymatically, and the adhesivity is lost when lectins are bound to the surface (*12*).

Seconds after the attachment of a pollen grain to the receptive surface, a meniscus is formed at the interface and hydration begins. In 2 minutes, a rye pollen grain may take up water at a rate of more than 5×10^{-10} cubic centimeters per second, or, assuming a meniscus 50 micrometers in diameter, of about 2.7×10^{-5} cubic centimeters per square centimeter per second (*5*). These high rates point to the unusual properties of the walls and membranes of stigma and pollen. Clearly, the discontinuous cuticle of the receptive zone of the stigma papilla offers very low resistance to water movement, and the high permeability of the underlying wall and the cell membrane is shown by the ease with which vital dyes penetrate (*12*).

In 1955 Watanabe (*13*) reported an unusual aspect of the behavior of grass pollen during the pregermination interval, namely that the hydrating grain actually exudes fluid during one period of water uptake from the stigma. The exudate is later resorbed, and the grain continues thereafter to enlarge until the tube tip emerges. Such behavior is not at all to be expected in a cell contained by a normal membrane. However, the events can be accounted for if it is assumed that the membrane of the partly dehydrated vegetative cell is initially ineffective as an osmotic barrier but undergoes a transition during hydration into a normal configuration, and in so doing regains the capacity for controlling the movement of solutes (*11, 14*).

The likelihood that the plasmalemma of the vegetative cell does not at first

Table 1. Pathway of the pollen tube in the grass pistil and comparison of pollen tube behavior in compatible and incompatible situations. In the self-incompatible (SI) response, the rejection occurs on the stigma surface or shortly after tube penetration; cross-incompatibility may be expressed in these sites, or later in the transmitting tracts or ovarial cavity.

Pollen tube pathway	Compatible behavior	Self-incompatibility	Cross-incompatibility
Receptive surface of stigma papilla	Germination governed by ambient humidity and water-carrying capacity of stigma branch	Site of rejection in strong SI systems	Rejection in these sites simulates SI response
----------------------------	Penetration of stigma cuticle		
Intercellular spaces of stigma branch	Tube guided by tract geometry	Site of rejection in weak SI systems	
Transmitting tract in stylodium	Tube guided by tract geometry; water uptake mainly from tissues of stylodium and ovary		Incompatibility expressed in retarded tube growth, often with tip inflation or bursting
Transmitting tract in upper ovary wall			
----------------------------	Penetration of cuticle of inner ovary wall		
Ovarial cavity	Tube guided by epidermal cells of ovule; probably also chemotropically		Loss of tube orientation, with random growth and failure to locate micropyle
Micropyle and nucellus	Micropyle accommodates single tube, which penetrates nucellus and enters embryo sac		

form a fully effective barrier to the movement of solutes carries further implications. Given that the exine itself is penetrated by micropores, the rapid inflow immediately following the attachment of the grain to the stigma may convey materials from the stigma surface up to and through the plasmalemma, while the later outflow associated with the exudation phase could, in turn, transfer pollen-borne materials onto the stigma in the reverse direction. During this latter phase the intine-held proteins are discharged (*15*); loss from the vegetative cell itself probably accounts for the massive release of sugars observable during the first minute of hydration (*5*). A mechanism therefore exists for the rapid and turbulent admixture of pollen-borne and stigma-borne components. On the pollen side, at least, this mechanism may not only involve materials derived from wall sites, but also from the cytoplasm of the vegetative cell.

Germination of the grain is preceded by rupture of the sporopollenin membrane at the germination aperture and lifting of the operculum. This is achieved by the rapid hydration and gelation of the pectins of the zwischenkörper, which push up the operculum and begin to diffuse away during the exudation phase (*5, 9*). This mechanism is set up during development of the spore wall in the anther, and its operation at the time of hydration does not depend on the presence of a viable vegetative cell. Similarly, the first emergence of the apertural intine, the precursor of the pollen tube tip, does not require the intervention of a living protoplast. However, the succeeding events marking germination proper clearly do (*5*).

The emerging tube tip releases several acid hydrolases and transferases derived at first from the intine sites (*7, 15*). The thin cuticle of the stigma papilla is eroded in the vicinity of the tip, which then effects entry. Thereafter the tube may continue to grow beneath the cuticle or may pass immediately into the intercellular spaces in the core of the secondary branch to continue growth through the interstitial material. The tube does not enter the stigma cells, but its passage leads almost immediately to the death of the adjacent cells, much as in a hypersensitive-type reaction to a fungal pathogen (*12*).

The architecture of the grass stigma, adapted as it is to the capture of airborne pollen, imposes certain constraints. The ultimate branches are necessarily attenuated, and this sets a limit to the number of tubes that can be accommodated. In the unpollinated stigma of rye, the only grass for which reasonably accurate dimensional data are available, the interstitial material occupies about 10 percent of the internal volume of the secondary pollen-receiving branches (*12*). A single pollen tube traversing the axis occupies more than 20 percent of the volume of the branch before pollination, implying that the branch is dilated substantially by the entry and passage of the tube. A single branch can accommodate no more than three tubes, and it is rare for more than one of these to enter the stylodium.

A further limitation is imposed by the water-carrying capacity of the stylodia. Calculations for rye reveal that perhaps as much as 0.5 mm of tube growth can be supported by the transfer of water acquired by the grain during the initial hydration (*12*); further growth must depend on water uptake from the tissues of the transmitting tract. Water availability evidently is governed by numerous factors, some of which can be identified but not as yet evaluated. They include the water potential of the tube itself, affected by the metabolic release of osmoticum and the passage of water and solutes across membranes, and the water potential of the medium in the transmitting tract, controlled in part by the water status of the plant and the composition of the interstitial secretion, which is likely to be affected by enzymes released by the advancing tube.

The grass ovary is uniovulate, and the micropyle can accommodate only a single tube; after a heavy pollination, the number achieving passage must therefore be reduced dramatically. Competition is the major factor in this. With a linear growth rate of 2 μm per second, a single tube of rye has a water demand of 12×10^{-11} cm^3 per second. Taking into consideration the water uptake during hydration, it can be estimated that supporting the germination of 100 pollen grains and the growth of the tubes throughout the tract would require the transfer over 30 minutes of an amount of water equivalent to almost half the volume of the stigma before pollination, assuming that the pistil is the sole source. It is improbable that such a load could be sustained. Also, after a heavy pollination those tubes that do effect entry compete for space and water. The consequences are diverse: tubes may be displaced onto the stigma surface or may be blocked in the tissues of the stylodia or upper ovary wall, ceasing growth or fusing to form a plasmodium. The final elimination occurs in the ovary cavity itself, when the micropyle provides the ultimate sieve (*16*).

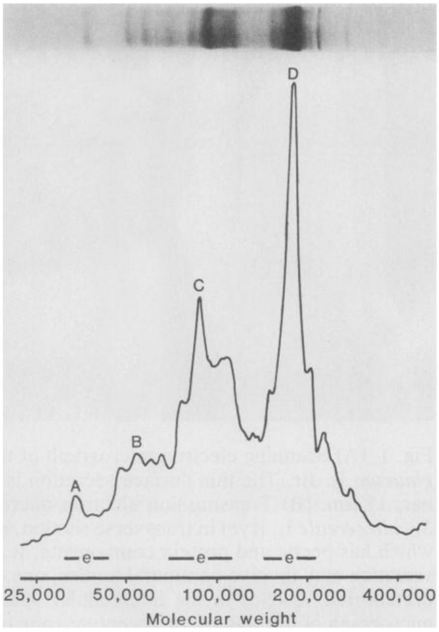

Fig. 2. Separation of the stigma-surface proteins of *Pennisetum glaucum* on a microgradient gel covering an approximate molecular weight range of 25,000 to 400,000. The bars (*e*) mark the location of esterase activity. Bands A, B, C, and D react as glycoproteins.

The Self-Incompatibility System

The basis of the genetic control of self-incompatibility in the grasses was established in 1956 by Lundquist (*17*) and Hayman (*18*), the former working with rye and the latter with *Phalaris caerulescens*. Two polyallelic loci are involved, *S* and *Z*. Each combination of alleles establishes a specificity in the pollen, and rejection occurs when this specificity is matched by one of the four possible combinations of *S* and *Z* in the pistil. These circumstances imply that the two loci act in a complementary manner but that there is no interaction between different alleles at the same locus. Subsequent investigations with other genera indicated that the system worked out by Lundquist and Hayman is the general one for the family, but the conclusion has not gone unchallenged; thus it was suggested that at least three loci are involved in *Lolium perenne* (*19*). This finding was refuted by later work on this species, which tended to reaffirm the two-locus system (*20*), but the demonstration of multiple-locus control in other families (*21*) indicates that the question is far from settled.

The self-incompatibility system in the grasses is of the "gametophytic" type in the sense that the behavior of the pollen is determined by its own genotype and not by that of the pollen parent (*22*). However, in many respects it is functionally somewhat anomalous (*23*). In most families with gametophytic systems the pollen is bicellular at the time of dispersal, with one vegetative cell and one generative cell; the generative cell does not divide to produce the two male gametes until after germination. In the grasses the pollen is tricellular, with a vegetative cell and two gametes, these being formed by division of the generative cell before pollen dispersal. This characteristic is typical of families with sporophytic determination of pollen specificity, a relation pointed out by Brewbaker (*24*). Also, grass pollen is short-lived compared with the binucleate pollen of most gametophytic families, another point of resemblance with the pollen of sporophytic systems (*25*). On the pistil side, whereas most genera with gametophytic self-incompatibility systems have stigmas with a copious surface secretion, the grass stigma is of the dry type. This feature, again, is found in families with a sporophytic system (*26*).

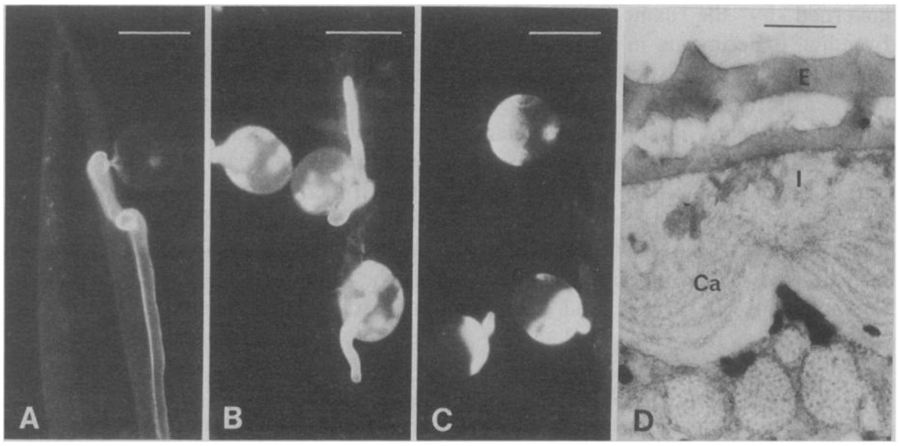

Fig. 3. (A) Fluorescence micrograph showing self-pollination in a genotype of *Anthoxanthum odoratum* L. The pollen tube is made visible by staining of its inner lining of callose (a glucan rich in β-1,3 linkages) by the decolorized aniline blue technique of Linskens and Esser (*35*). Scale bar, 50 μm. (B) Self-pollination in self-incompatible *Hordeum bulbosum* L., showing blockage of the tubes in a secondary stigma branch and deposition of callose in the tubes and grains. Scale bar, 50 μm. (C) Self-pollination in self-incompatible *Gaudinia fragilis* Beauv. The tubes are arrested immediately after emergence, and callose occlusion quickly follows. Scale bar, 50 μm. (D) Transmission electron micrograph of a pollen grain of *G. fragilis* from a pollination comparable to that shown in (C), illustrating the layered deposition of callose (*Ca*) within the intine (*I*) and exine (*E*). Scale bar, 3 μm.

Earlier work (*18*) indicated that in some grass species the inhibition of incompatible pollen tends to be immediate, at or near the surface of the stigma, and not during the growth of the pollen tube in the style, as in most gametophytic systems. This still holds as a generalization, but recent detailed investigations of the rejection response have shown that the behavior is far more variable than was hitherto supposed (*27*). The strength of the rejection reaction varies among species, and even among individual genotypes in a population.

Gaudinia fragilis is an example of a species with an exceptionally early acting self-incompatibility system. In a self-pollination, inhibition may be observed as soon as 30 seconds after the emergence of the tube tip (*28*). It seems, however, that the tip must touch the stigma surface before growth is blocked, which suggests that exine contact alone is inadequate to provoke the response. The tube tip does not penetrate the stigma cuticle, and rejection is followed within minutes by the deposition of callose, which continues to accumulate in the tube and eventually in the grain during the succeeding 90 minutes (Fig. 3, C and D). *Hordeum bulbosum* has a self-incompatibility system that is fully effective in the genetic sense, but physiologically the rejection response is a good deal slower than in *G. fragilis*. Arrest of incompatible tubes occasionally occurs on the stigma surface as in *G. fragilis*, but more commonly the tubes penetrate the stigma cuticle first and are then arrested during their passage through the intercellular material in the secondary stigma branch. Again, callose occlusion follows (Fig. 3B). Figure 3A shows, in contrast, an example of selfing in a self-compatible genotype of *Anthoxanthum odoratum*.

Examples like *G. fragilis* indicate that the primary interaction in the self-incompatibility system can be extremely rapid. The recognition event must occur within less than 2 minutes of the first contact between pollen and stigma. It could be in the vegetative cell following the initial uptake of stigma surface materials, but the fact that arrest is generally seen to occur when the emerging and actively growing tube touches the stigma suggests that it is the contact between the tube tip and the surface secretion of the receptive zone that matters. Thereafter the ordered deposition of wall microfibrils in the tube-tip region ceases and the inordinate thickening of the inner callosic wall begins. The reaction is essentially the same in *H. bulbosum*, but the tube grows farther and the stigma cuticle is breached, so that the tip lies in contact with the intercellular material of the stigma branch at the time of arrest.

The incompatibility response may be thought of as embracing a minimum of three elements: the self-recognition step governed by the *S* and *Z* loci, the rejection, and a superimposed control that determines the rate at which the growth of the tube is brought to a stop (Fig. 4). For the pistil side the most plausible hypothesis is that the *S* and *Z* factors are proteins present in the surface secretion of the receptive zone, which itself appears to be a sample of the intercellular secretion in the transmitting tract. Quantitative variation in the response would then be related not to any specific properties of the secretion, but to the rate at which it is produced by the stigma cells. On the pollen side, the *S* and *Z* factors must remain throughout very closely associated with the tube walls; otherwise it is difficult to see how independent control of the growth of each tube could be established. One possibility is that the interaction at the tube surface generates a secondary mobile stimulus that passes to the adjacent plasmalemma and affects enzyme activity there. An alternative interpretation is that the binding affects wall growth directly by interfering with the extension, or pattern of deposition, of polysaccharide microfibrils (*29*). If the *S*, *Z* specificity on the pollen side is carried in the sugar sequence of a polysaccharide wall component, the role of the *S* and *Z* pistil constituent may be to bind with the complementary sugar sequence, in the manner envisaged by Mirelman *et al.* (*30*) for the function of lectins in inhibiting the growth of fungal hyphae.

Primary Interspecific Barriers and the Self-Incompatibility System

Since the self-incompatibility system in the grasses works by governing pollen tube growth into and through the stigma, it is reasonable to inquire whether cross-incompatibilities—interracial, interspecific, or intergeneric—for which the blockages are in these same sites may be governed by the same mechanisms. Breeding experiments in various angiosperm families have shown connections between intra- and interspecific incompatibility systems, notably in the Solanaceae, where the self-incompatibility system is gametophytic and governed by a single polyallelic *S* locus. A common manifestation is the occurrence of so-called unilateral incompatibility between taxonomically closely related species, the general rule being that when one parental species is self-compatible and the other self-incompatible, the pollen of the self-compatible species is rejected by the pistil of the self-incompatible species; in the reciprocal cross, the pollen of the self-incompatible is accepted by the pistil of the self-compatible (*31*). Various models have been suggested to account for the relation. Some are ingenious and suggestive, but it is difficult not to concur with de Nettancourt's conclusion (*22*) that most "are extremely speculative and, as often the case in incompatibility research, not supported by any well-established fact."

Table 2. Incompatibility barriers in intergeneric grass crosses. The species are ranked in order of effectiveness of the self-incompatibility response, as judged from the site of pollen inhibition. Abbreviations: SI, self-incompatible; (SI), weakly self-compatible; SC, self-compatible; —, early inhibition with little or no tube penetration; – –, inhibition somewhat delayed, with some tubes penetrating; +, many tubes penetrating, but most then inhibited; + +, tubes not inhibited in the stigma branch; NT, not tested.

Female	Male					
	Gaudinia fragilis	*Dactylis glomerata*	*Secale cereale*	*Alopecurus pratensis*	*Anthoxanthum odoratum*	*Elymus arenarius*
Gaudinia fragilis (L.) Beauv.	SI	—	– –	—	—	—
Dactylis glomerata L.	– –	SI	—	– –	—	– –
Secale cereale L.	– –	+	SI	+	+	NT
Alopecurus pratensis L.	+	+ +	+	(SI)	+ +	+ +
Anthoxanthum odoratum L.	+	+ +	– –	+ +	SC	– –
Elymus arenarius L.	+	+ +	NT	+ +	+	SC

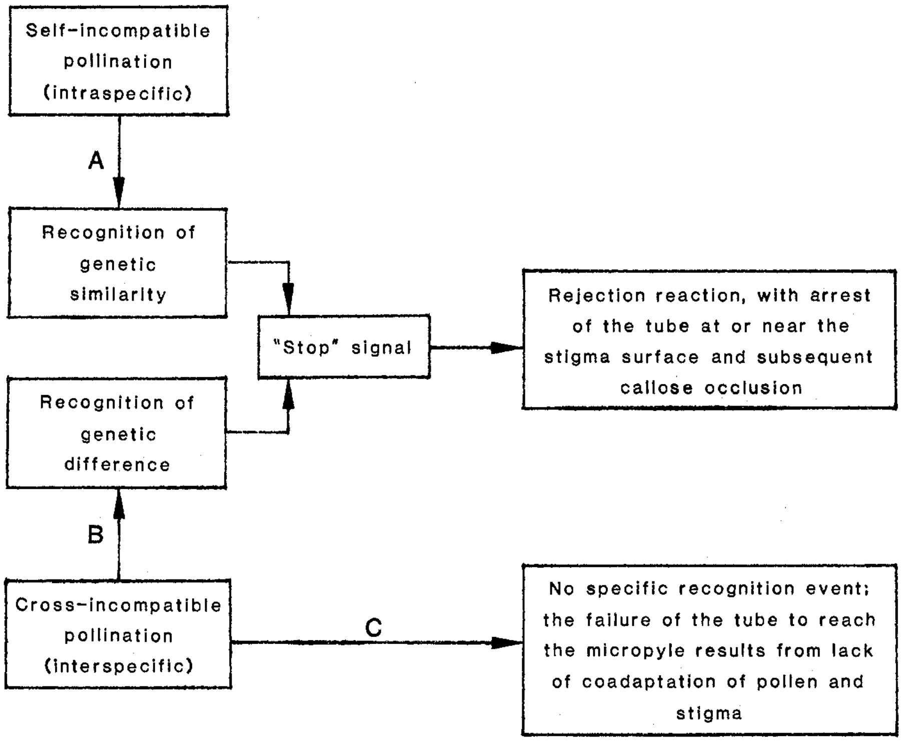

Fig. 4. Possible relations between self- and cross-incompatibility. (A) Self-incompatibility sequence; (B) cross-incompatibility sequence in which the rejection response simulates that of self-incompatibility; (C) cross-incompatibility sequence in which no specific recognition event is involved and failure results from physiological maladjustment of the pollen tube and stigma.

Yet there is no doubt that there can sometimes be a relation between the incidence of cross-incompatibility and the functioning of the self-incompatibility system. Although most interpretations have accepted that when this is true the *S* locus must be directly involved in the genetic control of cross-incompatibility, an apparent interaction could arise without the participation of *S* allele specificities. The distinctions made above between recognition, response, and rate in the *S* and *Z* self-incompatibility system of the grasses suggest that the response mechanism—including the metabolic changes that check pollen germination or arrest tube growth and the factors that determine the rate of the reaction—might be common to the self- and cross-incompatibility systems, but that the initial recognition events are independent. The requirement in the intraspecific system is to recognize and reject likenesses; in the interspecific system, to identify and reject differences. Distinct molecules may be involved in the primary interaction, or distinct parts of the same molecule, as proposed by Sampson (*32*) for the Cruciferae. The two control pathways would then anastomose in the enabling or disabling of the response system (Fig. 4). In such a circumstance one might expect epistatic effects, as well as parallel variation in effectiveness according to the genetic background and the efficiency of the rejection reaction itself.

What relations, then, are to be discerned between intra- and interspecific incompatibility systems in the grasses? Hitherto there appear to have been no detailed investigations of the kind carried out with the Solanaceae, although examples of unilateral interspecific incompatibility are on record (*31*). Table 2 sets out some of the variations we have observed in stigma receptivity in a set of intergeneric crosses and relates them to the strengths of the self-incompatibility responses in the individual species. Pollination tests with small numbers of plants may not provide a basis for applying the findings to the species as a whole, but the results are positive enough in that they show unequivocal reciprocal differences and general obedience to the rule that self-incompatible species tend to reject the pollen of self-compatibles, while producing pollen that functions on the stigmas of self-compatibles.

An important feature of the behavior

of blocked pollen in instances of cross-incompatibility (Table 2) is that, cytologically, it often matched that of incompatible pollen from the same plant. In crosses with the strongly self-incompatible *G. fragilis* as pistillate parent, rejection of pollen usually occurred on the stigma surface, with early inhibition of germination and associated callose blockage. When *Dactylis glomerata* was the pistillate parent, the response was almost as extreme, but some of the foreign tubes did penetrate the cuticle of the stigma papilla before arrest and occlusion, as in a selfing in this species. In the weakly self-incompatible *Alopecurus pratensis*, rejection occurred with the pollen of only two of the foreign genera, *Gaudinia* and *Secale*, both strongly self-incompatible, but the response was somewhat delayed, again as in a selfing.

It may be concluded that cross- and self-incompatibility systems often have some fairly direct interrelations in the Gramineae. Whether *S* and *Z* specificities are involved in the results shown in Table 2 is not known, and until the recognition factors in the intraspecific system are isolated and characterized, it is improbable that evidence will be forthcoming. Nevertheless, it is plausible that segregation at the *S* and *Z* loci has nothing to do with the intergeneric recognition system, the latter being governed by "species identification" loci; in each instance the same rejection or acceptance systems are activated following the initial "dialogue" (Fig. 4). The parallelism in the intensity of the responses may then be accounted for as the consequence of differences in the strengths of the stigma-side controls.

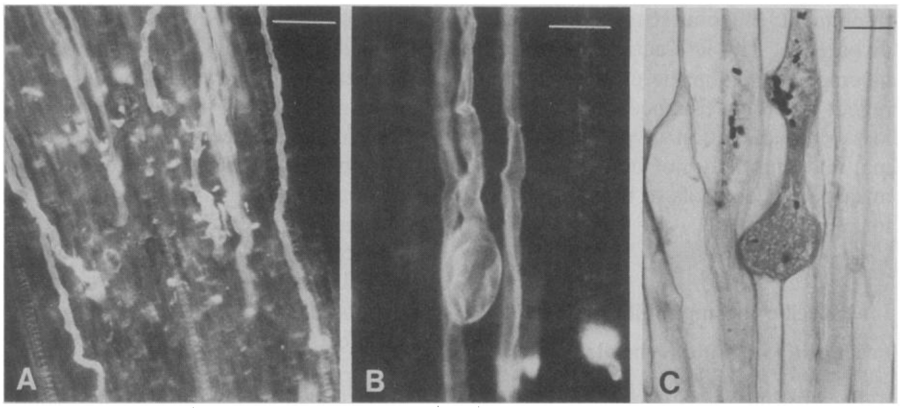

Fig. 5 (A) Fluorescence micrograph showing arrest of the pollen tubes of *Sorghum bicolor* (L.) Moench. in the lower part of the stylodia of *Pennisetum glaucum*. Scale bar, 100 μm. (B) Detail of arrested tube tip. Scale bar, 25 μm. (C) Optical micrograph showing arrested and swollen pollen tube in the transmitting tract of a stylodium of *Secale cereale* following compatible pollination of a detached stigma held in high humidity over agar (*34*). Stain, toluidine blue; scale bar, 25 μm.

Late-Acting Interspecific Barriers

Table 2 shows that in the grasses, controls acting with the same rapidity and in the same sites as those of the self-incompatibility system may provide efficient barriers to interspecific crossing in one or both directions. It also shows that controls of this kind are not by any means universal; even in such crosses as between *Dactylis* and *Anthoxanthum*, genera in different tribes, there may be ready acceptance of pollen in one direction. Yet this initial acceptance is not necessarily followed by growth of the tube into the embryo sac and fertilization; in most of the intergeneric crosses in which tube penetration occurs (Table 2) there is later an arrest, and hybrid zygotes are never formed.

Many examples of late-acting bars to fertilization have been described in interspecific and intergeneric crossings among the small-grain cereals (*33*). The recorded cytological details show that the tubes most commonly are arrested in the transmitting tracts of the stylodia or upper ovary wall and that the blockage does not usually involve the development of massive callose plugs (as is commonly found in the self-incompatibility reaction controlled by the *S* and *Z* loci), although there may be some thickening of the inner callosic wall near the tip. The crossing of barley and rye may be taken as an example. The rye tubes are not usually excluded from entry in this cross, and they grow freely through the secondary stigma branches into the stylodia. According to the interpretation given earlier, there would be no massive water uptake during this phase of tube growth. After the tubes have entered the stylodia, growth is retarded, and ultimately the tips enlarge at a lower level in the stylodia or in the transmitting tract of the upper ovary wall. The gametes and vegetative nucleus eventually pass into the tip region, but there is no massive accumulation of callose. The tubes may ultimately burst, and, if several are present in the tract together, they may then form a plasmodium.

The manifestations of cross-incompatibility between the tropical cereals millet (*Pennisetum glaucum*) and sorghum (*Sorghum bicolor*) are quite similar, but, in this instance, with millet as the pistillate parent, there is a tendency for the sorghum tubes to be arrested in a stretch of the transmitting tract which coincides with the abscission zone in the fused portion of the stylodia (Fig. 5, A and B). Again, the arrest is often associated with enlargement of tube tips but with little or no callose deposition. In the bizarre example of the pollination of rye by maize, we have found that although hydration may be much delayed, a tube is ultimately formed. It penetrates the stigma and may even grow to the base of the secondary branch, but it is physically unable to enter the transmitting tract of the stylodium.

Taken together, these aspects of late-acting cross-incompatibilities indicate that there is no direct link with the self-incompatibility system. How, then, are they to be interpreted?

A feature of delayed tube arrest is that its manifestation is often sensitive to environmental influence. Related to this is the fact that similar syndromes can be induced experimentally in otherwise compatible, intraspecific pollinations. In rye, for example, tubes are commonly arrested in the transmitting tracts of the stylodium or ovary wall in pollinations of excised pistils maintained on agar in high humidity; arrest may be accompanied by the enlargement of the tube tips or bursting (Fig. 5C) (*34*).

These facts strongly suggest that the delayed cross-incompatibilities are nonspecific and are the consequence of a disharmony between the tube and the transmitting tract. In some instances the disharmony is related to the osmotic balance between the tube and the cells of the tract; in other cases, as in crossing rye and maize, it is due to physical maladjustment.

This conclusion accords with a theory of species intersterility which states that reproductive failure commonly arises from lack of mutual adjustment in the reproductive systems of the partners. Within a breeding population, selection will necessarily maintain functional compatibility between the sexes; but this will not necessarily be preserved between individuals of different populations throughout the course of evolutionary divergence. The late-acting interspecific incompatibilities in the grasses probably reflect the consequences of fortuitous loss of coadaptation of pollen and stigma, and thus may not be related to the intraspecific controls of breeding behavior.

Interspecific Incompatibilities and the Practicality of Wide Hybridization

The distinction between primary and late-acting cross-incompatibility barriers (Table 1 and Fig. 4, B and C) may have some significance for the practical business of obtaining interspecific and wider hybrids, even if only in helping to identify potential problems. Since pollen tubes can be identified readily enough by the callose-fluorescence method (*35*), there generally is no difficulty in establishing the site of inhibition in the tissues of the stigma and ovary or in recognizing surface blockage of the kind associated with the self-incompatibility reaction.

Figure 4B suggests that when a cross-incompatibility response simulates self-incompatibility, a specific recognition event is involved. The problem of surmounting this kind of barrier is comparable with that of overcoming self-incompatibility. Yet the probability that the strength of the rejection reaction is determined by the genetic background—perhaps, as suggested here, through the control of the rate of stigma secretion—does mean that variation in the response is to be expected; obviously it will always be desirable to acquire and test as wide a range of female genotypes as possible. Pollen-side variation is less likely to be significant, although it is not to be ignored. Direct manipulation aimed at modifying stigma properties during development remains to be explored in the grasses; at present, however, we know too little about the factors that control stigma secretion for any procedures to be specified (*36*).

Figure 4C covers a wide range of possible causes of failure. Setting aside morphological disparities that are bound to be involved in some instances, it is evident from the foregoing discussion just how closely coadapted pollen tube and stigma physiology must be for success. Yet here, also, the behavior of the partners presumably will always be subject to genetic variation. Recent work on the action of the so-called crossability genes of wheat which affect the capacity for hybridization with rye as pollen parent has shown that they modify the number of pollen tubes successfully negotiating the transmitting tract, the weeding out being more rapid and complete in the genotypes with low crossability (*16*). It will be of interest to discover what physiological parameters these genes control, and whether—as is predictable—they also affect crossability with other species.

It may also be possible to manipulate stigma properties experimentally. The rate of water movement into and through the pistil, an important determining factor, is likely to be affected by the water status of the plant and by the relative humidity, both of which are open to manipulation. Furthermore, chemical treatment may have the potential for modifying water movement, although the approach must be largely empirical in the absence of more detailed knowledge of the controls in the pistil. Hormonal applications to the female parent have been shown to modify crossability among cereals (*37*), and, although the basis of their effect is unknown, one target might be the membranes of the transmitting tract cells.

References and Notes

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