Plant Physiol. (1997) 115: 1221–1230

# Pollen-Stigma Adhesion in Kale Is Not Dependent on the Self-(In)Compatibility Genotype[1]

**Doan-Trung Luu, Philippe Heizmann*, and Christian Dumas**

Reproduction et Développement des Plantes, Unité Mixte de Recherche, Centre National de la Recherche Scientifique, Institut National de la Recherche Agronomique, Ecole Normale Supérieure de Lyon, 46 allée d'Italie, F-69364 Lyon cedex 07, France

**The adhesion of pollen on the stigmas of flowering plants is a critical step for the success of reproduction in angiosperms, long considered to present some specificity in terms of self-incompatibility. We carried out quantitative measurements of the pollen-stigma adhesion (expressed in Newtons) in kale (*Brassica oleracea*), using the flotation force of Archimedes exerted by dense sucrose solutions (50%, w/v) to release pollen grains fixed on the surface of stigmas. We demonstrate that pollen adhesion varies with the genotypes of the plants used as partners, but increases with time in all cases for about 30 to 60 min after pollination. There is no correlation with the self- or cross-status of the pollinations, nor with the self-compatible or -incompatible genotypes of the parents. Only late events of pollination, after the germination or arrest of the pollen tube, depend on compatibility type. Biochemical and physiological dissection of pollen-stigma adhesion points to major components of this interaction: among male components, the pollen coating, eliminated by delipidation (or modified by mutation in the case of the *cer* mutants of the related species *Arabidopsis thaliana*), plays a major role in adhesion; the genetic background of the pollen parent is also of some importance. On the female side, the developmental stage of the stigma and the protein constituents of the stigmatic pellicle are critical for pollen capture. The SLG and SLR1 proteins are not involved in the initial stages of pollen adhesion on the stigma but one or both may be involved in the later stages.**

In angiosperm mating, the lack of mobility of individual plants is compensated for by mobility of pollen grains mediated by physical agents such as water or wind (anemophily) or by animals such as birds or insects (entomophily). Whatever the vector, the efficiency and specificity of capture of pollen grains by stigmas is of importance for the reproductive success of plant species.

The secretions of some stigmas classified as "wet" by Heslop-Harrison and Shivannah (1977) include all of the components of a perfect universal "glue" capable of fixing pollen grains efficiently (Clarke et al., 1975). But these stigmas also often permit indiscriminate pollination by foreign species as well as infection by the spores of pathogens (Dickinson, 1995). In addition, families with wet stigmas, such as the Solanaceae, Liliaceae, or Rosaceae, allow partial development of self- or interspecific incompatible pollen tubes down to the mid-style at the expense of female resources (Dickinson, 1995). In families considered to be more evolutionarily advanced, such as the Asteraceae, Brassicaceae, Gramineae, or Papaveraceae, the stigmas are classified as dry and appear more efficient since they do not present the aforementioned drawbacks, rejecting pathogens and incompatible pollen tubes immediately at the surface of their stigmas (Hodgkin et al., 1988).

The chemical and physical structure of the pollen cell wall coating co-evolved in these families with the structure and composition of the stigmatic surface to maintain efficient pollen-stigma adhesion and to improve recognition and rejection of incompatible pollen.

In *Brassica oleracea*, self-incompatible pollen has been shown to undergo germination inhibition soon after capture on the stigma. Recognition must therefore occur within minutes of pollination (Ferrari and Wallace, 1975). The events immediately following the first contact between the pollen grain and the stigma have thus drawn much interest. Adhesion was shown to depend on the (in)compatibility status of mating partners, with incompatible crosses generating lower pollen-stigma adhesion than compatible ones (Roggen, 1972, 1975; Stead et al., 1979; Kerhoas et al., 1983), suggesting that the products of the self-incompatibility genetic system, the *S*-locus, might be located at the interface between pollen and stigma. To investigate this possibility, the surfaces of both interacting partners have been analyzed extensively before, during, and after the initial pollen-stigma adhesion (Gaude and Dumas, 1984, 1986; Elleman and Dickinson, 1986, 1990, 1994, 1996).

In pollen the cavities of the external ornamented cell wall layer, the exine, contain a tapetally derived pollen coat lipoproteic complex that includes many different enzymes (Knox et al., 1970), proteins (Gaude and Dumas, 1987; Doughty et al., 1993; Ross and Murphy, 1996), and lipid droplets (Preuss et al., 1993), but no characteristic *S*-locus-specific products have been detected (Gaude et al., 1988). Two successive lamellar membrane-like layers have been observed at the surface of the pollen wall; the most internal one, the exinic outer layer, invests the exine itself (Gaude and Dumas, 1984), and the most external one, the coating superficial layer, seals the pollen coat in the baculae of the exine (Elleman and Dickinson, 1986).

In the stigma the existence of a protein pellicle, which

[1] Supported by grants from the French Ministeries of Agriculture, Environment, and Education.

* Corresponding author; e-mail philippe.heizmann@ens-lyon.fr; fax 33-4-72-72-86-00.

probably first interacts with the pollen coating and was proposed to be the recognition site in incompatibility reactions (Mattsson et al., 1974), was demonstrated. Anhydrous fixation of stigmas allowed the characterization of the pellicle as an electron-opaque layer covering the electron-transparent cuticle (Elleman and Dickinson, 1986). Using various cytochemical methods, Gaude and Dumas (1986) demonstrated that, although it does not possess a trilamellar structure, the pellicle possesses many characteristics of biological membranes, including esterase, ATPase, and adenylate cyclase activities. The question of the secretion of *S*-locus glycoproteins into this outer membrane-like structure remains open. The presence of *S*-locus glycoproteins in the pectocellulosic cell wall itself has been clearly demonstrated by immunolocalization (Kandasamy et al., 1989, 1991), but it is not known whether these proteins are present at the very surface of the papillar pellicle and exposed to the incoming pollen grains, at least initially.

Both male and female partners present to each other membrane-like external surfaces potentially carrying signals for the self-incompatible interaction. Such signals are likely to explain the *S*-phenotype dependence of pollen-stigma adhesion but proof of the presence of these *S*-signals is still lacking. Moreover, the events following the first contact of the pollen with the stigma do not seem to differ markedly according to the compatibility status of the crossing: compatible as well as incompatible pollen grains hydrate after a lag phase of about 20 to 40 min. Hydration lasts 20 to 30 min in both cases, but only quantitative measurements of the small and large axes of the pollen grains demonstrate that compatible grains hydrate more rapidly and more extensively than incompatible ones (Dickinson, 1995). This is consistent with the recent finding that an aquaporin-like protein is involved in self-incompatibilty mechanisms (Ikeda et al., 1997). Ultramicroscopic observations have also demonstrated that self-compatible grains only complete the cytological reorganization of the pollen-stigma interface, leading to full germination (Dickinson and Elleman, 1985; Elleman and Dickinson, 1986, 1990, 1994, 1996).

We have reexamined in this work the physiology of pollen-stigma adhesion in *B. oleracea*, using an original biomechanical test to measure quantitatively the forces necessary to detach pollen populations from stigmas. We have "dissected" the kinetics of the process and the various female and male elements of this adhesion. We have confirmed the importance of the participation in pollen adhesion of pellicular proteins and of the pollen coating, namely those affected in the *cer* mutants of the related species *Arabidopsis thaliana*. We have shown that, although every single pair involved in pollination has its individual adhesion properties, the adhesion strength itself is not dependent on the *S*-haplotypes of the partners, and that incompatible pollen grains do not adhere less efficiently than compatible grains. The *S*-specific pollen recognition and rejection must occur after the initial adhesion event sensu stricto, affecting the later stages of hydration and pollen tube germination.

## MATERIALS AND METHODS

The self-incompatible lines $S_{29}$ and $S_{25}$ of Chinese kale (*Brassica oleracea* L. var *alboglabra*) were obtained from Dr. D.J. Ockendon (HRI, Wellsbourne, UK). The two near-isogenic biannual lines *P57Si* (self-incompatible) and *P57Sc* (self-compatible) were produced at the Institut National de la Recherche Agronomique (INRA) station in Rennes, France (Gaude et al., 1993). $S_{29}$ and *P57Si* are pollen-dominant class I haplotypes, whereas *P57Sc* is a pollen-recessive class II haplotype, according to the classification proposed by Nasrallah et al. (1991). The two rapid-cycling lines *RC-Sc* and *RC-Si* were obtained by crossing the rapid-cycling line $S_{25}$ with the lines *P57Sc* and *P57Si*, respectively, to associate the rapid-cycling character with the *Si* and *Sc* haplotypes. The F1 were back-crossed with the $S_{25}$ line as a male parent, to reinforce the rapid-cycling character. In the F2 and in their self-crossed F3 and F4 progenies, segregants were selected carrying the homozygotic *Si/Sc* haplotypes in the *RC* background. The self-compatible line *CGS43* of *B. oleracea* was from the INRA station in Versailles, France. The Landsberg *erecta* ecotype of *Arabidopsis thaliana* Schur., the *cer1*, *cer2*, *cer3*, and *cer6–1* mutants (Preuss et al., 1993; Hülskamp et al., 1995) were obtained from the Arabidopsis Biological Resource Center (Columbus, OH) and the Nottingham Arabidopsis Stock Center (UK).

### Physical Characteristics of Pollen Grains

The size of the mature pollen grains was estimated by videomicroscopy, both for dry, dehiscent pollen grains and for pollen grains suspended in pure water or in 2% (v/v) formalin-Suc solutions (formalin was added to freeze the rapid evolution of pollen-stigma adhesion at given instants after the initiation of pollination). For videomicroscopy, we used a charge-coupled device color video camera (model DXC-107P, Sony) mounted on an inverted microscope and a videocassette recorder (model NV-FS 100 HF Panasonic, Matsushita Electric Industrial, Osaka, Japan). The density of pollen grains was determined by equilibrium centrifugation using a step gradient of Suc solutions ranging in concentration from 20 to 60% (w/v) in 5% increments (Fig. 1A). We checked that the grains reached the same equilibrium Suc layer whether they had been deposited on the top of the gradient as a 20% (w/v) Suc suspension layer or at the bottom of the gradient as a 60% (w/v) Suc suspension layer.

The average weight of the pollen grains was determined by hemocytometer counting of known masses (10–100 mg) of pollen; the fresh weights were first measured after collection. The batches were then dried in an infrared oven (Sartorius) until all of the free water was lost, the corresponding dry weights were measured, and the pollen was then suspended in 0.1% (v/v) Tween 20 (to avoid aggregation) and 40% (w/v) Suc (to avoid rapid sedimentation during pipettings) for counting under the microscope. In *A. thaliana* the pollen was harvested by vortexing inflorescences in 15% Suc according to the method of Preuss et al. (1993), eliminating plant debris by filtration through

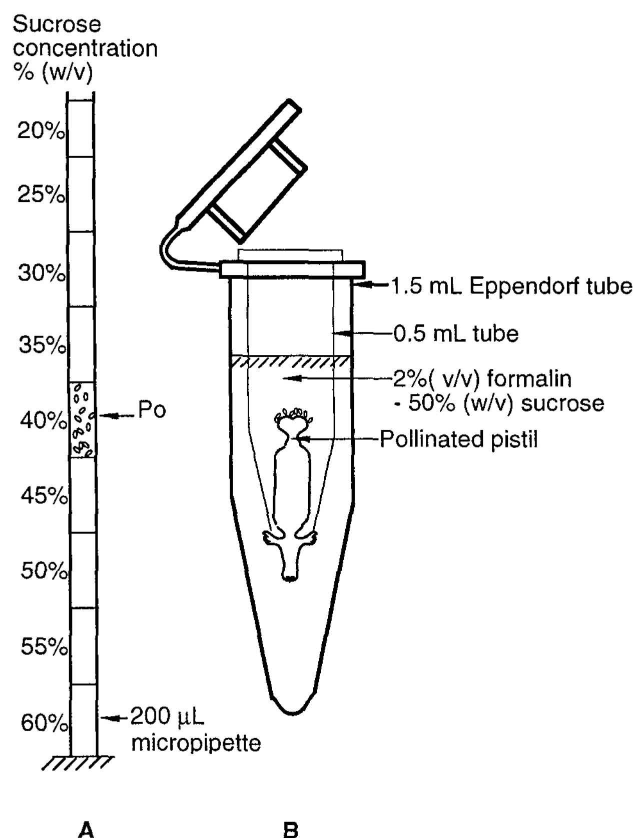


**Figure 1.** Experimental device for pollen-stigma measurement. A, Determination of the pollen grain density by equilibrium centrifugation on a Suc gradient. B, Pollinated pistil maintained in a 50% (w/v) Suc solution (d = 1.19 g $cm^{-3}$) and centrifuged as described in "Materials and Methods."

cheesecloth and collecting pollen grains on a glass fiber filter. Only the dry weight was determined.

### Experimental Device for Adhesion Measurement

Pollinated pistils were maintained in 1.5-mL Eppendorf tubes filled with 2% (v/v) formalin, 50% (w/v) Suc solution (or 60% [w/v] in the case of delipidated pollen grains) using 0.5-mL tubes cut at the bottom (Fig. 1B). These were centrifuged (Sigma) at a constant acceleration for 10 min at 20°C to remove pollen grains by Archimedes flotation. After centrifugation, the pistils were rinsed in water and observed with a binocular microscope (Nikon, SMZ-2T). If the papillar tips of all five pistils analyzed for each determination were not completely devoid of pollen grains, a new batch of five pollinated pistils was treated in the same way and centrifugation was increased in increments of 500*g* until the superficial monolayer of pollen was totally eliminated from the five stigma surfaces.

Pollen adhesion is broken when the flotation forces (directed toward the top of the tube) overcome the centrifugal and the adhesion forces (directed toward the tube bottom). The adhesion force, $F_{adhesion}$, expressed in Newtons (N), was deduced from the following relationships:

$$\begin{aligned}F_{adhesion} &= (\text{flotation force of Archimedes}) \\ &\quad - (\text{centrifugal force}) \\ &= (V_{pollen} \times d_{50\%sucrose} \times \text{acceleration}) \\ &\quad - (V_{pollen} \times d_{pollen} \times \text{acceleration}) \\ &= V_{pollen} \times (d_{50\%sucrose} - d_{pollen}) \times \text{acceleration} \\ &= 2.0 \times 10^{-13}\ \text{kg} \times 9.81\ \text{m s}^{-2} \times \text{acceleration}_{(g)} \\ &= 19.62 \times 10^{-13} \times \text{acceleration}_{(g)}\end{aligned}$$

where $V_{pollen}$ is the average intrinsic volume of a pollen grain ($5.0 \times 10^{-9}$ $cm^3$), $d_{50\%Suc}$ and $d_{pollen}$ are the densities of the 50% centrifugation medium and of the pollen grains (1.19 and 1.15 g $cm^{-3}$, respectively); and the acceleration is expressed in *g* (9.81 m $s^{-2}$).

### Pollen-Coating Extraction by Organic Solvents

The pollen coating was removed using standard treatments with organic solvents (acetone, hexane, or cyclohexane) (Jain and Shivanna, 1988; Doughty et al., 1993). The delipidated pollen grains were checked for their capacity to germinate in vitro on liquid media, and their density was found to be significantly increased ($d$ = 1.20 g $cm^{-3}$ versus 1.15 g $cm^{-3}$ in native conditions) after elimination of light lipids. The pollen-stigma adhesion measurements were therefore made in 61% Suc solutions ($d$ = 1.23 g $cm^{-3}$). In vitro pollen germination was performed in media previously described (Luu et al., 1997).

### Proteinase K Treatment of the Stigmas

Fresh stigmas were surface treated with proteinase K (1 mg $mL^{-1}$), or with 20 mM Tris solution, pH 7.5, as a control, for 10 min at 20°C. The stigmas were then pollinated and adhesion measurements were performed. To address the question of in situ degradation of SLR1 proteins by the proteinase K treatment, we performed western analysis (SDS-PAGE) of soluble proteins extracted from stigmas. Immunodetection of proteins was carried out as previously described (Gaude et al., 1993), with the exception that the primary antibody was a polyclonal anti-SLR1 produced by immunization of a rabbit with the peptide TNTLSPNEALTISSN cross-linked to ovalbumin (Ruffio-Chable et al., 1997).

### Scanning Electron Microscopy

The tissues were settled on copper plates (model 35 CF, Jeol) with a conductive C glue, frozen in liquid $N_2$, freeze-dried, coated with Pt and C in an evaporator, and observed in a scanning electron microscope (model 35 CF, Jeol).

## RESULTS

### Physical Properties of the Pollen Grains

The size of the mature ellipsoid pollen grains of *B. oleracea*, estimated by videomicroscopy, depended greatly on their hydration status: the dimensions of dry dehiscent grains were measured as 37 ± 1 μm × 19 ± 1 μm for the large and small diameters, respectively, with the corresponding volume of $57 \times 10^{-9}$ $cm^3$ and the ratio of the major/minor axis about 2.1 (similar dimensions were found for pollen grains of lines $S_{29}$, *RC-Sc*, and *CGS43*). Pollen grains suspended in 2% formalin, 50% Suc solutions measured 34 ± 1 μm × 25 ± 1 μm (with a volume = $93 \times 10^{-9}$ $cm^3$ and an axis ratio of 1.4); in pure water the dimensions were 35 ± 2 μm × 26 ± 1 μm and the volume was $104 \times 10^{-9}$ $cm^3$. The morphological changes induced by hydration correspond closely to those described in the literature (Stead et al., 1979; Dickinson, 1995) and high Suc concentrations did not substantially affect the apparent size of pollen grains.

The density of *B. oleracea* pollen grains, determined by equilibrium centrifugation on Suc, was equal to that of the 40% Suc layer ($d = 1.15$ g $cm^{-3}$) for mature pollen, and 55% Suc ($d = 1.20$ g $cm^{-3}$) for pollen treated with cyclohexane and devoid of exinic light lipidic components.

The fresh pollen weight (6.4 to $7.2 \times 10^{-9}$ g per grain) depended on the atmospheric RH at the moment of harvest. The measurement of the dry weight of pollen grains ($5.75 \times 10^{-9}$ g) allowed estimation of the range of their water content (10–20% [w/w] of the fresh weight) in the various humidity conditions tested.

The intrinsic volume of *B. oleracea* pollen grains (i.e. the volume of their dry materials) is given by the formula volume = mass/$d$ ($5.75 \times 10^{-9}$g/1.15 g $cm^{-3}$ = $5.0 \times 10^{-9}$ $cm^3$), whereas the apparent volume of the fresh pollen grains visualized by videomicroscopy is $57 \times 10^{-9}$ $cm^3$, and the corresponding apparent density ($d$ = mass/volume) is about 0.12 g $cm^{-3}$.

In *A. thaliana* the pollen grains were slightly smaller than those of *B. oleracea* in size (30 × 14 μm) and dry weight ($2.6 \times 10^{-9}$g per grain), with the same average buoyant density ($d = 1.15$ g $cm^{-3}$); we did not record significantly higher densities for the *cer* pollen grains.

### Visual Aspects of Pistil Pollination/Depollination

*B. oleracea* pistils pollinated either by immersion in a deep layer of freshly harvested pollen or by brushing with dehiscent anthers from open flowers appeared overloaded with masses of pollen grains (Fig. 2A). Dipping the pistils in water or in 50% Suc solutions disrupted the unstable masses and produced a strict monolayer of pollen grains firmly attached to the stigma surface (Fig. 2B). Centrifugation of such pistils fixed to the bottom of centrifugation tubes in 50% Suc solutions ($d = 1.19$ g $cm^{-3}$) depleted the monolayer of pollen grains, because of the flotation forces exerted ($d = 1.15$ g $cm^{-3}$).

With sufficient acceleration all of the pollen grains adhering at the tips of papillar cells were detached (Fig. 2, C and D). However, numerous grains trapped between individual papillae resisted detachment, because their number of contact points with the papillae was higher (>2) and

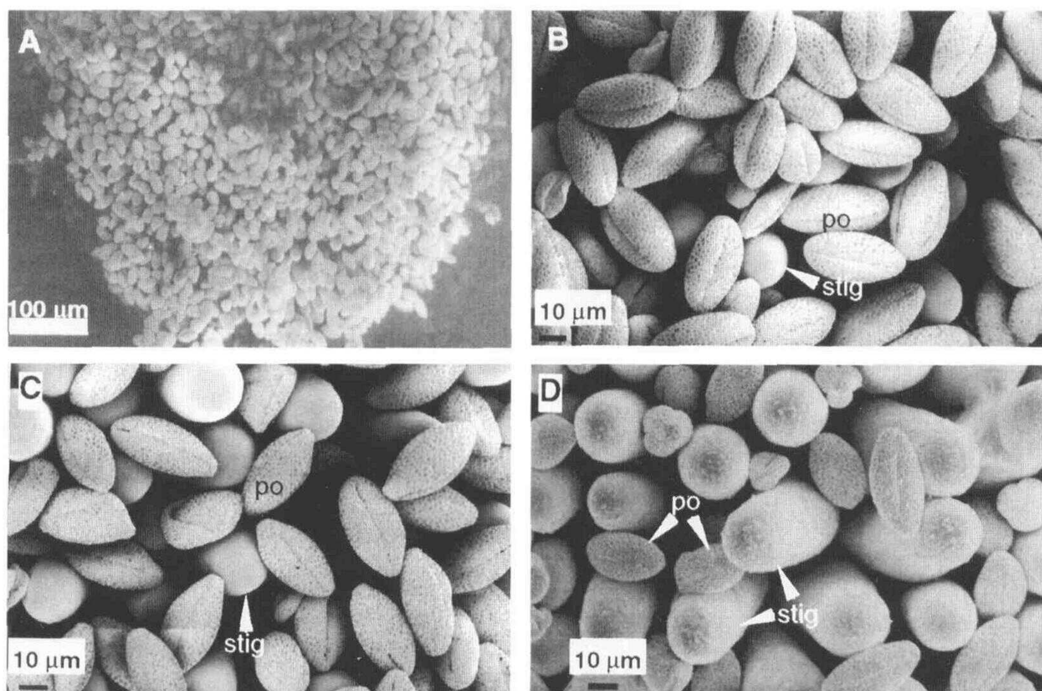


**Figure 2.** Observation of self-pollinated $S_{29}$ stigmas by scanning electron microscopy. A, Overloaded stigmas with stacks of pollen grains. B, Monolayer of pollen grains after dipping the stigma in water or in 50% (w/v) Suc solution. C, B centrifuged at 1000$g$ for 10 min, with many pollen grains still attached to the tips of the papillae. D, B centrifuged at 2500$g$ for 10 min, with no more pollen grains adhering at the tips of the papillae, only between them (diameter of the stigma, about 1.5 mm).

**Table I.** *Centrifugation parameters and adhesion disruption*

Combination of length × acceleration required to release the superficial pollen grains in $S_{29}$ self-pollinations on batches of six flowers.

| Time after Pollination | Length of Centrifugation (min) | | |
|---|---|---|---|
| | 5 | 10 | 15 |
| 5 min | 7000*g* | 2000*g* | 1000*g* |
| 10 min | 8000*g* | 3000*g* | 2000*g* |

because the direction of the "shearing" attachment forces (parallel to the meniscus of contact of these grains with the stem of papillae) provide much higher mechanical resistance than the "tearing" forces exerted perpendicular to the meniscus of the pollen adhering only at the tip of the papillae. The pollen grains maintained between the papillae could be released neither in 50% Suc ($d$ = 1.19 g cm$^{-3}$) nor in 60% Suc ($d$ = 1.225 g cm$^{-3}$) at the highest acceleration obtainable in the centrifuge (18,000*g*) and therefore resisted forces at least 8 to 10 times higher than the pollen grains attached at the tip of the papillae. The use of CsCl solutions of higher density ($d$ = 1.5 g cm$^{-3}$) allowed much larger flotation forces and thus released all pollen grains from the stigmas, but it is quite possible that such high salt concentrations might destabilize the pollen-stigma complexes by dissolving or extracting some of their components.

## Mechanical Aspects of the Disruption of Pollen-Stigma Complexes

The procedure used here to break down the pollen-stigma adhesion forces is based on simple physical laws of hydrostatics (the principle of Archimedes). We noticed, however, that various combinations of centrifugation length times acceleration force could achieve the same release of pollen grains from the stigmas (i.e. short, high-speed centrifugations were equivalent to longer, low-speed runs) (Table I). Our interpretation is that a certain amount of mechanical work (i.e. force times displacement or power times time) rather than a simple amount of force is required to disrupt the pollen-stigma attachment. A corollary of this observation is that a pollinated pistil, centrifuged under a given combination of acceleration times time and not completely cleared of pollen grains, could not be used as a "new" pistil for a second centrifugation to determine the conditions required for pollen elimination because its previous "history" modified the adhesion complex.

We have therefore determined standard conditions for the estimation of adhesion. In Figure 2D (presented here as a scanning electron microscope image for aesthetic reasons, but routinely observed with a binocular microscope), the stigmas centrifuged at 2500*g* after 10 min of pollination we consider depollinated superficially, corresponding to the force needed to release the pollen grains ($4.9 \times 10^{-9}$ N). We performed 20 independent repetitions of the procedure. The sigmoidal curve of pollen release and the plateau finally reached indicate that an accurate estimation of the adhesion forces can be obtained using 500*g* increments (Fig. 3).

## Kinetics of Pollen-Stigma Adhesion: Effects of SI/SC Genotypes

The initial adhesion, measured after the delay necessary to pollinate a pistil and to dip it rapidly in the 2% formalin-50% Suc solution (i.e. few seconds), was high for all crosses. However, when the $S_{29}$ line was used as the pollinator, the initial adhesion was found to be significantly lower than in other pollinations, both in self-pollinations and in cross-pollinations with other lines as female partners. This prompted us to systematically measure the initial adhesion in several reciprocal crosses for specific effects of male pollinators, female recipients, the *S*-genotype, and the genetic background (Table II). The variance analysis of male and female factors indicated strongly that differences exist between pollinators ($P < 0.01$; $F_{(5, 30)} = 20.4$); the line $S_{29}$ has pollen that adheres much less than that of other genotypes. No significant differences were found among female recipients ($P > 0.01$; $F_{(5, 30)} = 0.24$).

The change of pollen-stigma adhesion forces as a function of time after pollination was analyzed for several pairs of *B. oleracea* lines. A significant increase of adhesion with time was observed in all pollinations. Variations in the adhesion depended on the genotypes involved in the pollinations, but no correlations could be established with either the self- or cross-status of the pollinations, or on the self-compatible or -incompatible genotypes of the plants used (Fig. 4).

After the initial contact of the pollen with the stigma surface, the adhesion increased and reached a maximum after approximately 1 h. This increase was significant for all of the pollinations analyzed: only the self-pollination *CGS43* × *CGS43* (self-compatible) showed a period of constant adhesion for 10 min, before the increase in pollen retention. In all other cases the adhesion forces had signif-

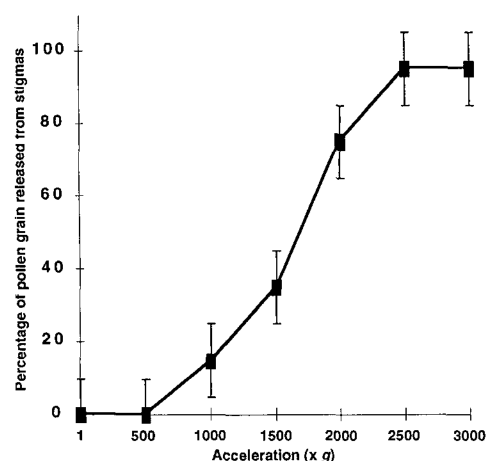


**Figure 3.** Sigmoidal curve of pollen grain release. For each acceleration, 20 $S_{29}$ flowers were self-pollinated for 10 min and centrifuged, and the number of stigmas totally devoid of superficial pollen grains was determined.

**Table II.** *Genotypical effects on initial adhesion*

Variations of the initial value of pollen-stigma adhesion are expressed in Newtons ($\times 10^{-9}$) and in *g* values for various reciprocal crosses.

| Male | $S_{29}$ | *RC-Si* | *P57-Si* | *RC-Sc* | *P57-Sc* | *CGS43* |
|---|---|---|---|---|---|---|
| Female | | | | | | |
| $S_{29}$ | 1.96–1000*g* | 4.90–2500*g* | 9.81–5000*g* | 3.92–2000*g* | 6.86–3500*g* | 4.90–2500*g* |
| *RC-Si* | 2.94–1500*g* | 7.84–4000*g* | 7.84–4000*g* | 4.90–2500*g* | 8.82–4500*g* | 5.88–3000*g* |
| *P57-Si* | 3.92–2000*g* | 5.88–3000*g* | 7.84–4000*g* | 6.86–3500*g* | 7.84–4000*g* | 4.90–2500*g* |
| *RC-Sc* | 2.94–1500*g* | 4.90–2500*g* | 10.78–5500*g* | 5.88–3000*g* | 7.84–4000*g* | 3.92–2000*g* |
| *P57-Sc* | 3.92–2000*g* | 6.86–3500*g* | 8.82–4500*g* | 6.86–3500*g* | 6.86–3500*g* | 5.88–3000*g* |
| *CGS43* | 1.96–1000*g* | 4.90–2500*g* | 7.84–4000*g* | 7.84–4000*g* | 6.86–3500*g* | 3.92–2000*g* |

icantly increased by 5 min after the initial capture of the pollen grains. These results suggest very rapid physical and/or chemical modifications of the interacting surfaces that do not depend on the self-(in)compatibility status of the plants.

In the case of the $S_{29} \times CGS43$ pair, a significant drop in adhesion was observed 1 h after pollination. This unexpected decrease occurred at the time of germination of many pollen tubes, as shown by scanning electron microscopy (Fig. 5). The growth of turgid tubes meant that many pollen grains were lifted up from the papillae. The menisci formed between pollen coating and pellicle were therefore disrupted and the pollen grains remained anchored to the stigma only by their growing tubes. It is evident from our observations that such anchorage is weaker than the attachment provided by the pollen-stigma menisci, explaining the decrease of adhesion observed in this compatible cross. Many other compatible pollinations were found to behave in this way, provided the humidity at the time of pollination was adequate (i.e. pollinations had to be performed in the greenhouse rather than in the laboratory, where centrifugations and microscopic observations were carried out). In contrast, the incompatible crosses, performed with early-acting *S*-genotypes, never showed this sudden drop in pollen adhesion during the time course of pollination, confirming that the reduction of pollen retention is correlated with the active germination of pollen tubes in compatible crosses.

The developmental stage of the stigma was also found to have a major effect on the initial adhesion of the pollen grains. In the case of *CGS43* self-pollinations, four stages of flower maturation were analyzed: closed buds about to open, buds with petals beginning to appear between the sepals, freshly opened flowers, and flowers that had been open for 1 d. The corresponding accelerations required to release pollen from these pistils increased with their developmental stage and were, respectively, 2000*g* ($3.9 \times 10^{-9}$ N), 3000*g* ($5.9 \times 10^{-9}$ N), 3500*g* ($6.9 \times 10^{-9}$ N), and 4000*g* ($7.8 \times 10^{-9}$ N) for the four stages. This is in agreement with the increase of the stigmatic waxes described during flower maturation (Roggen, 1972, 1975).

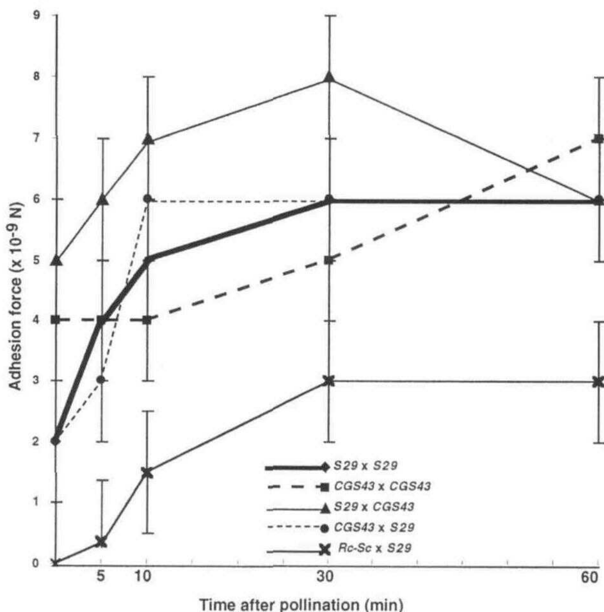


**Figure 4.** Kinetic evolution of pollen adhesion in some lines after self- and cross-pollinations. The first point was taken after a few seconds. In the cross *Rc-Sc* $\times$ $S_{29}$ the $S_{29}$ pollen had been delipidated with cyclohexane.

## The Role of Lipids in Adhesion

Several biochemical components of the meniscus formed at the contact of pollen grains and stigmas have been implicated in adhesion (see the introduction). We analyzed

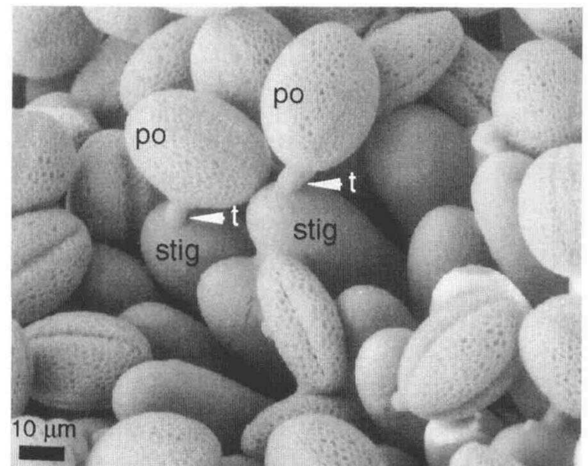


**Figure 5.** Pollen grains lifted from the stigmatic surface by germination of their pollen tubes in the cross $S_{29}$ $\times$ *CGS43* 1 h after pollination. po, Pollen grain; t, pollen tube; stig, stigmatic papillae.

**Table III.** *Pollen-stigma adhesion in A. thaliana cer mutants*
Data from previously published works.

| Plant | Effect of *cer* Mutation on Coating of Pollen Grain | Reference |
|---|---|---|
| *cer3* | Lipid drops in the tryphine with no obvious difference from the wild type | Hülskamp et al. (1995) |
| *cer2* | Lipid drops in the tryphine reduced in number and size | Preuss et al. (1993) |
| *cer6-1* | Lipid drops in the tryphine reduced in size | Preuss et al. (1993) and Hülskamp et al. (1995) |
| *cer1* | No detectable lipid drops in the tryphine layer | Hülskamp et al. (1995) |

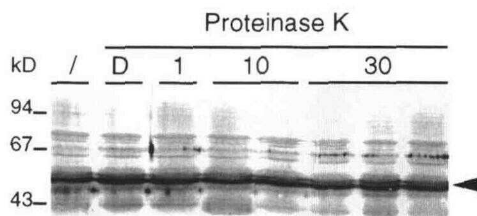


**Figure 6.** Immunodetection of SLR1 proteins. Western analysis (SDS-PAGE) of soluble proteins extracted from stigmas after proteinase K treatment. The arrow points to SLR1 proteins. /, Untreated; D, digestion with denatured proteinase K; 1, 10, and 30, 1, 10, and 30 min of proteinase K digestion.

particularly the role of components of the pollen coating and of the stigmatic pellicle.

The adhesion was found to be dramatically reduced after elimination of the pollen coating with organic solvents. The pollen grains adhering at the tips of stigmatic papillae were detached at accelerations as low as 50 to 100*g* ($7.5$ to $15 \times 10^{-11}$ N). However, after the initial contact, an increase in pollen-stigma adhesion could be observed, reaching considerable intensity after 30 to 60 min of contact (Fig. 4) and demonstrating that the stigmatic pellicle is important in the interaction between the stigma and pollen grains devoid of their coatings.

To address the question of the involvement of lipids in the pollen coat (tryphine) in adhesion, we investigated pollen-stigma adhesion measurements with *cer* wax mutants of *A. thaliana*. Because of the small size of the Arabidopsis flowers, the experimental device (Fig. 1B) could not be used with only one flower per tube, so observations were carried out on whole pollinated inflorescences fixed to the bottom of the 1.5-mL tube. The pollen-stigma adhesion for *cer1*, *cer2*, and *cer6–1* mutants was found to be significantly weaker than that of the wild type or *cer3* mutants. For wild type and *cer3*, the adhesion increased as a function of time after pollination, whereas for the other mutants, the adhesion was constant, suggesting a reduced interaction between the pollen grains and the stigmatic papillae (Tables III and IV).

## Effect of Water Treatment on the Stigmatic Pellicle

As a control for enzymic treatments of the stigmatic surface (see next paragraph), pistils were immersed in distilled water or in Tris solutions (20 mM, pH 7.5) for 10 min, allowed to dry by simple evaporation (10 min), and pollinated as usual. This simple water treatment of the pistils considerably increased the initial adhesion of pollen to the stigmas, at least doubling the centrifugation forces required to release the pollen grains.

Videomicroscopic analysis of the pollen grains in contact with prewetted pistils showed an almost immediate hydration after capture, since the major/minor axis ratio observed (1.3) was close to that of completely hydrated pollen grains. However, the adhesion forces did not increase further as observed for pollinations on dry stigmas. The usual pollination sequence could be observed only if the pistils were allowed to recover for 2 h after water treatment.

**Table IV.** *Pollen-stigma adhesion in A. thaliana cer mutants*
Adhesion is given in Newtons; *g* values are given in parentheses. Density = 1.15.

| Plant | Pollen-Stigma Adhesion | | |
|---|---|---|---|
| | Few seconds | 10 min | 30 min |
| Wild type | 1.76 (900) | 1.76 (900) | 2.55 (1300) |
| *cer3* | 1.37 (700) | 1.76 (900) | 2.16 (1100) |
| *cer2* | 0.39 (200) | 0.39 (200) | 0.39 (200) |
| *cer6-1* | 0.39 (200) | 0.39 (200) | 0.39 (200) |
| *cer1* | 0.39 (200) | 0.39 (200) | 0.39 (200) |

## Role of Proteins of the Stigma Pellicle

The surface of the stigmas (the *CGS43* line) was digested by dipping the pistils in proteinase K. We observed a considerable reduction in the initial adhesion of pollen on the digested pistils: adhesion forces resisted 4500*g* accelerations ($8.8 \times 10^{-9}$ N) for untreated wetted stigmas and only 2500*g* ($4.9 \times 10^{-9}$ N) for proteinase K-treated stigmas (nonwetted control stigmas resisted 2000*g* [ $3.9 \times 10^{-9}$ N]) (see Table II).

In parallel, we checked the status of SLR1 and SLG proteins after proteolytic treatments. When proteinase K activity was inhibited by $Ca^{2+}$ chelation using EGTA (Sambrook et al., 1989) before extraction, no degradation, signal reduction, or even slight smearing of SLG or SLR1 bands on western blots was observed using specific antibodies against SLG or SLR1 proteins (Fig. 6). Also in parallel, clear tissue printings were obtained with these antibodies on polyacrylamide gels using pistils from which part of the stigmas had been removed with a scalpel blade. In contrast, with printings made with intact pistils, we could not detect the proteins. We suggest that SLG and SLR1 glycoproteins do not diffuse freely through the pellicle at an appreciable rate and do not participate in the initial phase of pollen-stigma adhesion, in contrast to other stigma peptides accessible to the protease and significantly affected in their adhesive function by its action.

## DISCUSSION

### Pollen-Stigma Adhesion Is Independent of *S*-Phenotype

Our observations do not support potential correlations between pollen-stigma adhesion and the self-(in)compatibility phenotype of pollen acceptance or rejection. We showed that the line $S_{29}$ of *B. oleracea* var. *alboglabra* produces pollen with significantly lower adhesion potentials than the other lines analyzed, but this effect was observed in self-incompatible as well as in cross-compatible pollinations and so is not linked to the *S* incompatibility haplotype itself, but rather to other aspects of the genetic background of the $S_{29}$ plant. Adhesion itself is not *S*-specific, at least during the first stages of pollen-stigma interaction including contact and hydration. After germination in compatible crosses, the pollen grains are lifted from the stigma surface but remain anchored to the pistil through their turgid pollen tubes.

In contrast to the classical assumption that germinating pollen grains are firmly fixed to the stigma, we measured an attachment force that was significantly reduced compared with the adhesion of the pollen through the meniscus formed by the pollen coat and the stigmatic pellicle. From a biological point of view, it must be remembered that at this stage of pollination, the male gametes are already safely inside the stigma, moving toward the ovules in the pollen tube tip and isolated from the dying (or dead) pollen grain envelope by a succession of callose plugs. Our results thus contradict the conclusions previously reported by Roggen (1972), Stead et al. (1979), and Kerhoas et al. (1983) that pollen-stigma adhesion is stronger in compatible pollinations than in incompatible ones.

Roggen (1972) observed that following incompatible pollinations, more pollen grains became detached from the papillae than in compatible pollinations. He presented scanning electron microscope images of compatible pollinated stigmas bearing scars after detachment of the pollen grains, and complementary marks on these detached grains, apparently caused by the broken contact points of coalescence between the pollen wall and the papilla cuticle. The scars and marks were absent in self-incompatible crossings, indicating less recognition. Roggen neither illustrated the traces of incompatible pollen-stigma contacts nor explained how he had been able to estimate their increased number compared with the compatible situation. In our experiments the number of pollen grains forming the monocellular layer at the surface of the stigma papillae was estimated by recovery and counting at about 800 for buds about to open and 1500 for flowers opened for 36 h.

We were not able to see any mark at the tips of the papillae after removal of the pollen grains by our flotation procedure, although there should be hundreds of them on a single stigma. In contrast, when pistils pollinated with delipidated pollen were observed, we noticed that even after prolonged contact, the pollen grains were not firmly attached to the stigma surface and were frequently removed in the microscope itself during freeze drying of the samples by the beam of electrons (Fig. 7). Many scars were evident under these circumstances, but they appeared much lighter than those presented by Roggen, since the pollen coating removed by delipidation did not coalesce with the papillar stigma surface. It seems to us that scars and marks appear only when pollen grains are removed from stigmas in the solid state, after fixation and/or freezing of the samples, but not when they are detached from the stigma surface in its in vivo dynamic state, which allows remodeling of the pellicle and cuticle.

In the experiments undertaken by Stead et al. (1979), adhesion was estimated by a hydrodynamic method whereby pollinated stigmas were stirred with a magnetic bar in mannitol solutions (the laws of hydrodynamics are more complex and this test is thus more prone to artifacts than the simple hydrostatic test used in the present work). The pollen grains released after three steps of progressive stirring were counted and relative adhesion forces were estimated from the ratio of pollen counts released at the three steps. It was concluded that stronger pollen adhesion occurs in cross-pollinations than in self-pollinations, whether self-compatible or self-incompatible, which seemed paradoxical to the authors and was not confirmed in later work by the group.

Kerhoas et al. (1983) counted as adhering the pollen grains remaining attached to stigmas following fixation with FAA starting 6 to 24 h after pollination. In fact, their conditions did not strictly take into account only the stage of pollen adhesion itself but also included those of hydration, germination, and anchoring into the stigma surface, which are of course dependent on self-compatibility. Therefore, positive correlations were shown between self-compatibility and adhesion defined in this way. We have shown previously (Luu et al., 1997) that pollinated pistils treated with FAA were completely depleted of pollen grains, whether compatible or incompatible, if fixation occurred before any significant germination (i.e. during the first 30 min after pollination); in contrast, if fixation began after germination, the status of compatibility/incompatibility was found to be dependent on the number of pollen grains remaining attached to the stigma surface.

The results obtained using the simple hydrostatic technique presented herein are largely artifact-free and include observations from a significant number of genotypes and crosses. We are therefore confident in our conclusion that

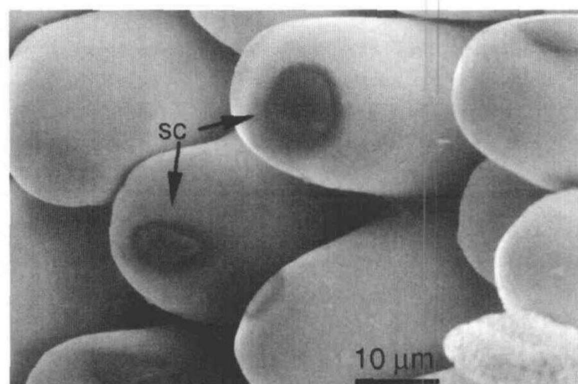


**Figure 7.** Scars (sc) on the papillae after the removal of delipidated pollen grains in the scanning microscope by the beam of electrons.

self-incompatible recognition and rejection of the pollen are not made at the stage of early adhesion onto the stigmatic pellicle.

### Dissection of the Components and of the Kinetics of Pollen-Stigma Adhesion

Our observation of the primary importance of the pollen coating for pollen adhesion is in agreement with previous reports (Jain and Shivanna, 1988; Doughty et al., 1993). The very low initial adhesion forces observed using delipidated pollen grains and the reduced adhesion of the *cer* mutant pollen confirmed these data. We obtained a remarkable correlation between the reduction of lipidic droplets of the tryphine described for the *cer* mutants and the decrease in their pollen-stigma adhesion (Tables III and IV). We do not understand, however, why the adhesion of the *cer* pollen grains did not evolve at all after the first contact with the stigmas of *A. thaliana*, whereas in the case of *B. oleracea*, the cyclohexane-treated pollen showed some increase in adhesion.

The developmental gradient of initial pollen adhesion along the inflorescence demonstrates the participation of stigmatic molecules produced during flower maturation. Roggen (1972) correlated the increase of wax bumps with aging (and hypothesized that waxes could play a role in the acquisition of self-incompatibility during bud maturation). We observed a similar increase of waxes on the papillar surface during development, but found that wax accumulation occurred much earlier in some lines ($S_{29}$) than in others (*CGS43*). In addition, the evolution of the adhesion of delipidated pollen grains after the initial contact shows the dynamic role of the stigmatic surface in pollen adhesion. The reduced adhesion observed following digestion of pellicular proteins with proteinase K confirmed this, and is in agreement with similar observations by Stead et al. (1980). SLR1 and SLG proteins resisted protease action, probably because they were not accessible to the enzyme, being mainly located in the cell wall but not in the pellicle itself. In this respect, the SLG proteins have been immunolocalized essentially in the stigma cell wall (Kandasamy et al., 1989, 1991).

Previous results in our laboratory (Luu et al., 1997) showed that SLR1 proteins participate in pollen adhesion as soon as 10 min after pollination. We therefore propose three stages in the kinetics of adhesion: during the initial stage following pollen capture, neither SLR1 proteins nor any of the self-incompatibility elements would play any role because they are not accessible to pollen components; a second stage (from a few minutes to about 30 to 60 min after initial contact) showing increasing adhesion and beginning when the formation of the pollen-stigma meniscus allows the coalescence of male and female surface components, including the participation in adhesion of secreted SLR1 and possibly SLG glycoproteins (Luu et al., 1997); and a third stage (about 60 min after initial pollination) when incompatible pollen is recognized and rejected after interaction between the pollen signal (still unidentified) and the transmembrane *S*-receptor protein-kinase.

These stages coincide with those defined on the basis of pollen hydration (Dickinson, 1995), including an initial variable lag phase during which hydraulic continuity is established between pollen and stigma, a second phase involving rapid transfer of water to the grain, and, finally, a third phase of pollen tube germination in cases of compatible pollination. During the first two stages, selfed and crossed pollen grains behave similarly and are not easily distinguished morphologically, both forming a pollen grain "foot" resulting in conversion and flow of the dry coating over the stigmatic surface and profound reorganization of both surfaces and establishment of bonds between them (Elleman and Dickinson, 1986, 1990). Quantitative measurements of pollen axis ratios indicate that compatible pollen hydrates more extensively (Zuberi and Dickinson, 1985). A clear distinction between SI and SC pollinations is evident only during stage III, when self-incompatible pollen tubes are arrested by the stigma.

### Biological Significance of Pollen-Stigma Adhesion

Taken as a whole, these results indicate that the various elements of the sporophytic SI system are not present at the immediate surfaces of the male and female partners (the EOL/CSL and the pellicle). Pollen-stigma adhesion is not under the control of the *S*-locus, but allows close contact between the pollinic signals and stigmatic receptors probably located deeper in the cell walls and/or plasma membranes, and their subsequent translocation and functioning in rejecting or accepting the selfed or crossed pollen grains.

Taking into account the mass of *B. oleracea* pollen grains ($6.4–7.2 \times 10^{-9}$ g depending on RH), the adhesion forces demonstrated in this work can be broken by accelerations of about 80 to 200*g* for superficial pollen grains and perhaps 10 times more for those trapped between papillae. From the biological point of view, these are huge accelerations, confirming that pollen adhesion counteracts any natural force (e.g. wind, gravity, or rubbing by insects) that could oppose pollination (Woittiez and Willemse, 1979). In the case of *A. thaliana*, the adhesion is lower than in most *Brassica* lines. This feature might be related to the fact that in Arabidopsis pollination occurs before anthesis in the protected environment of closed flower buds.

Interspecific incompatibility rejection among Brassicaceae and, more specifically, unilateral incompatibility, has been shown to be largely predictable on the basis of the self-incompatible phenotype of interacting partners (Lewis and Crowe, 1958; Hiscock and Dickinson, 1993), suggesting that the *S*-locus might also be implicated in the genetic control of interspecific pollinations. We have analyzed some interspecific crosses and have shown that interspecific pollen-stigma adhesion forces are very similar to those of intraspecific pollinations in the species *B. oleracea*, irrespective of the self-incompatibility status of the species concerned. This confirms the idea that pollen-stigma adhesion has co-evolved with the biological structure of the interacting partners, but has no specific function in sexual compatibilities and pollen rejection.

ACKNOWLEDGMENTS

The scanning electron microscope observations were made at the microscopy center of the University of Lyon (CMEABG). The anti-SLR1 serum was kindly provided by Dr T. Gaude. We would like to thank R. Blanc, H. Leyral, B. Moyroud, and E. Ferreira for technical assistance. D-T.L. received financial support from the Ministery of Education, P.H. by the Centre National de la Scientifique Recherche, and C.D. by the Ecole Normale Supérieure de Lyon.

Received May 16, 1997; accepted July 24, 1997.
Copyright Clearance Center: 0032-0889/97/115/1221/10.

## LITERATURE CITED

**Clarke AE, Gleeson P, Harrison S, Knox RB** (1975) Pollen-stigma interactions: identification and characterization of surface components with recognition potentials. Proc Natl Acad Sci USA **76:** 3358–3362

**Dickinson H** (1995) Dry stigmas, water and self-incompatibility in *Brassica*. Sex Plant Reprod **8:** 1–10

**Dickinson HG, Elleman CJ** (1985) Structural changes in the pollen grain of *Brassica oleracea* during dehydration in the anther and development on the stigma as revealed by anhydrous fixation techniques. Micron Microscopia Acta **16:** 255–270

**Doughty J, Hedderson F, McCubbin A, Dickinson H** (1993) Interaction between a coating-borne peptide of the *Brassica* pollen grain and stigmatic-S (Self-Incompatibility)-Locus-Specific Glycoproteins. Proc Natl Acad Sci USA **90:** 467–471

**Elleman CJ, Dickinson HG** (1986) Pollen-stigma interaction in *Brassica*. IV. Structural reorganisation in the pollen grains during hydration. J Cell Sci **80:** 141–157

**Elleman CJ, Dickinson HG** (1990) The role of the exine coating in pollen-stigma interactions in *Brassica oleracea* L. New Phytol **114:** 511–518

**Elleman CJ, Dickinson HG** (1994) Pollen-stigma interaction during self-incompatibility in *Brassica oleracea*. *In* EG Williams, RB Knox, AE Clarke, eds, Genetic Control of Self-Incompatibility and Reproductive Development in Flowering Plants. Kluwer Academic Publishers, Dordrecht, The Netherlands, pp 67–87

**Elleman CJ, Dickinson HG** (1996) Identification of pollen components regulating pollen-specific responses in the stigmatic papillae of *Brassica oleracea*. New Phytol **133:** 197–205

**Ferrari TE, Wallace DG** (1975) Germination of *Brassica* pollen and expression of incompatibility *in vitro*. Euphytica **24:** 757–765

**Gaude T, Dumas C** (1984) A membrane-like structure on the pollen wall surface in *Brassica*. Ann Bot **54:** 821–825

**Gaude T, Dumas C** (1986) Organization of stigma surface components in *Brassica*: a cytochemical study. J Cell Sci **82:** 203–216

**Gaude T, Dumas C** (1987) Molecular and cellular events of self-incompatibility. Int Rev Cytol **107:** 333–366

**Gaude T, Friry A, Heizmann P, Mariac C, Rougier M, Fobis I, Dumas C** (1993) Expression of a self-incompatibility gene in a self-compatible line of *Brassica oleracea*. Plant Cell **5:** 75–86

**Gaude T, Nasrallah ME, Dumas C** (1988) Use of monoclonal antibodies to determine putative S products of pollen in *Brassica oleracea*. Heredity **61:** 317–318

**Heslop-Harrison Y, Shivanna KR** (1977) The receptive surface of the angiosperm stigma. Ann Bot **42:** 1233–1258

**Hiscock SJ, Dickinson HG** (1993) Unilateral incompatibility within the Brassicaceae: further evidence for the involvement of the self-incompatibility (S)-locus. Theor Appl Genet **86:** 744–753

**Hodgkin T, Lyon GD, Dickinson HG** (1988) Recognition in flowering plants: a comparison of the *Brassica* self-incompatibility system and plant pathogen interactions. New Phytol **110:** 557–569

**Hülskamp M, Kopczak SD, Horejsi TF, Kihl BK, Pruitt RE** (1995) Identification of genes required for pollen-stigma recognition in *Arabidopsis thaliana*. Plant J **8:** 703–714

**Ikeda S, Nasrallah JB, Dixit R, Preiss S, Nasrallah M** (1997) An aquaporin-like gene required for the *Brassica* self-incompatibilty response. Science **276:** 1564–1566

**Jain A, Shivanna KR** (1988) Storage of pollen grains in organic solvents: effects of organic solvents on leaching of phospholipids and its relationship to pollen viability. Ann Bot **61:** 325–330

**Kandasamy MK, Paolillo DJ, Faraday CD, Nasrallah JB, Nasrallah ME** (1989) The *S*-locus specific glycoproteins of *Brassica* accumulate in the cell wall of developing stigma papillae. Dev Biol **134:** 462–472

**Kandasamy MK, Parthasarathy MV, Nasrallah ME** (1991) High pressure freezing and freeze substitution improve immunolabeling of *S*-locus specific glycoproteins in the stigma papillae of *Brassica*. Protoplasma **162:** 187–191

**Kerhoas C, Knox RB, Dumas C** (1983) Specificity of the callose response in stigmas of *Brassica*. Ann Bot **52:** 597–602

**Knox RB, Heslop-Harrison J, Reed C** (1970) Localisation of antigens associated with the pollen grain wall by immunofluorescence. Nature **225:** 1066–1068

**Lewis D, Crowe LK** (1958) Unilateral incompatibility in flowering plants. Heredity **12:** 233–256

**Luu D-T, Heizmann P, Dumas C, Trick M, Cappadocia M** (1997) Involvement of *SLR1* genes in pollen adhesion to the stigmatic surface in Brassicaceae. Sex Plant Reprod **10:** 227–235

**Mattsson O, Knox RB, Heslop-Harrison J, Heslop-Harrison Y** (1974) Protein pellicle of stigmatic papillae as a possible recognition site in incompatibility reactions. Nature **247:** 298–300

**Nasrallah JB, Nishio T, Nasrallah ME** (1991) The self-incompatibility genes of *Brassica*: Expression and use in genetic ablation of floral tissues. Annu Rev Plant Physiol **42:** 393–422

**Preuss D, Lemieux B, Yen G, Davis RW** (1993) A conditional sterile mutation eliminates surface components from *Arabidopsis* pollen and disrupts cell signaling during fertilization. Gene Develop **7:** 974–985

**Roggen HP** (1972) Scanning electron microscopical observations on compatible and incompatible pollen-stigma interactions in *Brassica*. Euphytica **21:** 1–10

**Roggen HP** (1975) Stigma application of an extract from rape pollen (*Brassica napus*) effects self-incompatibility in Brussels sprouts (*Brassica oleracea* L. *var. gemmifera* DC). Incompat Newslett **6:** 80–83

**Ross JHE, Murphy, DT** (1996) Characterization of anther-expressed genes encoding a major class of extracellular oleosin-like proteins in the pollen coat of Brassicaceae. Plant J **9:** 625–637

**Ruffio-Chable V, Herve Y, Dumas C, Gaude T** (1997) Distribution of *S*-haplotypes and relationship with self-incompatibility in *Brassica oleracea*. 1. In inbred lines of cauliflower (*B. oleracea*. var. *botrytis*). Theor Appl Genet **94:** 338–346

**Sambrook J, Fritsch EF, Maniatis T** (1989) Molecular Cloning: A Laboratory Manual, Vol 3, Ed 2. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, p B16

**Stead AD, Roberts IN, Dickinson HG** (1979) Pollen-pistil interaction in *Brassica oleracea*: events prior to pollen germination. Planta **146:** 211–216

**Stead AD, Roberts IN, Dickinson HG** (1980) Pollen-stigma interaction in *Brassica oleracea*: the role of stigmatic proteins in pollen grain adhesion. J Cell Sci **42:** 417–423

**Woittiez RD, Willemse MTM** (1979) Sticking of pollen on stigmas: the factors and a model. Phytomorphology **29:** 57–63

**Zuberi MI, Dickinson HG** (1985) Pollen-stigma interaction in *Brassica*. III. Hydration of the pollen grains. J Cell Sci **76:** 321–336