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Pollen-Pistil Interaction in *Linum grandiflorum:* Stigma-surface Proteins and Stigma Receptivity

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Stigma-surface proteins in *Linum grandiflorum*, a dimorphic taxon, were present at all the stages of development examined (0-3 days before anthesis). Coomassie blue staining material was found only on the surface of the thrum stigma. Non-specific esterases were found on the stigmas of both the forms. Stigmas of all stages supported good germination of compatible pollen, but pollen tubes reached the ovary only in pistils on the day of anthesis and 1 day before anthesis. The responses of incompatible pollinations were similar in buds of all stages. Bud pollinations were ineffective in overcoming intramorph-incompatibility.

Intramorph thrum pollinations permitted pollen germination, but pollen tubes were inhibited in the stigma. Following intramorph pin pollinations, although a majority of the pollen grains failed to adhere to the stigma, the response of the few that did adhere was similar to that following intramorph thrum pollinations. It is inferred that physiological mechanisms, similar to those operating in homomorphic systems, are also involved in pollen tube inhibition in this taxon.

Key Words: *Linum grandiflorum*, **Bud pollination, Heteromorphic incompability, Pollen-pistil interaction, Stigma receptivity, Stigma-surface esterases**

Introduction

Recent investigations on pollen-pistil interaction have revealed the presence of extracellular proteins on the surface of the stigma (Mattsson et al. 1974, Heslop-Harrison et al. 1975, Y Heslop-Harrison & Shivanna 1977). There is considerable evidence to indicate that stigma-surface proteins are involved in stigma receptivity and incompatibility reactions (Heslop-Harrison 1978, Heslop-Harrison & Y Heslop-Harrison 1975, Knox et al. 1976, Shivanna 1979, Shivanna et al. 1978). However, investigations along these lines have been thus far largely confined to homomorphic systems. Studies on heteromorphic systems have been limited to the morphological differences of

the stigmas and pollen grains of the various forms (Dulberger 1974, 1975 a, b) and the behaviour of pollen on the stigmas of mature pistils (Lewis 1943, Baker 1966, Dickinson & Lewis 1974). In this paper, we report some of our observations on stigma-surface proteins and stigma receptivity, at different stages of development, in a dimorphic taxon, *Linum grandiflorum*.

Material and Methods

Investigations were carried out on flowers of both thrum and pin morphs from field grown plants of *Linum grandiflorum* Desf. Mature as well as young pistils at various stages of development were used.

Stigma-surface proteins were localized by staining the stigmas with 0.25% coomassie brilliant blue R for 10 min. Non-specific esterases on the stigma surface were localized by using α-naphthyl acetate as the substrate in a coupling reaction with fast blue B (Mattsson et al. 1974).

For all experiments involving pollen germination and pollen tube growth, flowers were field-pollinated as it was observed that pollen tube growth in pistils excised and implanted on 1% agar in petri plates, was not normal. Where mature pistils were used, the buds were emasculated and bagged the evening before anthesis and pollinated the subsequent morning. For bud pollinations, buds were emasculated at the time of pollination. In all pollinations fresh pollen from just-dehisced anthers was used. To study pollen germination and pollen tube growth, pollinated pistils were fixed in acetic-alcohol (glacial acetic acid: ethanol, 1 : 3, v/v) 0.5, 1, 2, 4, 8 and 24 hr after pollination, cleared in 8N NaOH for 8-10 hr and washed in water to remove all traces of the alkali. The pistils were then stained in 0.005% decolourised aniline blue in 0.05M Na_2HPO_4, *p*H 8.2, and observed under a Reichert Zetopan-Binolux fluorescent microscope using exciter filter No. 2 and absorption filter No. 1.

For bud pollinations, pistils at 3 stages of development, i.e., 24, 48 and 72 hr before anthesis (corresponding to bud lengths of 15-16, 11-12, and 8-9 mm respectively) were used. Sixteen pollinations were carried out on buds of each stage (in 2 replicates of 8 each); of these 6 were fixed 8 hr after pollination and processed as above to observe pollen germination and pollen tube growth, while the remaining 10 were left on the plant to record seed set.

Observations

Stigma-surface proteins

Coomassie blue staining material was observed only on the surface of thrum (short styled) stigma papillae. Though present at all stages of development, the intensity of staining was highest on mature stigmas. Coomassie blue staining material was absent from the pin (long styled) stigma surface at all stages examined. On the other hand, non-specific esterases were found on the stigma surface of both morphs in the form of a distinct pellicle (figures 1A-D). The intensity of esterase activity increased with the maturity of the stigma but could be detected even in the youngest buds. At maturity, esterase activity appeared to be somewhat higher in the thrum morph as compared to the pin morph. In addition, esterases were more intense towards the papilla tip on thrum stigmas but were more evenly distributed on pin stigmas (figures 1C, D). The pellicle at the tip of the thrum papilla was raised and ruptured at places because of the accumulation of secretion products between the pectocellulosic wall and the pellicle (figure 1C).

Receptivity of mature stigma

On mature compatibly (intermorph) pollinated pistils, pollen grains germinated within 30 min and pollen tubes could be observed in the style 1 hr after pollination (figure 2A).

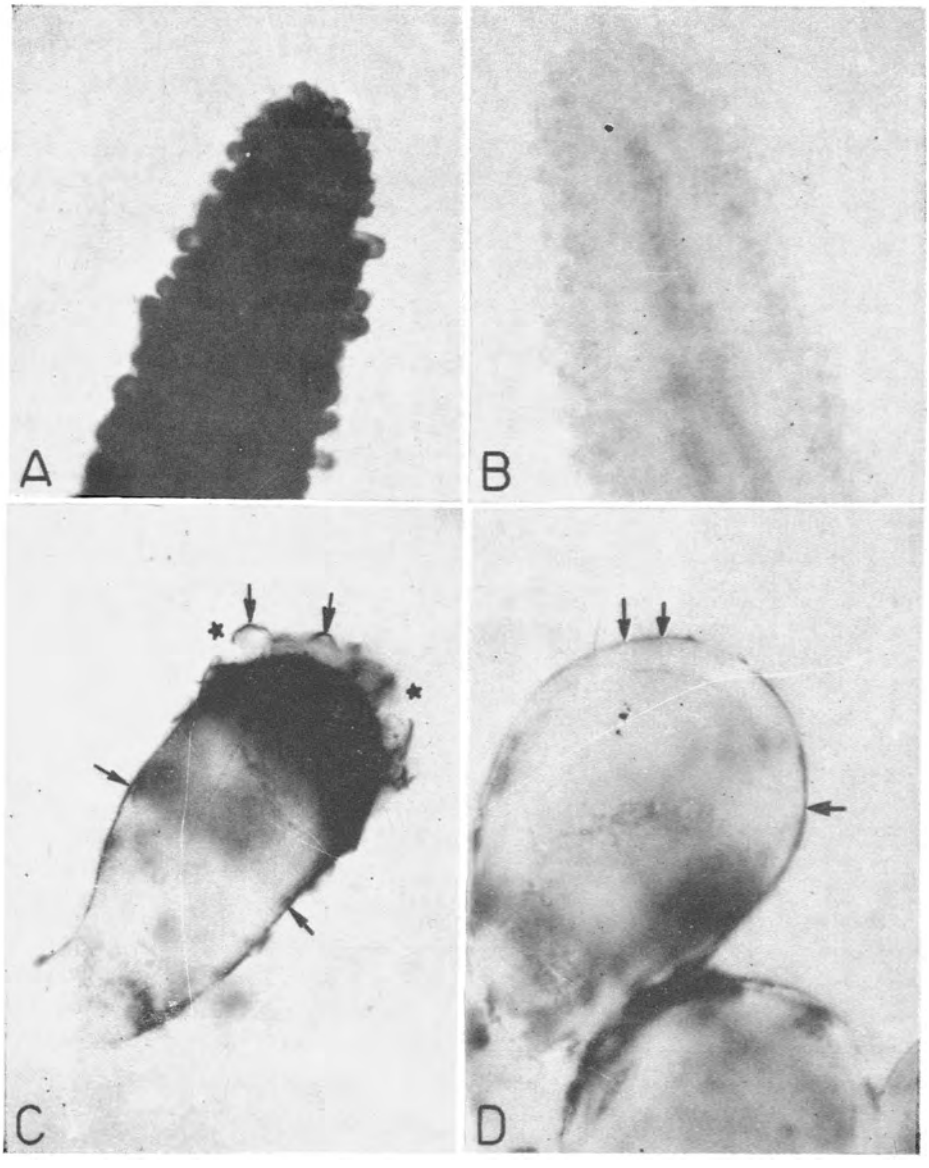

Figure 1 Cytochemical localization of non-specific esterases on the stigma-surface in *Linum grandiflorum*. *A*, Part of thrum stigma treated with substrate; *B*, Similar to *A* but treated without substrate (control for *A*). Surface esterases are apparent in *A*; *C,D* Individual papillae from thrum (*C*) and pin (*D*) stigmas treated with substrate. Pellicle (arrows) is clear in both the papillae. In *C*, surface esterase activity is more intense towards the tip; pellicle layer at the tip is raised and ruptured at places (*) because of the accumulation of secretion products just below the pellicle. A, B(×ca. 105); *C*, *D*(×ca. 1000).

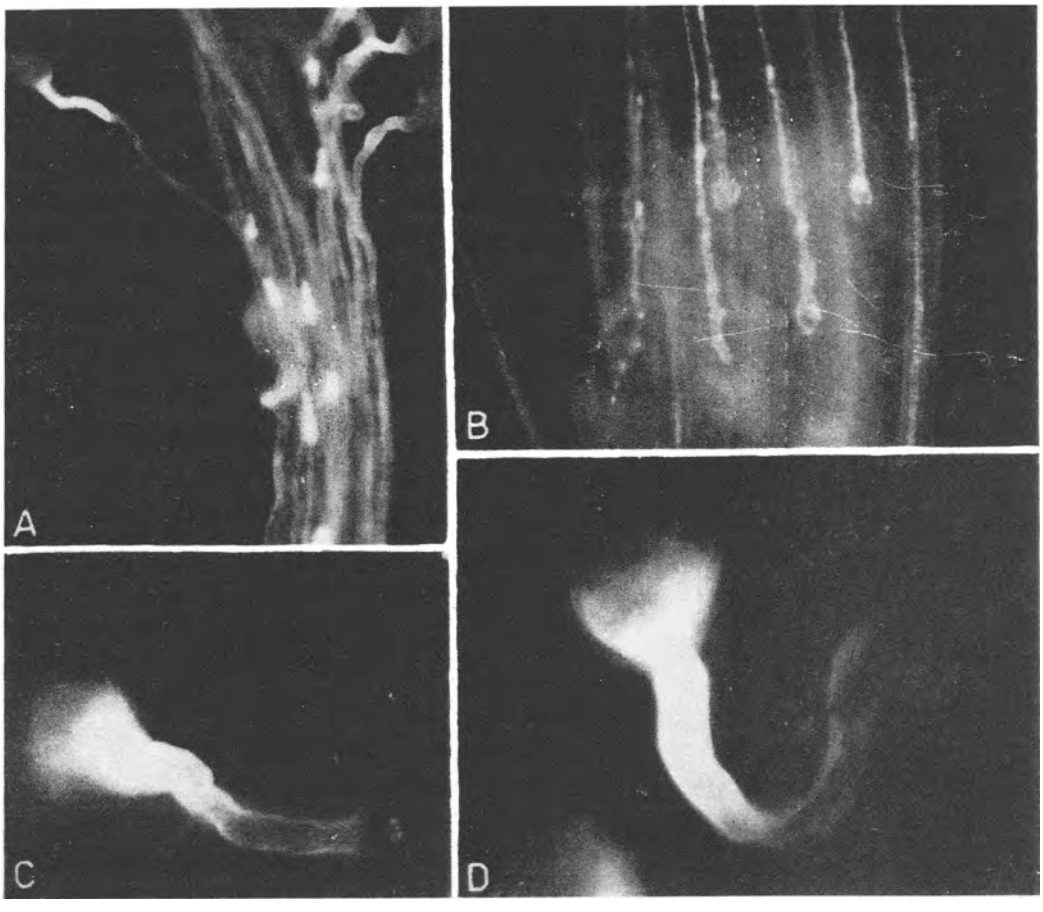

Figure 2 Flourescent micrographs of pistils 8 hr after pollination following staining with aniline blue. *A*, Part of a mature thrum stigma and style following compatible pollination. Observe normal growth of pollen tubes; *B*, Part of a thrum style compatibly pollinated 48 hr before anthesis. Pollen tube growth is arrested in the style. Many of the tubes show swollen tips; *C*, Part of incompatibly pollinated pin stigma (pin × pin). One of the pollen grains which had adhered to the stigma is shown; *D*, Part of incompatibly pollinated thrum stigma (thrum × thrum). Only one pollen grain is shown. In both *C* and *D* the pollen tube is inhibited soon after entering the stigma. Observe intense callose deposition and swelling of tube tip in both. A, B (× *ca* 400); C, D (× *ca*. 1000)).

4 hr after pollination, pollen tubes were visible near the base of the style and 8 hr after pollination in the micropylar region of the ovules.

In incompatibly (intramorph) pollinated thrum pistils oollen germination was profuse, the pollen tubes penetrated the stigmatic tissue but ceased growth soon after (figure 2D). When pin pistils were incompatibly pollinated, most of the pollen grains failed to adhere to the stigma; the few pollen grains that did adhere to the stigma, germinated and entered the stigmatic tissue (figure 2C). The possibility of contamination was ruled out by checking the morphology of the exine of the pollen grains (Dulberger 1974) under the microscope. The pollen tubes were effectively inhibited in the stigma soon after their entry. In both cases, many of the tubes showed swelling and rupture (figures 2C, D).

Bud receptivity

Stigmas were found to be receptive even 3 days before anthesis and supported pollen germination. Pollen tube growth was normal in pistils 24 hr before anthesis. In the two younger stages (48 hr and 72 hr before anthesis), however, growth of pollen tubes was arrested after they had grown a short distance in the style (figure 2B). The pollen tubes showed marked fluorescence upon staining for callose and appeared convoluted and swollen at the tips. Following incompatible pollinations, the response in buds of all three stages was essentially the same as that following incompatible pollination of mature pistils. Pollen tubes were inhibited soon after entering the stigma.

Seed set data (table 1) following bud pollinations supported observations on pollen germination and tube growth. Compatible pollinations of mature pistils and of those 24 hr before anthesis resulted in seed set. There was no seed set following compatible pollinations of pistils of the two youngest stages and incompatible pollinations at all the stages.

Table 1 *Seed set following bud pollination*

Stage of buds hr before anthesis	Number of seeds/pollination†			
	Thrum × Pin	Thrum × Thrum	Pin × Thrum	Pin × Pin
0 (mature)	8.0	0	9.2	0.2††
24	6.8	0.3††	5.5	0
48	0	0	1.0	0
72	0	0	0	0

†Average of 10 pollinations
††The only fruit that developed in each case may be the result of contamination

Discussion

Present investigations are the first attempts to study the details of stigma-surface proteins in a heteromorphic taxon. In *Linum grandiflorum*, stigma-surface proteins are present in the form of a pellicle in both morphs and are comparable to those found on dry stigmas of homomorphic forms (Mattsson et al. 1974, Heslop-Harrison et al. 1975, Shivanna et al. 1978). The surface proteins of the two morphs seem to differ both qualitatively and quantitatively. Coomassie blue staining material was found only on the stigma of the thrum morph (see also Dulberger 1975b). Although non-specific esterases were present on stigmas of both morphs, their activity was invariably higher on thrum stigmas.

The responses of mature pistils of this taxon to compatible and incompatible pollen have been described earlier by Lewis (1943). He reported that following intramorph pin pollinations most of the pollen grains fail to adhere to the stigma; a few that adhere, fail to undergo imbibition and germination. Based on these observations and studies on

osmotic pressures of pollen grains and styles, he proposed that intramorph incompatibility in *L. grandiflorum* is controlled by differences in osmotic pressures and the nature of cell colloids of pollen grains and styles of the two morphs. The differences are such that they do not permit pollen tube growth after intramorph thrum pollinations and prevent even hydration of the pollen grains following intramorph pin pollinations. Thus, no physiological mechanisms comparable to those of homomorphic taxa were implicated in the operation of incompatibility in this taxon.

We have, however, observed imbibition and germination of the few pollen grains that normally adhere to the stigma after intramorph pin pollinations. The number of pollen grains that are able to adhere to the stigma and germinate can be increased markedly by wetting or injuring the stigma before pollination. Tube growth of these pollen grains is effectively inhibited, following their entry into the stigma, as in intramorph thrum pollinations. Thus, intramorph incompatibility cannot be explained satisfactorily by simple differences in osmotic pressures and the nature of cell colloids. Apart from the inability of pin pollen to adhere to the pin stigma, physiological mechanisms similar to those operating in homomorphic taxa seem to inhibit the growth of incompatible pollen tubes.

In many homomorphic systems, bud pollination has been one of the most effective methods of overcoming intraspecific incompatibility (see Nettancourt 1977). To our knowledge there have been no studies on the efficacy of this technique in any heteromorphic taxon. Present investigations have shown that in *L. grandiflorum* bud pollinations cannot be used to overcome incompatibility. The factor(s) responsible for intramorph incompatibility appear to be present from a very early stage of development of the pistil.

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