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Unilateral incompatibility within the Brassicaceae: further evidence for the involvement of the self-incompatibility (S)-locus

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Abstract. Unilateral pollen-pistil incompatibility within the Brassicaceae has been re-examined in a series of interspecific and intergeneric crosses using 13 self-compatible (SC, Sc) species and 12 self-incompatible (SI) species from ten tribes. SC × SC crosses were usually compatible, SI × SC crosses showed unilateral incompatibility, while SI × SI crosses were often incompatible or unilaterally incompatible. Unilateral incompatibility (UI) is shown to be overcome by bud pollination or treating stigmas with cycloheximide – features in common with self-incompatibility. Treating stigmas with pronase prevents pollen tubes from penetrating the stigma in normally compatible intra- and interspecific pollinations. The results presented show that the presence of an incompatibility system is important in predicting the outcome of interspecific and intergeneric crosses and, combined with the physiological similarities between UI and SI, would suggest an involvement of the S-locus in UI.

Key words: Brassicaceae – *Brassica oleracea* – Unilateral incompatibility – Self-incompatibility

Introduction

Interspecific pollen-pistil incompatibility has been defined as "any post-pollination event preventing the fusion of gametes from two fertile individuals of different species" (de Nettancourt 1977). Such incompatibility restricts gene flow and maintains species identity. It is likely that many cases of interspecific incompatibility, especially those involving distantly related species, are the result of physiological and biochemical mismatch of partners, termed 'incongruities' by Hogenboom (1972, 1975). However, as early as 1955, a relationship between interspecific incompatibility and intraspecific self-incompatibility was indicated with the description of unilateral incompatibility by Harrison and Darby (1955).

Unilateral incompatibility (UI) occurs when the pollen of one species is rejected by the pistil of another, while the reciprocal cross is fully compatible (de Nettancourt 1977). UI most commonly manifests itself in crosses where a self-incompatible (SI) species is the pistillate partner and a self-compatible (SC) species donates the pollen (Lewis and Crowe 1958). Lewis and Crowe (1958) showed that SI × SC UI is widespread and possibly the norm within the angiosperms and may, in some cases, transgress generic and even familial boundaries. They also identified a class of self-compatible species (Sc) which are incompatible with SC pollen but compatible with SI pistils and proposed that these Sc species had recently evolved from SI species. They further proposed that SC evolves from SI by a series of mutations at the S-locus.

Since the extensive study of UI by Lewis and Crowe, a great deal of genetical evidence has accumulated (mainly from families possessing gametophytic SI) suggesting an involvement of the S-locus in UI. In work with *Nicotiana*, Pandey (1968–1970) has considerably developed the Lewis and Crowe hypothesis, such that all exceptions to the basic SI × SC UI 'rule' can be explained (notably SC × SC, SI × SI and SC × SI UI) in terms of the S-gene complex. According to Pandey (1979, 1980), the S-gene complex consists of many different elements, some controlling interspecific incompatibility ('elements of primary specificity'), while others control SI ('elements of secondary specificity'). The model proposes that different elements of primary and secondary specificity are active in pollen and pistil, and only when such active elements are matched in male and female cells does incompatibil-

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ity result. While Pandey assumes an omnificent role for the S-locus in UI, genetical evidence from *Lycopersicon* (Martin 1963, 1964, 1967; Hardon 1967) suggests the involvement of other genes. Recently it has been shown by UI segregation studies and restriction fragment length polymorphism (RFLP) analysis that two genes, unlinked to the S-locus, play an essential role in UI in *Lycopersicon* (Chetelat and De Verna 1991).

Alternative theories to account for UI in gametophytic systems assume no S-locus involvement. Abdalla (1974) has suggested that in *Solanum* SI species also possess 'UI genes' that are solely responsible for inhibition of SC pollen. Hogenboom (1975) presents an incongruity-based polygenic control involving an unspecified number of dominant genes controlling aspects of the pollen-pistil interaction. Interspecific barriers, some of which can be unilateral, have been explained in terms of incongruities (Williams and Rouse 1988, 1990; Ellis et al. 1991), but a large body of evidence points to a role for the S-locus in UI in genera where gametophytic SI occurs (de Nettancourt et al. 1974; de Nettancourt 1977; Pandey 1980; Chetelat and De Verna 1991).

While UI in genera possessing gametophytic SI has been well documented, there have been fewer studies of UI in genera with sporophytic SI. Lewis and Crowe (1958) showed that the basic SI × SC UI was true for crosses within the Brassicaceae (= Cruciferae) and that certain species fell into the Sc category. Sampson (1962) re-examined UI within the Brassicaceae in a series of intergeneric crosses involving SI and SC species, and while his findings broadly agreed with those of Lewis and Crowe, they differed in that SI × SI crosses, instead of always being compatible, showed a high degree of incompatibility that, in a number of cases, was unilateral. Sampson suggested that pollen-pistil interaction depends upon the combination of stigmatic and pollen molecules at complementary sites. In his model there were two 'areas' in which complementation could occur: the 'S-allele area' and the 'species area'. Complementation at the S-allele area would result in intraspecific incompatibility, while complementation at the species area but not at the S-allele area would result in interspecific compatibility. Complementation at both areas or none of the areas would result in incompatibility. SI × SI incompatibility thus results from a lack of complementation at the species area, but Sampson stressed that this is often 'imperfect', leading to UI. In order to explain SI × SC UI Sampson proposed that SI stigmas present a fundamental barrier to pollen tubes that SC stigmas do not. This hypothesis has found favour with a number of reviewers (Burnet 1971; Heslop-Harrison 1975) and can explain certain features of the pollen-stigma interaction (Shivanna et al. 1978), but it stands alone as a study of UI within the Brassicaceae.

While progress is being made in the elucidation of the biochemistry and molecular biology of SI (for reviews see Dickinson 1990; Nasrallah et al. 1991; Gray et al. 1991; Franklin-Tong and Franklin 1992), these aspects of UI remain totally unexplored. Certain structural and physiological similarities exist between SI and UI (de Nettancourt 1977; de Nettancourt et al. 1974). The site, and apparent nature, of pollen rejection in UI generally reflects that of the SI system possessed. Thus, in sporophytic SI species UI is stigmatic, whereas in gametophytic SI species UI is stylar but rejection is often earlier than the SI response (de Nettancourt 1977).

The data presented here result from a re-examination of UI within the Brassicaceae and a comparison of certain basic aspects of the physiology of SI and UI within members of this family. The evolution and importance of UI are discussed and a simple model for UI is proposed.

Materials and methods

The species investigated were chosen from 10 of the 19 tribes of the Brassicaceae (= Cruciferae) (Schulz 1936) previously surveyed for the presence of SI by Bateman (1955). Tribal relations and sources of material are:

VII Brassiceae
- *Brassica oleracea* (Wellesbourne)
- *Brassica nigra* (Botanical Garden Stuttgart-Hohenheim)
- *Hirschfeldia incana* (Museum of Natural History Paris)
- *Raphanus sativus* (Reading University)
- *Rapistrum rugosum* (Museum of Natural History Paris)
- *Cakile maritima* (University of Reading)
- *Moricandia arvensis* (Botanical Garden Munich-Nymphenberg)
- *Conringia orientalis* (Botanical Garden University of Göttingen)

VIII Heliophileae
- *Heliophila longifolia* (St. Gallen)

X Lepidieae
- *Iberis sempervirens* (University of Reading)
- *Biscutella didyma* (University of Reading)
- *Capsella bursa-pastoris* (University of Reading)
- *Capsella grandiflora* (University of Reading)

XI Euclidieae
- *Neslia paniculata* (Botanical Garden Munich-Nymphenburg)

XIV Alysseae
- *Alyssum maritima* (Service de l'urbanisme et de construction Monaco)

XV Drabeae
- *Erophila verna* (University of Reading)

XVI Arabideae
- *Cardamine pratensis* (University of Oxford)
- *Cardamine bulbifera* (Index Seminum 1983)
- *Cardamine hirsuta* (University of Reading)

XVII Matthioleae
- *Aubretia deltoidea* (University of Reading)
- *Matthiola parviflora* (University of Reading)

XVIII Hesperideae
- *Malcolmia africana* (Botanical Garden Munich-Nymphenburg)

XIX Sisymbrieae
- *Alliaria petiolata* (University of Reading)
- *Sisymbrium officinale* (University of Reading)
- *Arabidopsis thaliana* (University of Reading)

Plants grown from seed were maintained in a heated (15°–25 °C) insect-proof greenhouse with no supplementary illumination during the flowering period.

Pollinations and observation of pollen germination and pollen-tube growth

Large, pre-anthesis buds were brought into the laboratory where their petals, sepals and anthers were removed. Pistils were then maintained in the wells of microtitre plates (Titertec Flow Labs, USA) containing tap water. Pollinations were carried out by gently brushing the stigma with a newly dehisced anther. Small self-compatible species were checked for the presence of self-pollen before pollination under a binocular microscope (M8 Wild Heerbrugg).

Table 1. Compatibility and incompatibility of the crosses tested

♀	♂	SC											Sc		SI											
		Cardamine hirsuta	*Arabidopsis thaliana*	*Capsella bursa-pastoris*	*Erophila verna*	*Alliaria petiolata*	*Biscutella didyma*	*Conringia orientalis*	*Neslia paniculata*	*Sisymbrium officinale*	*Heliophila longifolia*	*Alyssum maritima*	*Malcolmia africana*	*Brassica nigra*	*Cardamine pratensis*	*Capsella grandiflora*	*Cardamine bulbifera*	*Iberis sempervirens*	*Moricandia arvensis*	*Hirschfeldia incana*	*Cakile maritima*	*Matthiola parviflora*	*Aubretia deltoidea*	*Rapistrum rugosum*	*Raphanus sativus*	*Brassica oleracea*
SC	*Cardamine hirsuta*	++	++	++	−	++	++	++		++			++	++	++	++	++	++			++	++	++		++	++
	Arabidopsis thaliana	++	++	++	++	++	++	+	++	++		−	++	++	++	++	++	++	−	++		++	++	++	++	++
	Capsella bursa-pastoris	++	++	++		++	+	+		++			++		++	++	+	++			++	++	++		++	++
	Erophila verna	++	++		++									++	++			++								++
	Alliaria petiolata	++	++	+		++									+	++							++			++
	Biscutella didyma	++	++	−			++	+		++		+	++	++	−		+				++	++	+		+	+
	Conringia orientalis	++	++	−				++	++	++		++	+	++	++				−		++	++	++	−	++	++
	Neslia paniculata		++						++		++	+	+				++		++	+	++	−	++	−		+
	Sisymbrium officinale		+						+	++		−	+				++		+	++	+	++	+			
	Heliophila longifolia										++	+							++					−		++
	Alyssum maritima	+						+	++	++	++	+	++	++					++		+	++		++		
Sc	*Malcolmia africana*	−	+	−			−	+	−	+		−	++	+							−	−		+	−	++
	Brassica nigra	−					−	−	+	+	−	−	−	+				+	−		+	++	++	+	+	++
SI	*Cardamine pratensis*	+	−	−	−	−	−	+							−	++	++	++			+	+	++		+	++
	Capsella grandiflora	−	+	+		−									++	−		++				+	++		++	++
	Cardamine bulbifera	−	−	−			−	−	−								−		−	−				++	−	++
	Iberis sempervirens	−	−	−	−										−	−		−					+		−	+
	Moricandia arvensis			−				−	−	−	−	−		−			−		−	−	++			−	++	+
	Hirschfeldia incana											−	−				−		−	−						+
	Cakile maritima	−		−			−	−	−	−		−	−	−			−		−		−	−	+	−	+	+
	Matthiola parviflora	−	−	−		−	−	−				−	−		−	−	−	−	+		−	−	−		+	−
	Aubretia deltoidea	−	−	−		−	−	−					−	−	−	−	−	−			+	−	−		+	+
	Rapistrum rugosum			−				−	−	−	−	−		−			−		−	−	−			−	−	+
	Raphanus sativus	+	−	−		−	−	−				−	−	−	−	−	+	−			−	−	+		−	+
	Brassica olerocea	−	−	−	−	−	−	−			−	−	−	−	−	−	−	−			−	−	−	−	+	−

++, Full penetration >2% pollen tubes penetrate the stigma; +, low penetration <2% pollen tubes penetrate the stigma; −, no penetration

Bud pollinations involving *B. oleracea* (homozygous for the S63 incompatibility allele) were carried out using 3-mm buds. *Iberis sempervirens* and *Cardamine pratensis* buds less than half of the length of mature buds were selected for bud pollination.

To prevent wetting the stigmas of very small pistils microtitre plates were covered with 'Parafilm' and the pedicel inserted through a hole made with a syringe needle. Where possible a minimum of five pollinations per cross was carried out.

After pollination the pistils were maintained in the laboratory at 20°–25 °C in a moist atmosphere for 6–18 h, after which time the stigmas were removed and squashed in decolourized aniline blue (BDH) (0.1% in 0.1 *M* K_3PO_4, pH 7.0) (Linskens and Esser 1957) and assessed for pollen-tube germination and stigmatic penetration using a Dialux light microscope fitted with an incident UV illumination system (Leitz Wetzlar; excitation 450–490 nm, beam split 520 nm and suppression 575 nm) or a Zeiss Axiophot (excitation 365 nm, dichroic beam splitting 395 nm and long pass emission 420 nm). Pollen germination and penetration was scored on the basis of full compatibility/full penetration being when 2% or more of pollen tubes penetrate the stigma.

Cycloheximide treatments

Pistils were placed into the wells of microtitre plates containing 2×10^{-4} *M* cycloheximide (SIGMA) in distilled H_2O and allowed to equilibrate for 2 h before pollination.

Pronase treatments

Pistils from mature and immature buds were inverted in a solution of pronase (SIGMA) (0.1 mg ml^{-1} in 0.05 *M* TRIS-HCl 8% sucrose pH 7.2) in the wells of microtitre plates, briefly exposed to reduced pressure to remove any air bubbles trapped between papillae and incubated at 35 °C for 45 min. They were then washed twice in 0.05 *M* TRIS-HCl and once in distilled H_2O, dried for 15–30 min and pollinated.

Results

SC × SC pollinations

The majority of SC × SC pollinations were fully compatible or partially compatible in either direction (Table 1). In cases where an SC × SC pollination was incompatible (*Cardamine hirsuta* × *Erophila verna*, *Conringia orientalis* × *Capsella bursa-pastoris*, *Biscutella didyma* × *Capsella bursa-pastoris*, *Sisymbrium officinale* × *Alyssum maritima*) the reciprocal pollination was compatible.

The pollen of *Alyssum maritima*, while germinating well on many SC stigmas, showed low levels of penetration in 3 cases and no penetration in 2. When selfed, penetration was also low.

SC × SI and SI × SC pollinations

SC × SI pollinations were compatible – either fully (74%) or partially (16%). Only 10% of SC × SI crosses were incompatible, and no cases of SC × SI UI were found.

Of the SI × SC pollinations 93% were incompatible, and in no instance was a fully compatible SI × SC pollination observed although 7% of such crosses showed limited penetration. SI × SC UI therefore predominates in the species of the Brassicaceae studied.

SI × SI pollinations

The outcome of SI × SI crosses was variable. Only 18% were fully compatible, while 25% were partially compatible. The majority of crosses (58%) were incompatible. Of the 35 reciprocal SI × SI crosses carried out, 22 showed unilateral SI × SI incompatibility while 9 showed reciprocal incompatibility and 4 showed reciprocal compatibility.

There appeared to be a relationship between the ability of the stigma of an SI species to inhibit other SI pollen and the ability of its own SI pollen to grow on other SI stigmas (Table 2). From such information a crossability hierarchy can be constructed on the basis of a UI ratio (% ♀ inhibitions × % ♂ compatibilities). *B. oleracea* with a UI ratio of 0.82 can inhibit most SI pollens, while its own pollen will grow on and penetrate many SI stigmas. On the other hand, *Cardamine pratensis* with a UI ratio of 0.17 allows most SI pollens to grow on it, and in turn its own pollen is often inhibited by other SI stigmas.

'Sc Pollinations'

In this study *Brassica nigra* and *Malcolmia africana* behaved in a similar manner to the Sc category of plants so designated by Lewis and Crowe (1958): they were self-compatible when assessed for pollen-tube penetration and seed set, despite this self-compatibility they generally did not accept SC pollen, or did so only partially. SI pollen was accepted by *B. nigra* and to a

Table 2. Crossability hierarchy among SI species of the Brassicaceae based on a UI ratio: stigmatic incompatibilities with pollen of other species (% ♀ inhibitions) × pollen compatibilities with stigmas of other species (% ♂ compatibilities)

SI species	♀ inhibitions		♂ compatibilities		UI ratio
Brassica oleracea	9/10	0.90	10/11	0.91	0.82
Aubretia deltoidea	5/8	0.63	5/7	0.71	0.45
Raphanus sativus	5/8	0.63	7/10	0.70	0.44
Cakile maritima	4/7	0.57	3/7	0.43	0.25
Mathiola parviflora	7/9	0.77	2/6	0.33	0.25
Rapistrum rugosum	5/6	0.83	1/4	0.25	0.21
Iberis sempervirens	3/5	0.60	2/6	0.33	0.20
Capsella grandiflora	0/6	0.0	1/6	0.17	0.17
Cardamine pratensis	0/8	0.0	1/6	0.17	0.17
Cardamine bulbifera	3/5	0.60	2/9	0.22	0.13
Moricandia arvensis	3/6	0.50	1/6	0.17	0.09

lesser extent by *M. africana*. Both of their pollens grew and penetrated SC stigmas, but unlike the Sc plants of the Lewis and Crowe study they were not compatible with the stigmas of SI plants. It is therefore with reservation that *B. nigra* and *M. africana* are given Sc status, and primarily on the basis of incompatibility with SC pollen. The cross *B. nigra* × *M. africana* was unilaterally incompatible.

Bud pollinations

Stigmas from 2- to 3-mm-long buds of *Brassica oleracea* (S63) were brushed with pollen from SC and SI species, the pollens of which are normally inhibited on the stigmas of *B. oleracea* (Table 3). In all crosses pollen tubes were produced and penetration of the stigma was clearly visible (Figs. 1 and 2). Control pollinations of mature stigmas were all incompatible.

Stigmas from small buds of the SI species *Cardamine pratensis* and *Iberis sempervirens* also permitted growth and penetration of pollen from the SC species *Alliaria petiolata* and *Capsella bursa-pastoris*. Such pollen was inhibited by mature stigmas (Table 3).

Cycloheximide treatments

Pistils from the three SI species, *B. oleracea*, *I. sempervirens* and *C. pratensis*, were treated with cycloheximide and then self-pollinated or crossed with pollen from SC or SI species, the pollens of which are normally incompatible (Table 4). Self-pollination after cycloheximide treatment resulted in full compatibility with tubes clearly penetrating the stigma (Fig. 3). In all cases, the intergeneric incompatibility was overcome by cycloheximide treatment.

In the majority of cycloheximide-treated crosses pollen germinated, but the frequency of tubes penetrating the stigma was lower than in similarly treated selfs. The tubes that did penetrate appeared to cease growing soon after penetration – earlier than they did in self-pollinations following cycloheximide treatment (Fig. 4).

Pronase treatments

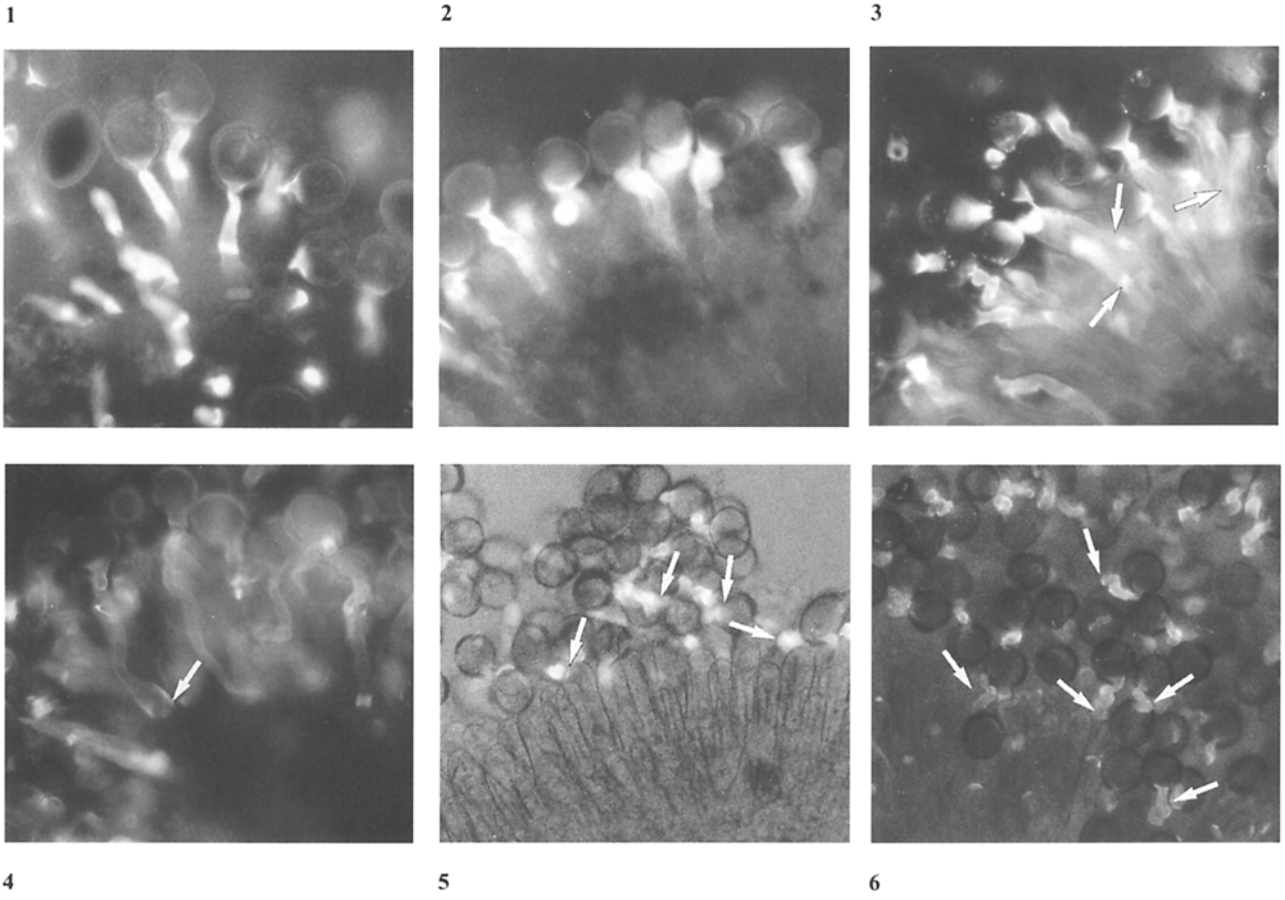
Treating stigmas of SI and SC species with pronase reduced their capacity to allow germination and especially penetration by pollen tubes with which they would normally be compatible (Tables 3 and 5). In SC selfs and SI crosses pollen germinated (although germination was often lower than in untreated controls) producing tubes, the majority of which were unable to penetrate the stigma (Fig. 5). In SC × SI crosses and compatible SI × SI crosses, again there was a slight reduction in pollen germination but a great reduction in the number of pollen tubes penetrating the stigma.

On stigmas treated with buffer alone (as a control) the ability of compatible pollen tubes to penetrate the cuticle was reduced when compared to the untreated

Table 3. The effect of pollination at the bud stage on normally incompatible interspecific crosses

		Self	*Alliaria petiolata*	*Arabidopsis thaliana*	*Capsella bursa-pastoris*	*Cardamine pratensis*	*Iberis sempervirens*
Brassica oleracea	Mature stigma	− > +	−	−	−	+ > −	−
	Bud stigma	+ + +	+ + + > + +	+ + + > +	+ + +	+ + + > + +	+ + + > + +
	Bud stigma pronase treated	+ > −	+ > −	+ > −	+ / −	+	+
	Bud stigma buffer treated	+ + + > + +	+ + > + + +	+ + > + + +	+ + > + + +	+ + + > + +	+ + > + + +
Iberis sempervirens	Mature stigma	+ > −	−		− > +		
	Bud stigma	+ + +	+ + +		+ + + > + +		
Cardamine pratensis	Mature stigma	+	+ > −		−		
	Bud stigma	+ + +	+ + +		+ + +		

+ + +, Full penetration > 2% tubes penetrate stigma; + +, low penetration < 2% tubes penetrate stigma; +, Pollen tubes produced, but do not penetrate stigma; −, pollen does not germinate



Figs. 1–6. **1** *Brassica oleracea* (S63) bud stigma × SC *Alliaria petiolata*. × 200. **2** As Fig. 1 × SC *Capsella bursa-pastoris*. × 200. **3** *B. oleracea* (S63) mature stigma treated with cycloheximide × self. Note long pollen tubes (*arrows*). × 160. **4** As Fig. 3 × SC *C. bursa-pastoris*. Note pollen tube stops growing at base of papilla (*arrow*). × 180. **5** *B. oleracea* (S63) mature stigma treated with pronase × *B. oleracea* (S29). Note that S29 pollen germinates but that the pollen tubes do not penetrate the stigmatic papillae (*arrows*). × 130. **6** *B. oleracea* (S63) bud stigma treated with pronase × SC *C. bursa-pastoris*. Note that tubes are produced but that penetration of the stigma does not occur (*arrows*). × 160

Table 4. The effect of the cycloheximide treatment of pistils on incompatible interspecific crosses

		Self	*Alliaria petiolata*	*Abidopsis thaliana*	*Capsella bursa-pastoris*	*Cardamine pratensis*	*Iberis sempervirens*
Brassica oleracea	Cycloheximide	+ + +	+ + > +	+ +	+ +	+ +	+
	Control	− > +	−	−	−	+ > −	−
Iberis sempervirens	Cyloheximide	+ + +	+ +	+ > + +	+ + +	+ +	
	Control	+	−	−	− > +	+	
Cardamine pratensis	Cyloheximide	+ + +	+ + > +	+ +	+ + +		+ + > +
	Control	+	+ > −	−	+		+ > −

See Table 3 for scoring

Table 5. The effect of the pronase treatment of stigmas on compatible interspecific pollinations

	Pronase			Buffer			Untreated		
	Self	*Brassica*	*Iberis*	Self	*Brassica*	*Iberis*	Self	*Brassica*	*Iberis*
Alliaria petiolata	+>−	+/−	+/−	++>+	++>+	++>+	+++	+++	+++
Arabidopsis thaliana	−/+	−>+		++	++>+++		+++	+++	+++
Capsella bursa-pastoris	+>−	+>−	+	++	++	++>+	+++	+++	+++

See Table 3 for scoring

controls but not to the same extent as on pronase-treated stigmas.

When stigmas from small buds of *B. oleracea* were treated with pronase before pollination, self pollen and pollen from other species, normally compatible at the bud stage, failed to penetrate the stigma (Fig. 6). Bud stigmas treated with buffer alone, while always allowing pollen tube penetration, did so to a lesser extent than untreated controls.

Discussion

The results of the interspecific and intergeneric crosses presented here support the earlier findings of Sampson (1962) by confirming the existence of SI × SC UI within the Brassicaceae and demonstrate that the outcome of SI × SI crosses, while unpredictable, was often incompatible or partially compatible, rather than all compatible as reported by Lewis and Crowe (1958). Only 18% of SI × SI crosses were fully compatible and of the 35 reciprocal SI × SI crosses carried out, 22 showed unilateral incompatibility, 9 showed reciprocal incompatibility and only 4 showed reciprocal compatibility. In common with the findings of Sampson (1962) these results suggest an involvement of the S-locus in UI.

The events accompanying SI × SC UI resemble very closely those of SI in that pollen often fails to germinate or if tubes are produced they fail to penetrate the stigmatic cuticle with the concomitant deposition of callose in the stigmatic papillae (S. Hiscock, personal observation). Our physiological data also highlight the similarities between UI and SI, namely the lack of an incompatible response when pollinations are carried out using pistils from small buds or pistils treated with cycloheximide. In SI species, compatibility in bud pollinations is held to result from the late formation of S-gene products during bud development. Interestingly, the onset of SI has been correlated with the appearance of S-locus-specific glycoproteins – SLSGs (Roberts et al. 1979; Nasrallah et al. 1985) – the function of which still remains unknown. Although SI has been shown to be overcome by applying inhibitors of protein synthesis, such as cycloheximide, to the pistils (Sarker et al. 1988), at present there is no evidence that it is the suppression of SLSG synthesis that induces pseudocompatibility. The data presented here shows that UI can also be overcome by cycloheximide, although to a slightly lower extent than for SI. These two pieces of data provide very strong circumstantial evidence for the involvement of S-locus products in the events of UI.

The results from the pronase treatments, although being clear-cut in that penetration of the stigmatic cuticle by pollen tubes is regularly prevented, are more difficult to interpret. The stigmatic cuticle of many species possessing 'dry stigmas' (Heslop-Harrison and Shivanna 1977) is known to be invested by a thin proteinaceous pellicle (Mattson et al. 1974; Heslop-Harrison et al. 1975) that can be disrupted by certain detergents and proteinases, such as pronase (Heslop-Harrison and Heslop-Harrison 1975; Knox et al. 1976). The pellicle of *Brassica* contains four prominent non-specific esterases and three glycoproteins (S. Hiscock unpublished data), some, or all of which, may be affected by pronase treatment. The integrity of these proteins within the pellicle may be of importance in recognition events between pollen and stigma or, as has been suggested by Heslop-Harrison (Heslop-Harrison and Heslop-Harrison 1975; Shivanna et al. 1978), the activation of a pollen-held cutinase needed for penetration of the stigmatic cuticle. Despite thorough washing of the stigmas after pronase treatment, it remains possible that some enzyme may remain on the surface and degrade pollen-secreted enzymes needed for successful penetration. Likewise, because of the porous nature of the stigmatic cuticle (Roberts et al. 1984; Elleman et al. 1988) the pronase may be exerting its effect on proteins in the cell wall, or even within the protoplasm. Further, proteinases are also known to stimulate a wound response in certain species (Swinburne 1975), so the effects of the pronase may well exceed merely the disruption of the pellicle. Interestingly, the treatment of stigmas with phosphate buffer as a control also slightly reduced pollen-tube penetration, confirming previous observations (Zuberi and Dickinson 1985) that were interpreted as resulting from the disruption of the stigma surface. Pronase treatment

may thus reasonably be assumed to affect a stigmatic component involved in tube penetration during both intra- and interspecific crosses.

There is increasing genetical evidence that the S-locus is involved in UI in groups featuring gametophytic SI (GSI) (Martin 1964; Hardon 1967; Pandey 1981) and that other genes, unlinked to the S-locus, may also be implicated (Martin 1963; Chetelat and De Verna 1991). In sporophytic SI (SSI) systems, however, no such segregation studies have been carried out to follow the inheritance of UI and SI, so there has been little direct evidence implicating the S-locus in UI in these species.

This study and others reported in the literature (Lewis and Crowe 1958; Sampson 1962) suggest that SI stigmas differ fundamentally from SC stigmas in that they present a 'barrier' to pollen-tube growth and penetration (Sampson 1962). No clear structural differences have been found between closely related SI and SC species or between the stigmas of buds and mature stigmas from the same SI species (Christ 1959; Dickinson and Lewis 1973; Shivanna et al. 1978), so such differences must be at a molecular level. Stigmas of SSI species such as *Brassica* and styles of GSI species such as *Nicotiana*, *Solanum* and *Lycopersicon* possess S-glycoproteins encoded by the S-locus. Interestingly, the SLSGs of *Brassica oleracea* have been shown to be greatly reduced in SC mutants (Nasrallah et al. 1992). No function has yet been assigned to the *Brassica* SLSGs but many Solanaceous S-associated glycoproteins have been identified as RNases with potent pollen-tube inhibitory properties (McClure et al. 1989, 1990), and in *Nicotiana*, SI species possess 100 times more stylar RNase activity than SC species, a fact that may have implications for UI.

If it is assumed that SI stigmas/pistils possess a 'barrier' to pollen-tube growth and penetration that is lacking in SC plants, then any pollen which is able to grow on that stigma must possess a means of overcoming, or not stimulating, this barrier mechanism. So far no molecules associated with incompatibility in the pollen have been identified; however, a gene linked to the S-locus which codes for a protein kinase, the S-receptor kinase (SRK) (Stein et al. 1991), is expressed in anthers as well as stigmas of *Brassica*, and a 7-kDa peptide from the pollen coat of *B. oleracea* has recently been shown to interact with stigmatic SLSGs (Doughty et al. 1992). A simplistic model may thus be devised to explain SC × SC and SC × SI compatibility based on the assumption that no barrier to pollen tubes is present in SC stigmas. Thus, SI × SC incompatibility is explicable because the SC pollen cannot overcome the stigmatic barrier. However, SI × SI crosses, for which the result is not so predictable, cause problems. Most SI × SI pollinations were incompatible or only partially compatible; this can be clarified if it is assumed that the pollen of each SI species contains a specific molecule or molecules able to overcome the stigmatic barrier in a cross within that species but which, because of 'specific' differences in these molecules, cannot overcome the barrier in another SI species. SI × SI reciprocal compatibility, rare in this study, would therefore be due to similarities between the 'specific component' of pollen molecules of the two SI species. On the other hand, SI × SI UI can be explained by Sampson's 'lock and key' analogy whereby a small key may be able to open a large lock but a large key cannot open a small lock. These 'key-lock' size differences can be considered to be analogous to structural or functional differences between the pollen molecules that overcome the 'stigmatic barrier'.

It is not surprising that the mechanisms of SI and UI appear to be similar since their ultimate outcomes are the same – pollen-tube inhibition. SI provides a fast and efficient pre-fertilization barrier that promotes outbreeding. Such a 'pollen sieve' mechanism would be just as advantageous to the plant if it could be employed to act on pollen from foreign species. This would prevent illegitimate crosses that may lead to fertilization and zygote formation but which eventually break down as a result of embryo abortion or the production of sterile hybrids – and thus ovule wastage. SC × SC and SC × SI crosses, while showing pollen-pistil compatibility, often show post-zygotic incompatibility events (de Nettancourt 1977) such as embryo abortion or produce sterile hybrids. Heslop-Harrison (1982) has suggested that in the grasses the mechanism of pollen rejection in SI and UI is the same but that the recognition and subsequent signalling events that trigger the rejection process are different. The harnessing of an early, S-mediated rejection of illegitimate pollen gives SI species a selective advantage over SC species in that their ovules are protected from futile fertilizations.

Since UI also occurs in species exhibiting GSI it is possible that the study of UI may also increase our understanding of the workings and evolution of SI itself. Current evidence suggests that GSI and SSI operate through different mechanisms, with perhaps the presence of a third in *Papaver* (Franklin and Franklin-Tong 1992). The active process by which the pollen is rejected in many GSI species appears to be an RNase (McClure et al. 1990), while in SSI species a receptor kinase is involved (Stein et al. 1991). This diversity may be predicted owing to the intense selective pressure that must exist for the evolution of self-sterility mechanisms (Whitehouse 1950).

However, the study of UI establishes a number of incontrovertible facts. First, plants with SI systems possess a pistillar defence mechanism that can only be overcome by pollen carrying the correct molecular "key". Indeed, the term "defence" used in this context may well be highly appropriate, as it is conceivable that the GSI RNase evolved from an RNase once important

in the plants' defences against invasion by pathogens and that the *Brassica* SRK similarly evolved from a pre-existing receptor kinase involved in defence-related signalling events. In support of this last assumption, the *Brassica* SRK has considerable homology to two other plants protein kinase genes – ZMPK1 from maize (Walker and Zhang 1990) and ARK1 from *Arabidopsis* (Tobias et al. 1992) – that are expressed throughout these plants, especially in the leaves and therefore likely to be involved in different signal transduction pathways to the reproductive tissue-specific SRK. As discussed earlier, the molecular key carried by pollen from SI species must carry two specificities: one quite general that in some way suppresses the rejection response and another which is specific to the S-allele that overrides the suppression of the rejection either by preventing the original suppressive interaction or by some other method. Considered in molecular terms, in GSI the first (equivalent to the primary specificity) (Pandey 1981) could involve the binding and inactivation of the RNase by a component of the pollen-tube surface, while in *Brassica* the pollen key could modify the complex interaction between SLSGs, SLRs (S-locus related glycoproteins) and the SRK product which, on challenge by an invading organism, would normally induce a rejection response. The second (S-locus-regulated) specificity would result from the match or mismatch between S-allele-encoded domains of the pollen and pistillar molecules that in GSI would prevent the primary interaction – thus permitting the RNase to enter the pollen tube – and in *Brassica*, the modification of the primary interaction of the male determinant with the SLSG or SRK product, with the resultant activation of a local rejection response. Interestingly, although the SRK is expressed in the anthers of *Brassica* (Stein et al. 1991) UI provides evidence that homology between the SRK product carried by the pollen and the SLSG of the stigma is not a prerequisite for rejection; it is therefore likely that the very precise homology between the "S" domain of the SRK and the SLSG is important for events at the plasma membrane of the stigmatic papliiae – perhaps permitting "pseudo-dimerization" of the extracellular receptor region of the kinase. The genetics and physiology of the SI response suggest that the pollen-held key is situated at the pollen-tube surface of GSI species and may require gene expression at some point of its operation (Franklin-Tong and Franklin 1992), while in *Brassica* it must be present at the pollen/stigma interface – perhaps held in the sporophytically-synthesized pollen-grain coating.

Evolution is economical (Corner 1964), and just as SI may have evolved from a pre-existing pathogen-defence system (Hodgkin et al. 1988) into a highly-specialized recognition and signalling system enabling recognition and rejection of self-pollen, so UI may have evolved in concert with SI as a means of preventing ovule wastage by a change in the recognition and signalling system that switches on the rejection.

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