APPEARANCE OF SPECIFIC ANTIGENIC PROTEINS IN
THE MATURING SEXUAL ORGANS OF SINAPIS
FLOWERS

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SUMMARY

Three proteins specific to the flowering state were found in Sinapis by immunological techniques. Two of these are specific to the stamen and one to the pistil. By the use of a histoimmunofluorescence technique their localization in the developing flower primordia and in the apex was examined during the transition to flowering. These proteins are not detected in the apex at evocation. They all appear at a relatively late stage of stamen or pistil maturation. The stamen proteins are localized in both the intine and exine layers of pollen grains in stamens 2–3 mm long; at anthesis they are essentially in the exine. The pistil protein is found in the stigma and in the transmitting tissue of the style. All these proteins contain sugar residues. A possible implication of these proteins in the process of male–female recognition is discussed.

INTRODUCTION

The transition to flower initiation by shoot meristems might be viewed as requiring the expression of genes that are not expressed in vegetative plants (Zeevaart, 1962; Salisbury, 1963; Bonner, 1965; Searle, 1965). Alternatively, the transition to flowering (floral evocation) has been seen by some investigators as resulting simply from an unspecific activation of meristems without an early derepression of particular genes (Evans, 1975). According to this hypothesis, new genes come into play only at a late stage of the transition when they are required to specify the chemical and structural characteristics of the developing inflorescences and/or flowers.

Since genes determine the formation of proteins, the expression of genes specific to flowering should be accompanied by the occurrence of proteins also specific to flowering, and which are not found in vegetative plants. In the past, attempts to find qualitative changes in the protein complement of meristems at evocation have been generally unsuccessful (Marushige & Marushige, 1962; Nitsan, 1962; Stiles & Davies, 1976), but more recently changes in the protein composition of the apical bud or meristem of Sinapis and Rudbeckia during floral evocation have been described (Pierard, Jacqmard & Bernier, 1977; Pierard, Jacqmard, Bernier & Salmon, 1980; Milyaeva, Kovaleva, Lobova & Chailakhyan, 1979).

Using immunological techniques, two antigenic proteins characteristic of the

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reproductive bud have been shown to appear in the apical meristem of *Sinapis* relatively early during the transition to flowering. This supports the hypothesis that a change in gene expression occurs at evocation (Pierard *et al.* 1980). However, these two proteins were not specific to the flowering state since they were also found in some vegetative parts of the plant (Pierard, Jacqmard & Bernier, 1979).

Changes in the complement of proteins being synthesized were also detected in the meristem of *Sinapis* during evocation by two-dimensional electrophoresis (Lyndon, Jacqmard & Bernier, 1983).

The aim in the present work was to look for proteins specific to the flowering state. Such proteins might be expected in the floral parts themselves, particularly in the stamens and pistil because these sexual organs are specific to flowers. Proteins specific to floral parts have indeed been found in *Pharbitis* (Marushige & Marushige, 1962), *Tulipa* (Barber & Steward, 1968), *Mercurialis* (Durand-Rivieres, 1969; Kahlem, 1975) and *Prunus* (Raff, Hutchinson, Knox & Clarke, 1979). We compared the antigenic-protein compositions of the different floral parts of *Sinapis* in order to find out if there were proteins characteristic of one or another floral part. The antigenic proteins specific to stamens and pistil were then localized by an histoinmunofluorescence technique in sections of flower primordia and apical buds during the transition to flowering to determine whether these proteins might be detectable before the flowers themselves were initiated.

**MATERIALS AND METHODS**

**Growing conditions**

Plants of the long-day plant *Sinapis alba* were raised from seeds in 8-h short days in the growth rooms of the phytotron of the Botany Department at Liège as previously described (Pierard *et al.* 1977). Flowering was caused by subjecting 2-month-old plants to continuous long days.

**Preparation of extracts**

The different floral parts—sepal, petals, stamens and pistil—were collected from flowers at anthesis and separately from flower primordia just before anthesis. The term 'flower primordium' refers to the flower bud from initiation to anthesis. Buds 3 mm long were also collected from 2-month-old vegetative plants and from reproductive plants after exposure to 10 successive long days. The vegetative buds contained, in addition to the meristem, a number of leaf primordia 2 mm long and the upper 1 mm of the stem. The reproductive buds did not contain any leaves but had a great number of flower primordia at various stages of development. Plant materials were then frozen in liquid nitrogen and stored at −20°C until use. Buds and organs were homogenized in cold 10 mM-Tris-HCl buffer (pH 7.5), with 0.5 M-sucrose, 5 mM-MgCl₂, 50 mM-KCl and 5 mM-β-mercaptoethanol. After centrifugation at 19,000* g* for 20 min the precipitate was discarded and the protein concentration of the supernatant extract was estimated by the Bio-Rad Protein Assay (Bio-Rad Laboratories).

**Preparation of antisera**

Antisera against protein extracts only of floral parts collected at anthesis were raised in rabbits by the procedure described previously (Pierard *et al.* 1977). Four injections of 0.2–0.8 mg of protein each were made during 5 months. The first injection was with complete Freund's adjuvant while the others were with incomplete adjuvant. The production of antibodies was monitored by regularly checking the reaction of the antiserum against the plant extract. Antisera collected after the third
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injection, which were the richest in antibodies, were mostly used in this study. ASE, APE, AST and API refer to the anti-sepal, anti-petal, anti-stamen and anti-pistil antisera, respectively.

Ouchterlony double immunodiffusion. Antisera were compared by means of the double immunodiffusion agar plate and carried out according to the method described by Pierard et al. (1977). Cross reactions were tested by various antigen-antibody combinations, placing the antiserum in the centre well and the antigens in the outer wells. Diffusion was for 24 h at room temperature.

Immunoelectrophoresis. This technique was used to compare the electrophoretic and antigenic properties of the antigens and to check that the precipitating antibodies were immunoglobulins G (IgG). Immunoelectrophoresis was performed using the micro-slide method (Scheidegger, 1955). Antigen samples were analysed by electrophoresis in 0.85% agarose (Calbiochem Behring) or 1.5% agar (Difco-Noble) in 30 mM-barbital-acetate buffer (pH 8.6) at 30 V/cm for 45 or 50 min at room temperature.

Diffusion with antiserum placed in the trench was then allowed to proceed for 24 h in a moist chamber at room temperature. After washing in 0.85% NaCl with 0.02% azide, and drying the gel, the electrophoretograms were stained with 0.1% Amido Black in acetic acid/methanol (1:9, v/v) for 5 min and then destained in acetic acid/methanol.

Crossed-immunoelectrophoresis (Laurell, 1966)

The first-dimension electrophoresis of the antigen preparation was done as above in agarose on 7.5 cm x 7.5 cm slides pre-coated with 0.5% agarose as an adhesive. After this first electrophoresis, a cut was made in the gel parallel to the zone of protein migration, 4 mm clear of the sample hole. The unused gel beyond the cut was stripped from the plate and the agarose layer replaced by 0.9% agarose gel containing 10% antiserum (v/v). The separated proteins were then run, at right angles to the first direction into the antibody-containing gel. The second electrophoresis was performed at 4 V/cm for 5 h at room temperature. The slide was then washed and stained as above.

Immuonabsorption

To demonstrate the exclusive capacity of an extract to produce specific antibodies and therefore to contain specific antigens, immunoabsorption was carried out by incubating serum and extract at an adequate concentration (v/v) for 2 h at 37°C then overnight at 4°C followed by centrifugation at 750 g for 10 min.

Antigen characterization

Pronase was used to show the protein nature of antigens; the enzyme (E 70,000 PU/g, Merck, 20 mg/ml in 10 mM-Tris-HCl, pH 7.4) was added to the protein extract to reach a final concentration of 2 mg/ml. The mixture was incubated 3 h at 37°C. Two controls for this reaction were done simultaneously: (1) incubation in the presence of a large amount of bovine serum albumin (BSA) (50 mg/ml, final concentration) as a competitor; (2) separate preincubations of extract and Pronase, followed by combining them just before immunodiffusion to test the eventual effect of Pronase on the antibodies during the immunodiffusion reaction itself.

Galactosyl, mannosylpyranosyl or glucopyranosyl residues in antigens were tested for by immunoelectrophoresis and crossed-immunoelectrophoresis. Antigen extract was preincubated with soybean agglutinin (SBA) (Miles Yeda Ltd) or concanavalin A (ConA) (Calbiochem), with identical concentrations of protein and lectin for 2 h at 37°C then overnight at 4°C, and centrifuged at 750 g for 10 min.

Histoimmunological techniques

An indirect histoimmunofluorescence technique was used to test for the presence of specific stamen and pistil antigens during flower development. Plant material was immediately frozen and cut at 6 μm thickness using a cryostat at −20°C, according to the technique of Knox & Heslop-Harrison (1970). The sections were fixed for 5 min in methanol, dried and stained in 0.2% toluidine blue in methanol for 5 min. This staining step was necessary to remove much of the native fluorescence and to allow antigen localization. The dye should bind to phenolic compounds, which are present in Sinapis flowers as well as in most other plant tissues (Knox & Clarke, 1978; Knox, Vithanage & Howlett, 1980), and so prevent them reacting with rabbit IgG. IgG that contained the
specific antibodies were isolated from AST and API by DEAE-cellulose chromatography. Then
AST IgG and API IgG were made specific with respect to stamen or pistil by absorption with a floral
extract containing all the antigens common to stamen (ST) or pistil (PI) except the specific ones.
AST IgG was adsorbed by a pistil extract leaving only antibodies against three (ST1, ST2 and
ST3) antigens specific to stamens, and API IgG was absorbed by a sepal extract leaving only
antibodies against two (PI1 and PI5) antigens specific to pistils (see Results). Control IgG solutions
were also prepared: (1) AST IgG absorbed by stamen extract; (2) API IgG absorbed by pistil
extract. The first control solution did not contain any antibodies against stamen antigens and the
second one was free of antibodies against pistil antigens. Each tissue section was incubated with 10 μl
of IgG (6 mg/ml) in a damp and dark chamber at 37 °C for 60 min. They were then washed in three
changes, for 15 min each, of phosphate-buffered saline (PBS; 0.01 M-potassium phosphate, 0.15 M-
NaCl, pH 7.2). Goat anti-rabbit IgG (Nordic Pharmaceuticals, Antwerp, Belgium), hereafter
called GAR IgG, labelled with fluorescein (FITC), was used as the conjugate throughout.
GARFITC IgG had about two fluorochrome molecules per protein molecule. It was made up at a
concentration of 0.25 mg/ml and allowed to act upon the sections previously treated with the rabbit
IgG solutions. Incubation with GARFITC IgG was for another period of 60 min in the dark and
in a moist atmosphere at 37 °C. Slides were then washed in PBS, drained and mounted in PBS/
glycerol (9/1). They were stored in the dark at 4 °C until observation. Additional control sections
incubated with GARFITC IgG alone were also made.

Fluorescence microscopy and photography

The sections were examined with a Leitz Ortholux fluorescence microscope equipped with a
Ploem Opak appliance. A xenon XBO arc (75 W) or a mercury arc (100 W) was used as the light
source. S 370-3 mm, AL 405 and 433-20 exciting filters were chosen and a barrier filter adjustment
filtered out light of wavelength greater than 495 nm (K 490–K 495 emission filters). The slides were
observed using a 60X oil immersion objective (n.a. 1.3) and were photographed using Kodak Tri
X Pan film (400 ASA).

RESULTS

Organ-specific antigens

The antigenic relationships between the various floral parts at anthesis were deter-
mined by immunodiffusion and immunoelectrophoresis, using both homologous and
heterologous antisera before and after immunoabsorption. The results are sum-
marized in Table 1.

Sepal antigens. There was no specific antigen in the sepals. The three precipitin
arcs obtained with a sepal extract in the immunoelectrophoretogram of Fig. 1 were

Table 1. Antigenic relationships between floral parts and with the reproductive bud
of Sinapis

<table>
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<th>Antiser to</th>
<th>Sepal</th>
<th>Petal</th>
<th>Stamen</th>
<th>Pistil</th>
<th>Reproductive bud</th>
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<td>1</td>
<td>4</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Pistil</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>5</td>
<td>4</td>
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</table>

Numbers refer to the number of antigens detected by a combination of immunodiffusion and
immunoelectrophoresis, as described in the text.
Specific proteins of sexual organs in Sinapis also common to the pistils. Immunodiffusion reaction of the different floral parts with ASE confirmed that sepal antigens were in pistils (Fig. 2). Components with antigenic determinants similar to those precipitating in the innermost band were also present in the petals and possibly in the stamens (Fig. 2, arrow).

Petal antigens. The three precipitin bands (Fig. 3, arrows) given by a petal extract with its homologous antiserum cross-reacted with three pistil antigens, but with pistil extract the median and the outermost lines precipitated as one. In the stamens the innermost band only was present, while in sepals there were only the other two that precipitated in one band as with the pistils. There was therefore no precipitating antigen specific to the petals.

Stamen antigens. The homologous stamen–AST interaction showed essentially four different antigens in the stamens (Fig. 4, arrowheads), of which three were present only in the stamens. One (ST1) was a cathodic antigen with a high isoelectric point, above pH 8.6; the other two (ST2 and ST3) were anodic antigens. Antigen ST4 was common to pistils, sepals and petals. The agar plate of Fig. 5 provides confirmation that there was one antigen (the innermost band) common to the various floral parts and that there were two precipitin bands specific to the stamens (arrows). The absorption of AST with a pistil extract removed the capacity of the serum to precipitate the antigen common to the pistils and stamens as shown in Fig. 6 where the precipitin arc ST4 of Fig. 4 is absent. Immunoprecipitation of AST with a stamen extract resulted in the loss of all antibodies (not shown).

Pistil antigens. Immunoelectrophoresis of pistil antigens against API gave five arcs of precipitation (Fig. 7, arrowheads). Two of these arcs (PI1 and PI3) were developed as protracted lines and probably represented two families of antigens with identical immunological properties, but with different electrophoretic mobilities. Of the five pistil antigens, two were present only in the pistils: a major one, PI1, migrated towards the cathode and a minor one, PI5, towards the anode. The others were also common to the sepals and PI3 was also present in the petals and stamens. The immunodiffusion test of Fig. 8, although showing only four precipitin lines for the pistils, confirmed the occurrence of one antigen common to the different floral parts (arrowhead) and a strong precipitin line specific to the pistils (arrows). Assignment of two antigens as being characteristic of the pistils was supported by the following results. First, the absorption of API with a sepal extract suppressed the capacity of the serum to precipitate the three antigens common to the pistils and the sepals but did not affect the capacity of the serum to precipitate the two characteristic antigens (Fig. 9, arrows), while an immunoprecipitation of API with a pistil extract removed the capacity of the serum to precipitate all antigens (not shown). In addition, the two precipitin lines present with the pistil extract only did not appear with a sepal extract even when the protein concentration of the sepal extract was double (not shown).

Antigen characterization
Serological identification, by immunodiffusion of the stamen or pistil antigens with their homologous antisera, was greatly diminished by preincubating the floral antigens with Pronase even when BSA was added as a competitor (Figs 10, 11). Separate
Figs 1–8
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Preincubation of antigens and Pronase followed by combining them just before immunodiffusion did not alter the immunoprecipitation of the antigens (shown for ST in Fig. 10). This control indicated that the considerable loss of antigen–antibody precipitation caused by preincubation of antigens with Pronase was not due to Pronase-mediated inactivation of the homologous antibodies during the subsequent immunodiffusion test. Stamen and pistil antigens are thus proteinaceous.

Sugar residues in stamen and pistil antigens were tested for, using lectins, by immunoelectrophoresis and crossed-immunoelectrophoresis. Figs 12 and 15 show crossed-immunoelectrophoreses of stamen and pistil extracts with their homologous antisera. The pistil proteins all moved towards the anode because of the use of agarose (Figs 15, 16) rather than agar (Fig. 7). The peaks were numbered as their corresponding arcs in Figs 4 and 7. Peaks ST1, ST2 and ST3 corresponded to the antigens characteristic of the stamen (Fig. 12) and peaks PI1 and PI5 to the antigens characteristic of the pistil (Fig. 15). Preincubation of a stamen extract with SBA did not eliminate any peak but reduced the proportion of ST2 in the antigenic solution (compare Figs 13 and 12). Preincubation with ConA eliminated all antigens except ST2 (Fig. 14). Preincubation of a pistil extract with SBA did not precipitate any pistil antigen (Fig. 16). On the contrary, preincubation with ConA eliminated all antigens except PI4 (Fig. 17). Note that the strong precipitin arc on the cathodic side in Fig. 17 did not result from a precipitation of pistil antigens with corresponding antibodies but from a reaction between ConA and a glycoprotein of the serum. Thus among the three antigens characteristic of the stamens (ST1, ST2 and ST3), ST1 and ST3 were glycoproteins with manno- or glucopyranosyl residues and ST2 contained galactosyl residues.

As far as the antigens characteristic of the pistil (PI1 and PI5) were concerned, both were glycoproteins with manno- or glucopyranosyl residues.

Appearance and localization of antigens specific to floral parts

Since no antigen was specific to the sepals or the petals, histochemical localization of these in developing flower primordia was not attempted. Immunodiffusion reactions of bud extracts with ASE indicated that the vegetative and reproductive buds contained at least one antigen in common with the sepal (Fig. 2, arrow), but immunoelectrophoresis showed that there were in fact two common antigens (not shown). Using APE, extracts of reproductive (and vegetative) buds were shown to possess the three petal antigens (Fig. 3).

Stamen-specific antigens. ST1, ST2 and ST3 were localized together by histo-immunofluorescence using AST IgG absorbed by a pistil extract. The specific

Figs 1–8. Immunodiffusion tests and immunoelectrophoresis of antigens of sepals (SE), petals (PE), stamens (ST), pistils (PI), and reproductive (RB) buds against anti-sepal (ASE), anti-petal (APE), anti-stamen (AST) or anti-pistil (API) serum. In Fig. 6, AST was absorbed by PI (AST ↓ PI). In immunodiffusion tests, the protein concentration of extracts was 1 mg/ml, except in the cases designated by (.) where the concentration was 2.5 mg/ml or (b) 5 mg/ml. In Figs 1, 7, 6 μg of protein were electrophoresed in agar gel; in Figs 4, 6, 10 μg were used in agarose gel. Arrows and arrowheads point to precipitin bands or arcs (see the text).
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Figs 15–21. Figs 15, 16. Crossed-immunoelectrophoresis in agarose gel of PI (50 μg of protein) alone or preincubated with SBA (PI + SBA) against API. Figs 17, 18, 19, 20, immunoelectrophoresis in agar gel of PI (30 μg of protein) preincubated with ConA (PI + ConA), PI, stigmas (SA), ovaries (O), RB (10 μg of protein), against API. Fig. 21, immunodiffusion test of PI, VB and RB (1 mg of protein/ml), VBa and RBa (2–5 mg of protein/ml) against API.

Figs 9–14. Fig. 9, immunodiffusion test of SE and PI (1 mg of protein/ml), VBa (vegetative bud) and RBa (2–5 mg of protein/ml) against API absorbed by SE (API, SE). Figs 10, 11, immunodiffusion tests of ST and PI (2 mg of protein/ml) without incubation (ST, PI) or incubated with Pronase (STp, Pip), with Pronase and BSA (STpB, PipB) or alone and mixed with Pronase just before immunodiffusion (STp). Figs 12–14, crossed immunoelectrophoresis in agarose gel of ST (50 μg of protein) alone or preincubated with soybean agglutinin (ST + SBA) or concanavalin A (ST + ConA) against AST.
solution so obtained, contained a higher proportion of antibodies against ST1 and ST2 than against ST3 (Fig. 6). In the stamens at anthesis, a much higher fluorescence was found in the exine layer of the pollen wall in sections incubated with specific AST IgG (Fig. 22) than in sections treated with AST IgG absorbed with stamen antigens (Fig. 23). On some occasions activity was also detected in the immediate vicinity of the exine surface suggesting that the antigens had diffused out of the pollen grains. The wall of the anther was also fluorescent but this fluorescence was no higher in sections treated with specific IgG than in those treated with non-specific IgG (Fig. 24) and was thus considered to be due to non-specific binding of rabbit IgG with some components of the wall. No fluorescence was exhibited by control sections incubated with GARFITC IgG alone (not shown). The presence of the antigens, ST1, ST2 and ST3, in pollen grains of flowers at anthesis was confirmed by an immunoelectrophoretic analysis of an extract of pollen grains alone against AST (not shown). In flower primordia 6 mm long, collected just before anthesis, the localization of the fluorescence was the same as in freshly opened flowers (not shown). In younger primordia, 3–4 mm long, a specific fluorescence was detected in about 50% of the stamens and was localized in the exine layer of pollen grain but was also found in the intine (Fig. 25). There was almost no fluorescence in the control section treated by non-specific IgG (Fig. 26). In stamens smaller than 2 mm (collected from flower primordia smaller than 3 mm), no fluorescence was exhibited by sections treated with either the specific (Fig. 27) or the non-specific IgG solution (Fig. 28), although exine and intine were already differentiated in stamens only 1.5 mm long. These last results were confirmed by immunodiffusion and immunoelectrophoresis of a reproductive bud extract against AST, since reproductive buds contained flower primordia of 2 mm long or less. Antigens ST1 and ST2 were absent but antigen ST3 was present (Fig. 4). Furthermore, the same pattern was found for the vegetative buds (not shown). The discordance concerning the minor antigen ST3, shown by immunoelectrophoresis and immunofluorescence, was probably due, in the latter technique, to the pretreatment with toluidine blue, which may mask some antigenic determinants. Thus, of the three antigens (ST1, ST2, ST3) present only in stamens of flowers at anthesis, ST3 was already present in the bud at all developmental stages. It was therefore not, in fact, specific to the stamens, in contrast to antigens ST1 and ST2, which were. ST1 and ST2 were not present in the shoot apical meristem before and during floral evocation but appeared exclusively in the pollen wall during stamen development.

**Pistil-specific antigens.** A rough localization of PI1 and PI5 antigens in the pistils at anthesis was first made by an immunoelectrophoretic analysis of stigmas, styles and ovaries separately. PI1 was found exclusively in the stigmas (Fig. 18, arrowhead 1); PI5 was present in the styles and ovaries (Fig. 19, arrowhead 5) and possibly proportionately less in the stigmas (Fig. 18). A more precise localization was obtained using histoimmunofluorescence although both antigens PI1 and PI5 were tested for together. Note, however, that the specific API IgG solution (i.e. API IgG absorbed by a sepal extract) contained a higher proportion of antibodies against PI1 than PI5 (Fig. 7). Sections of pistils at anthesis treated with specific API IgG showed a high
Figs 22–28. Localization of the stamen-specific proteins using histoimmunofluorescence. Fluorescence is represented by white areas in the photographs. Pollen grain (po); exine (e); intine (i); wall (w). Figs 22, 23, 24, stamen at anthesis. Figs 25, 26, stamen 2 mm long. Figs 27, 28, stamen 1.5 mm long. Figs 22, 25, 27, sections incubated with AST IgG_{1PI} and then with GARFITC IgG. Figs 23, 24, 26, 28, control adjacent sections of Figs 22, 25, 27, respectively, incubated with AST IgG_{1ST} and then with GARFITC IgG. ×760.
Figs 29–34. Localization of the pistil-specific proteins using histoimmunofluorescence. Cytoplasm (c); stigma (sa); transmitting tissue (tt); ovule (o); placenta (pl). Figs 29–33, pistil at anthesis. Fig. 34, pistil 1.5 mm long. Figs 29, 30, 31, 34, sections incubated with API IgG and then with GARFITC IgG. Fig. 32, control adjacent section of Fig. 29 incubated with API IgG and then with GARFITC IgG. Fig. 33, another control adjacent section of Fig. 29 incubated with GARFITC IgG alone. ×760.
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level of fluorescence in the stigmatic tissue (Fig. 29) and some fluorescence in the transmitting tissue of the style (Fig. 30), but no fluorescence in the ovule and placenta (Fig. 31). In stigma cells the fluorescence was localized to the peripheral cytoplasm (Fig. 29). In the transmitting tissue more fluorescing material accumulated in the cytoplasm at the pole of the cells orientated towards the base of the pistil. Control sections of stigma (Fig. 32) and transmitting tissue treated with API IgG absorbed by pistil extract exhibited almost no fluorescence. Other controls incubated with GARFITC IgG alone were almost completely dark (Fig. 33). In younger flower primordia about 6 mm long, the pistil antigens, PI1 and PI5, were present in both the stigmatic and the transmitting tissues (not shown). In flower primordia 2–3 mm long, the pistil (1–5–2 mm long) was totally devoid of fluorescence even in the stigma (Fig. 34), although the latter was already well differentiated. Similarly, no fluorescence was found in smaller flower primordia. These last results were confirmed, in the same way as for the stamen antigens, by immunodiffusion and immunoelectrophoresis of reproductive bud extract against API. Antigen PI1 was absent but antigen PI5 was present (Figs 9, 20, 21). The situation was the same in the vegetative buds. The discordance concerning the minor antigen PI5, shown by immunoelectrophoresis and immunofluorescence, can be explained as above for ST3. Thus of the two antigens, PI1 and PI5, found only in pistils of flowers at anthesis, PI5 was already present in the bud at all developmental stages and was not specific to the pistil. Only the other antigen, PI1, was specific to the pistil. It was not detectable in the shoot apical meristem before and during the floral transition but appeared exclusively in the stigma and the transmitting tissue of the style during pistil development.

**DISCUSSION**

The results described in this paper indicate that the antigenic-protein composition of the different floral parts in *Sinapis* is qualitatively different, from one class of parts to another. Three proteins are present only in the stamens and two others only in the pistil. Different protein patterns have also been found in the floral parts of other species as mentioned in the Introduction.

The three proteins present only in the stamens and the two proteins present only in the pistil were localized using a histoimmunofluorescence technique in the developing flowers and in the apex during the transition to flowering, in order to determine whether these proteins could be detected before the flowers themselves are initiated. The three stamen antigens were tested for together, but it was shown that our histoimmunofluorescence procedure detects only the two major ones, ST1 and ST2. It was also observed that the minor stamen protein ST3 is not specific to the stamens since it is already present in the bud. The two pistil antigens, PI1 and PI5, were also localized together but our histoimmunofluorescence procedure detects only the major one, PI1. It was also established that PI1 is the only antigen specific to the pistil. Although the monospecific antibodies with respect to the antigens to be localized were not used in the present study, our localization procedure seems reasonably specific since the control experiments have yielded very weak, if any, non-specific background
fluorescence (Figs 23, 32). In addition, several results obtained with the immunofluorescence technique were supported by the analysis of extracts using immunodiffusion and immunoelectrophoresis.

None of the specific stamen or pistil proteins appears in the meristem during floral evocation. They only appear in the stamens or pistil at a late stage of maturation. It can be concluded that, whichever developmental stage is considered, the proteins specific to sexual organ maturation are never present in the shoot apical meristem. Thus, the changes in the protein complement that occur in the apical meristem of *Sinapis* at evocation (Pierard *et al.* 1980; Lyndon *et al.* 1983) do not involve the antigenic proteins that concern us here that are specifically related to maturation of the sexual organs.

The two stamen antigens are first found in both the intine and exine layers of pollen grains in stamens 2–3 mm long. Later on, they are localized essentially in the exine layer of the mature pollen grain. The pistil protein is localized only in the stigma and the transmitting tissue of the style. All these antigenic proteins of stamens and pistil appear just before anthesis and contain sugar residues. Their late appearance, their localization and their glycoproteinaceous nature suggest that they could be involved in the process of male–female recognition. In *Brassica oleracea*, a closely related species, specific glycoproteins appear in maturing stigmas concomitantly with the development of the self-incompatibility system (Nishio & Hinata, 1977; Roberts, Stead, Ockendon & Dickinson, 1979). In *Gladiolus*, similar observations were also made (Knox, 1971; Clarke, Gleeson, Harrison & Knox, 1979). However, if the antigens that appear specifically in the stamens of *Sinapis* are related to the male–female recognition process, it is surprising to find them in the intine at the beginning of stamen maturation, since the self-incompatibility system is sporophytically controlled in *Sinapis* (Knox & Clarke, 1980). Further experiments are needed to clarify this point.

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