UNILATERAL INTERSPECIFIC INCOMPATIBILITY IN FLOWERING PLANTS

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1. INTRODUCTION

The five well-established systems of sexual incompatibility in the flowering plants differ greatly in their genetical and physiological control. Each system during its evolution has accumulated its own special secondary differences in the genetic and floral structure of the species, culminating in a whole complex of integrated genetical and physiological characters. Because these complexes are very different in the different incompatibility systems, it is improbable that a switch in evolution from one fully developed system to another could occur.

A similar conclusion is inescapable from a study of the way the systems are distributed in taxonomic groups. Whole families have only one system as shown by heterostyly in the Primulaceæ and Plumbaginaceæ, gametophytic homomorphy in the Solanaceæ, Scrophulariaceæ and Rosaceæ, sporophytic homomorphy in the Compositæ and Cruciferæ. Even closely related families, the Solanaceæ and Scrophulariaceæ, have the same basic system.

In many species with gametophytic incompatibility, even when they come from different families, the genetics of the system is identical down to such details as the kinds of mutation of the controlling genes that occur. The physiology of incompatibility is also basically similar in such families : *Œnothera organensis* in the Onagraceæ and *Petunia hybrida* in the Solanaceæ, and probably others, have an antigen-antibody type of reaction between pollen and style (*cf.* Lewis, 1952 ; Linskens, 1953).

In closely related species the physiological control is actually identical in all its details. This is shown by hybrids of *Œnothera* species (Crowe, 1955) in which incompatibility works just as efficiently as in the species themselves. But widely different families, although they have the same basic antigen-antibody reaction, may differ in detail such as the effect of temperature. In *Œnothera* species the incompatibility reaction is accelerated by increased temperature, while in *Petunia* species it is not.

These comparisons between self-incompatible species are based on (1) a detailed analysis within the species, which is enough to characterise the basic genetic and physiological type and (2) an analysis of interspecific hybrids which shows whether or not the incompatibility

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systems in different species are identical in their genetic and physiological control.

The more distant relationships between species, genera and families, however, are outside the scope of such tests, which depend on the production of fertile hybrids, but the reaction of pollen and style provides a method of extending the analysis to groups that do not hybridise.

So far we have discussed only self-incompatible species. The majority of families and genera contain both self-incompatible and self-compatible species. Style-pollen relationships between a self-incompatible and a self-compatible species have been reported in the literature. In some examples a remarkable phenomenon was found. The pollen of the self-compatible (SC) species was inhibited in the styles of the self-incompatible (SI) species, while no inhibition occurred in the reciprocal cross. This unilateral incompatibility was totally unexpected on the current theories, and was therefore of great interest. But there were just as many exceptions in which the pollen of the SC species was not inhibited ; and therefore, interesting as the phenomenon was, it appeared not to be general and consequently not basic.

The data presented in this article show in fact that unilateral incompatibility of the type, SI ♀ × SC ♂, is general. The clue to its meaning and to the significance of the exceptions, which prove the rule, has come from mutation studies of the **S** locus.

The unilateral phenomenon is undoubtedly basic to the incompatibility system : it should prove to be a powerful tool in analysing distant phylogenetic relationships because it can be demonstrated without the production and fertility of a hybrid. It should be a means of estimating the age of incompatibility systems, and it is a fact to be considered in any theory of the genetics and physiology of the systems.

2. MATERIALS AND METHODS

The species were grown from seed obtained from various Botanical Gardens and seed firms as follows :

Cruciferæ	12 species, details in Bateman, 1955.	
Onagraceæ	10 *Œnothera* species, details in Crowe, 1955.	
	Epilobium angustifolium Linn.	Thompson and Morgan, Ipswich
	Epilobium hirsutum Linn.	Thompson and Morgan, Ipswich
	Epilobium lanceolatum Seb and Maur.	Thompson and Morgan, Ipswich
	Godetia amœna G. Don.	Chelsea Physic Garden
	Œnothera biennis Linn.	Chelsea Physic Garden
	Œnothera erythrosepala	Instituto Botanico, Coimbra, Portugal
	Œnothera lamarckiana	Thompson and Morgan, Ipswich
	Œnothera rosea	Portugal
Solanaceæ	*Capsicum annum* Linn.	Thompson and Morgan, Ipswich
	Capsicum frutescens Linn.	Thompson and Morgan, Ipswich
	Lycium chinense Mill.	Chelsea Physic Garden
	Lycopersicon esculentum Mill.	John Innes Hort. Instn.

Lycopersicon var. Best of All	John Innes Hort. Instn.
Lycopersicon hirsutum	Peru
Lycopersicon peruvianum	Peru
Lycopersicon pimpinellifolium	John Innes Hort. Instn.
Solanum capsicastrum Link.	John Innes Hort. Instn.
Solanum jasminoides Paxt.	John Innes Hort. Instn.
Solanum melongena Linn.	Thompson and Morgan, Ipswich
Solanum pseudocapsicum Linn.	Thompson and Morgan, Ipswich
Solanum rybinii Juz et Bük	John Innes Hort. Instn.

Several individuals of each species, at least four, were tested for self-incompatibility by seed setting and pollen-tube growth. Acid fuscin- light green mixture was the stain used in the Cruciferæ, Solanaceæ and Scrophulariaceæ ; the iodine-alcohol method was used in the Onagraceæ.

For measurement of pollen-tube growth flowers were pollinated, cut from the plant, placed in water and incubated at 25° C. for most of the tests ; when the effect of temperature was the object of the test the details are given in the text. Pollen-tube measurements were made on the longest pollen tube in the style.

3. INTERSPECIFIC RELATIONSHIPS

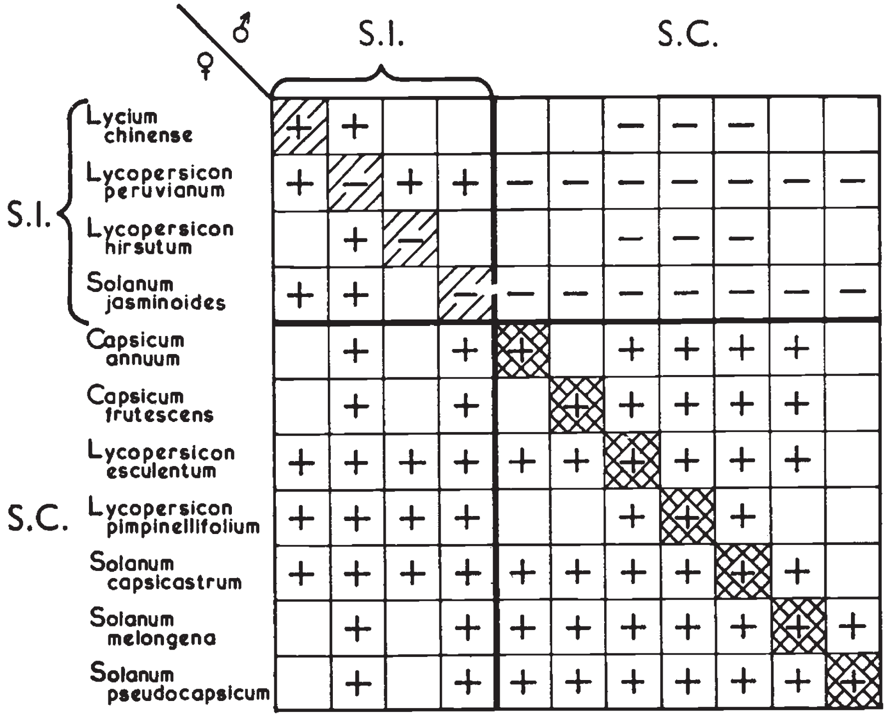
(i) *Gametophytic homomorphic system with one locus*

Solanaceæ. The genera and species in the Solanaceæ were selected to include a range of SI and SC species. In pollinations within species, pollen tubes of SC species and cross-compatible tubes of SI species grew to the base of the style, a distance of 6-8 mm. in 24 hours, and continued to grow into the ovary. The differences in growth rate of the pollen tubes between the species was not large and was correlated with the size of the pollen grain, which in turn was correlated with the length of the style. But these differences between the species are of no significance in the present study. Selfed pollen tubes of SI species grew 4·2 mm. in 24 hours in *Lycopersicon peruvianum* and *Lycopersicon hirsutum* and 3·0 mm. in *Solanum jasminoides* : no further growth occurred. Thus the difference between incompatible and compatible growth in these species was large and consistent. Selfed pollen tubes of *Solanum rybinii*, however, grew almost as fast as compatible tubes, attaining a length of 6-8 mm. in 24 hours.

The growth of the pollen tubes in interspecific and intergeneric crosses, given in fig. 1, are classified as +, when the tubes are 6-8 mm. long and into the ovary, or —, when they are less than 3 mm. long.

Fig. 1 shows that seven species of the three genera, *Capsicum*, *Lycopersicon* and *Solanum* are self-compatible (SC). The pollen of these species, when intercrossed on to any of the other SC species, is not inhibited. But when this SC pollen is placed on styles of the four SI species it is strongly inhibited. In contrast, pollen from SI species is not inhibited in styles of other species whether they are SI or SC. *Lycium chinense*, although it is SC in our material, has been included in the SI group for reasons that will be apparent later. *Solanum rybinii* is not included in the figure because it behaves in a unique way which we cannot explain. It is SI and its style behaves accordingly by inhibiting pollen of SC species. But its pollen on the contrary is inhibited in SI styles of other genera. This abnormal pollen behaviour may be associated with the fact that the self-pollen tube growth in *S. rybinii* is different from that in the other SI species of the Solanaceæ, for it continues to grow slowly to the base of the style.

Scrophulariaceæ. Eight species of *Antirrhinum*, including SI and SC species, have been investigated by Harrison and Darby (1955). As in the Solanaceæ, the two SC species *A. siculum* and *A. orontium* have their pollen tubes inhibited in the styles of the SI species. In all other combinations SC × SC, SC × SI and SI × SI the pollen tubes are not inhibited.



♀ \ ♂	S.I.				S.C.						
S.I. Lycium chinense	+	+					−	−	−		
Lycopersicon peruvianum	+	−	+	+	−	−	−	−	−	−	−
Lycopersicon hirsutum		+	−				−	−	−		
Solanum jasminoides	+	+		−	−	−	−	−	−	−	−
S.C. Capsicum annuum		+		+	+		+	+	+	+	
Capsicum frutescens		+		+		+	+	+	+	+	
Lycopersicon esculentum	+	+	+	+	+	+	+	+	+	+	
Lycopersicon pimpinellifolium	+	+	+	+			+	+	+		
Solanum capsicastrum	+	+	+	+	+	+	+	+	+	+	
Solanum melongena		+		+	+	+	+	+	+	+	+
Solanum pseudocapsicum		+		+	+	+	+	+	+	+	+

FIG. 1.—Results of intercrossing self-incompatible (SI) species with self-compatible (SC) species in the Solanaceæ : — pollen tubes are inhibited in top 3 mm. of the style, + pollen grows into the ovary.

Solanaceæ × *Scrophulariaceæ.* In view of the inhibition of SC pollen in SI styles in both the Solanaceæ and the Scrophulariaceæ, we made tests by cross-pollinating between the two families. If a similar inhibition existed between the families a common cause of the phenomenon could be established.

Pollen of Solanaceous species when applied to the *Antirrhinum* species did not germinate in most cases, and in none did the tubes penetrate the stigma. The results are given in table 1.

It is not surprising that the physico-chemical nature of the stigmas in the two families is apparently different. Such gross differences are probably independent of the differences between SI and SC styles.

However, *Antirrhinum* pollen germinated and grew normally in the styles of the Solanaceæ. Pollen tubes were frequently present in the ovary. The results of these inter-family tests are given in fig. 2. Despite some complications, the main features are unambiguous.

TABLE 1

Results of Intercrossing the Scrophulariaceæ *with the* Solanaceæ

Antirrhinum 4 species (SI and SC) × Solanaceæ 4 species (SI and SC)
Pollen does not germinate

Antirrhinum meonanthum × *Capsicum annuum*
Antirrhinum meonanthum × *Capsicum frutescens*
Tubes 2 × diameter of pollen

Antirrhinum meonanthum × *Lycopersicon esculentum*
Tubes 2 × diameter of pollen—burst

SCROPHULARIACEAE

SOLANACEAE

♀ \ ♂	A. majus	A. ramosissimum	A. meonanthum	A. glutinosum	A. orontium
Lycopersicon peruvianum	−	+	+	+	−
Solanum jasminoides	−	−	−	−	−
Solanum rybinii	−	+	+	+	−
Capsicum annuum	+			+	+
Capsicum frutescens	+		+	+	+
Lycopersicon esculentum	+	+	+	+	+
Solanum capsicastrum	+		+	+	+
Solanum melongena		+	+	+	+

FIG. 2.—Results of intercrossing pollen of *Antirrhinum* species on to the Solanaceæ.

The pollen tubes of the three SI species, *A. ramosissimum*, *A. meonanthum* and *A. glutinosum* were not inhibited in SI or SC species of the Solanaceæ. Pollen of the SC *A. orontium*, however, was inhibited in the SI species but not in the SC species of the Solanaceæ. Thus the inter-family crosses follow the pattern established by crosses within the family. One complication was that pollen tubes of *Antirrhinum* species were unable to grow in the style of *Solanum jasminoides*. This inability to grow is visibly different from the (SI × SC) inhibition since the tubes were extremely short, swollen and sometimes burst.

Exceptional behaviour was also shown by *A. majus* pollen. On the styles of other species of *Antirrhinum* it behaved like an SI species by growing uninhibited in styles of both SI and SC species. But on SI styles of the Solanaceæ it was inhibited. Because *A. majus* has only recently become self-compatible by mutation and selection this different reaction on the styles of its own genera and on the Solanaceæ may be significant. *A. majus* occupies an intermediate position between SI and SC species, and it shows a similarly intermediate state in regard to its reaction with other genera.

Onagraceæ. The species tested in the Onagraceæ can be conveniently grouped as follows :

8 SI species of Œnothera without established chromosome rings
6 Sc * species of Œnothera which have close relatives that are SI
3 Sc species of Œnothera, *O. biennis*, *O. lamarckiana*, *O. erythrosepela* which are self-fertilising and have established ring-forming chromosomes
3 species of Epilobium which is an SC genus
1 species of Godetia which is an SC genus

The results, given in fig. 3, show that in no combination was the pollen of either an SC or an SI species inhibited. The exceptions with *Epilobium hirsutum* and *Godetia amœna* are not relevant. These styles would not allow pollen of any foreign genera to germinate and therefore the negative results indicate gross physiological differences rather than incompatibility.

The actual growth of the pollen of different species varied greatly, but when a comparison was made of the growth of the pollen on its own and a foreign style, it was evident that there was no inhibition. Certain styles such as those of *Œnothera pallida* appeared to be less favourable for the growth of Epilobium pollen than those of other Œnothera species, but again little significance can be attached to this.

(ii) *Sporophytic homomorphic system*

The Cruciferæ have a system of incompatibility controlled by a single gene with many alleles as in the system found in the previous families. But the pollen reaction is under sporophytic control and not under gametophytic control. It is therefore of interest to determine whether the (SI × SC) inhibition, found in the gametophytic system, operates in other systems.

Bateman (1955) in his survey of the family for self-incompatibility found 80 SI and 102 SC and Sc species. Most of the genera have

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* See appendix, p. 254.

both SI and Sc species ; only one, *Iberis*, was wholly SI and four *Lepidium*, *Æthionema*, *Draba* and *Eriophila* were wholly SC as far as the species tests showed.

The interspecific and intergeneric crosses are shown in fig. 4. Six SI species and five Sc species taken from mixed SI, Sc genera, and one species each from the SC genera *Æthionema* and *Draba* have been tested.

	♀ \ ♂	O.p	O.l	O.d	O.r	O.rh	O.m	O.o	O.s	O.c	O.d *	O.t	O.rh *	O.a	O.ro	O.e	O.b	O.lm	G.a	E.l	E.a	E.h
S.I.	O.pallida	−	+	+	+	+	+	+	+	+	+	+			+	+	+	+	+	4	5	6
	O.latifolia	+	−	+	+	+	+	+	+	+	+	+			+	+	+	+	+	2	4	8
	O.deltoides	+	+	−	+	+	+	+	+	+	+	+			+	+	+	+	+	16	10	15
	O.runcinata	+	+	+	−							+										
	O.rhombipetala	+	+	+	+	−	+	+	+	+	+	+	+	+	+	+	+	+	+	14	14	
	O.missouriensis	+	+	+		+	−	+	+					+								
	O.organensis	+	+	+	+	+	+	−	+			+		+	+	+	+	+	+	9	13	
	O.speciosa	+	+	+	+	+	+	+	−		+	+										
Sc	O.cognata	+	+	+		+		+		⊕	+	+			+	+	+	+	+	9	11	12
	O.deltoides *	+	+	+		+		+		+	⊕	+			+	+	+	+	+	11	11	
	O.trichocalyx	+	+	+	+	+	+	+	+	+	+	⊕		+	+	+	+	+	+	4	1	18
	O.rhombipetala *					+							⊕		+	+	+	+	+			
	O.acaulis				+	+	+	+	+			+		⊕								
	O.rosea	+	+	+		+		+		+	+	+	+		⊕	+	+	+	+	5	12	7
	O.erythrosepala	+	+	+		+		+		+	+	+	+		+	⊕	+	+	+	4	2	
	O.biennis	+	+	+		+		+		+	+	+			+	+	⊕	+	+	11	7	8
	O.lamarkiana	+	+	+		+		+		+	+	+			+	+	+	⊕	+	9	10	
	G.amoena	/	/	/		/		/		/	/	/	/		/	/	/	/	⊕			1
	E.lanceolatum	+	+	+		+		+		+	+	+	+		+	+	+	+		⊕	+	
	E.angustifolium.	+				+						+			+					+	⊕	
	E.hirsutum		/					/		/	/	/			/		/		/			⊕

FIG. 3.—Results of intercrossing in the Onagraceæ ; *Sc forms of an SI species. The figures in the last three columns give the lengths in mm. to which pollen tubes grow in 24 hours.

It is clear from the figure that pollen was inhibited only when *Æthionema* pollen was placed on the style of other species. The styles of the other SC species *Draba verna* did not inhibit the pollen of *Æthionema*.

This result is therefore similar to the results in the preceding families with gametophytic incompatibility, suggesting that the (SI ×SC) inhibition is also a feature of sporophytic systems. The explanation for the non-conformity of the pollen of four Sc species in SI styles will be discussed later, along with the exceptional *A. majus* and *Lycium*.

In passing, however, we can note that Riley (1932) also recorded results in *Capsella* which are, at first sight, a violation of the (SI ×SC) rule. In crossing *C. grandiflora* SI reciprocally with *C. rubella* (Sc) and with *C. virginirri* (Sc) all crosses were compatible.

Karpechenko (1928) and many others obtained hybrids between *Raphanus sativus* (SI) and *Brassica campestris* (SI) only when the *Brassica* was used as pollen. The pollen tubes of the *Raphanus* were reported to be inhibited in the *Brassica* styles. Furthermore the allotetraploid of this hybrid, *Raphanobrassica*, was unexpectedly found to be self-compatible, and showed some unusual behaviour when back-crossed with the parents.

	♀ \ ♂	A.g	D.v	E.s	S.o	C.c	V.u	I.c	A.a	A.p	C.p	E.a	C.d
S.C.	Aethionema grandiflorum	▩ +	+	+	+	+	+	+	+		+	+	
	Draba verna	+	▩ +										
Sc	Erysimum suffrutuosum	−		▩ +	+	+	+	+	+		+	+	+
	Sisymbrium officinale	−		+	▩ +	+	+	+	+		+	+	+
	Cheiranthus cheiri	−		+	+	▩ +	+	+	+			+	+
	Vesicaria utriculata	−		+	+	+	▩ +		+		+	+	
S.I.	Iberis contracta	−		+	+	+		▨ −	+	+	+	+	+
	Alyssum argenteum	−		+	+	+	+	+	▨ −	+		+	+
	Arabis procurrens							+	+	▨ −	+		
	Cardamine pratensis	−		+	+		+	+		+	▨ −	+	
	Erysimum asperum	−		+	+	+	+	+	+		+	▨ −	+
	Cardaria draba			+	+	+	+	+	+			+	▨ −

FIG. 4.—Results of intercrosses between species and genera in the Cruciferæ; SC self-compatible species from genera that are wholly self-compatible, Sc self-compatible species from genera that contain SI species.

(iii) *Heteromorphic system*

The Linaceæ and Primulaceæ contain SI heterostyled and SC homostyled species. Tammes (1928) found that the SC homostyled species, *Linum usitatissimum*, was compatible when used as a female with pollen of five different SI heterostyled species. The reciprocal cross using the SC species as pollen was incompatible. A similar result was obtained with the SC homostyled, *Linum monogynum* (Vargas Eyre and Smith, 1916).

Khanna and Singh (1956), examined pollen tube growth in crosses between *Linum* species. They used two heterostyled species *L. grandiflorum* and *L. africanum* and crossed them with the homostyled *L. usitatissimum*. Pollen tubes were normal and uninhibited when the homostyled species was used as the female, but when used as the pollen parent the tubes were swollen and sometimes burst and never penetrated beyond the middle of the style. Khanna and Singh explain the inhibition on the difference in chromosome number of the species. *L. grandiflorum* has $n = 8$, *L. usitatissimum* with $n = 16$, *L. africanum*, $n = 15$. A more probable explanation based on (SI ×SC) inhibition which is concerned with pollen-tube growth appears to be more satisfactory.

In the Primulaceæ, Ernst (1953) distinguishes between primary and secondary homostyly. The secondary homostyles are recent mutants in an otherwise heterostyled species. Several of these mutants have occurred in experiments. These homostyles when crossed with heterostyled individuals obey the incompatibility rules which are based upon the known reactions of pollen and styles of the different morphological types. The homostyled species, however, do not conform with the rules. For example, crosses between the heterostyled SI *P. pulverulenta* and the homostyled SC species *P. cockburniana* and *P. chungensis* are compatible in all combinations. The evidence in the heterostyled species consists therefore of two examples of (SI ×SC) inhibition in *Linum* and no inhibition in *Primula*. Again the data support and do not conflict with the (SI ×SC) rule for the heterostyled system.

(iv) *Gametophytic homomorphic system with two loci*

Self-incompatibility is common in the Gramineæ, and in the only two species that have been examined, *Secale cereale* and *Phalaris cœrulescens*, the system is controlled by two loci S and Z both with multiple alleles and gametophytic control of the pollen (Lundqvist, 1954 ; Hayman, 1956). Many interspecific and intergeneric hybridisations have been made in the Gramineæ, and there are two examples of a seed failure in the (SI ×SC) cross where the (SC ×SI) is fertile. These are *Secale cereale* ×*Hordeum jubatum* (Quincke, 1940), and *Phleum alpinum* ×*P. nodosum* (Nordenskiold, 1937). But in neither of these cases is it known with certainty that the sterility of the (SI ×SC) cross is due to pollen-tube inhibition.

(v) *F_1 hybrids*

Another important aspect of the interspecific relationships is the behaviour of F_1 hybrids. In the gametophytic system, a cross SC ×SI such as *Lycopersicon esculentum* ×*L. peruvianum* (McGuire and Rick, 1954), assuming that the two species have an homologous incompatibility (S) locus, the F_1 will contain one S allele from each parent, and can be represented as SıSc. These alleles will segregate in the pollen of the hybrids giving Sı and Sc pollen grains. On self-pollination Sı pollen tubes will be inhibited because Sı is also present in the style. This inhibition is expected on the rules governing the behaviour in homomorphic incompatibility systems. Sc pollen tubes will also be inhibited by the presence of Sı in the style, but we attribute this reaction to the (SI ×SC) inhibition. The exceptional Sc *Antirrhinum majus* and the Sc *Œnothera* species, which have pollen that is not inhibited in an SI style, also differ in their F_1 hybrids with SI species. They are self-compatible (Shermann, 1939 ; Crowe, 1955). Predictable as this behaviour of the F_1 is in the gametophytic system, this is not so in sporophytic systems owing to the complication of dominance in the pollen. Even within a species, which is an uncomplicated relationship, an **Sc** allele can be either dominant or recessive according to the particular SI allele accompanying it (Bateman, 1954).

However, in crosses between species an **Sc** allele which shows (SI ×SC) inhibition should produce a self-incompatible F_1 hybrid whether the **Sc** allele is dominant or recessive. If **Sc** is dominant all the pollen will be phenotypically SC, and this will not function on an **S**1**Sc** style. If, on the other hand, **Sc** is recessive all the pollen will be phenotypically **S**1 and this will be opposed by the same **S**1 allele in the style. Only if the **Sc** allele has reversed dominance in pollen and style will the F_1 be self-compatible.

Capsella species, in which the **Sc** allele does not have the (SI ×SC) inhibition, produce F_1 hybrids that are self-compatible (Riley, 1932), as expected if **Sc** is dominant in the pollen.

The Compositæ also has the sporophytic system. Our limited tests in the family include *Cosmos bipinnatus*, *Cosmos diversifolium*, *Layia platyglossa*, *L. pentachadii* and *Senecio vulgaris*, but pollen in all crosses did not germinate. An example in the literature is found in the genus *Crepis*. *Crepis fœtida* has an incompatibility system with sporophytic control and many alleles of the **S** gene (Hughes and Babcock, 1950). This species exists in SI and Sc varieties (Babcock and Cave, 1938). The cross between these two varieties is compatible in both directions, the F_1 is SI and the F_2 segregates SI and Sc plants. This is in agreement with the Sc variety being a recent mutant of the SI variety, with the Sc allele dominant in the pollen. Two SC species *C. thompsonii* and *C. eritreensis*, however, will cross with the SI, *C. fœtida*, only in the direction SC ×SI. The reciprocal cross presumably shows (SI ×SC) inhibition. The F_1, with two exceptions, is SI. This result agrees well with the expectations. But before it can be said with any degree of certainty that the (SI ×SC) rule applies to the Compositæ, some critical tests of it must be made in this family.

4. PHYSIOLOGY OF INTERSPECIFIC INCOMPATIBILITY

(i) *Strength of the (SI×SC) inhibition*

In the gametophytic system, incompatible pollen tubes stop growing at varying times after pollination and at varying distances down the style, according to the species and conditions. But the time and place of inhibition is fairly narrowly defined and characteristic for each species.

The self-pollen tubes of *Lycopersicon peruvianum* at 26° C. reached a mean length of 4·2 mm. after 24 hours. The mean length of pollen tubes of six SC species of the Solanaceæ growing in *L. peruvianum* was only 1·7 mm. The maximum was 3·0 for *Solanum capsicastrum* and 0·1 for *Capsicum frutescens.*

McGuire and Rick (1954) found the same difference between SI × SC inhibition of *L. peruvianum* × *L. esculentum* and self-inhibition of *L. peruvianum.* Bateman (1943) found this difference in *Petunia* species. All these examples are in the Solanaceæ, but in the Scrophulariaceæ a similar difference exists. *Antirrhinum meonanthum*, SI, when selfed at 26° C. produces pollen tubes 8·5 mm. long in 26 hours. The SI × SC cross *A. meonanthum* × *A. orontium* produces tubes only 1 mm. long. *A. glutinosum*, however, shows equal and strong inhibition in selfing and in crossing with *A. orontium* pollen. The pollen tubes grow only 1·5 mm. at 15° C.

We can conclude that for the gametophytic system, at least in the Solanaceæ and Scrophulariaceæ, the SI × SC inhibition is stronger or earlier in action than the inhibition of selfing.

A similar comparison could not be made for the Onagraceæ because there was no example of (SI × SC) inhibition, or in the sporophytic system of the Cruciferæ because the self and (SI × SC) inhibitions are so immediate as to prevent germination.

(ii) *Temperature and the inhibition reaction*

In all SI species tested in the Onagraceæ the strength of the inhibition on selfing is increased with increasing temperature; $Q^{10} = 2{\cdot}5$ (Lewis, 1942; Crowe, 1955). A similar temperature effect is found in the Rosaceæ and Primulaceæ (Modlibowska, 1945). Tests at 15°, 26°, 30° on the SI species of the Solanaceæ, given in fig. 1, revealed no effect of temperature either in the self-pollen tubes or in the (SI × SC) pollen tubes. It would appear, therefore, that the effects of temperature on rate of growth and on the inhibition reaction cancelled out in the Solanaceæ. But in the Scrophulariaceæ the temperature experiments were more informative. The means of lengths of pollen tubes in 24 hours are given below:

	Selfed	(SI × SC)
Temperature	*Antirrhinum glutinosum*	*A. glutinosum* × *A. orontium*
15°	1·5 mm.	1·3 mm.
26°	0·05 mm.	0·1 mm.

There is therefore a corresponding effect of temperature on the two types of inhibitions.

(iii) *Unitary nature of the inhibition*

Perhaps the most remarkable aspect of the (SI ×SC) inhibition is its general nature in contrast to the highly specific nature of self-inhibitions of the (SI ×SI) type. Not only does the (SI ×SC) inhibition occur between widely different species and genera, but any of the different SI alleles in the style have the power to inhibit SC pollen. McGuire and Rick (1954) have shown in *Lycopersicon* crosses that S1.2, S1.c, and S2.c styles all inhibit SC pollen. Mather (1943) found in *Petunia* hybrids that both the **S** alleles he used from the SI species, when present in the style, either singly, together, or with an **SC** allele in the F_1, inhibited SC pollen.

This unitary action of the SI alleles is such a widespread phenomenon that it cannot be ignored and must be considered with the other facts bearing on the biochemistry of the incompatibility reaction.

The inhibition of pollen by the style is caused by an endothermic reaction between a specific protein, antigen, in the pollen and an antibody in the style (Linskens, 1953 ; Lewis, 1952). To explain incompatibility in *Petunia*, Straub (1947) has invoked a hypothesis in which an antigen-antibody reaction occurs but the antigen is itself a vital growth substance for the pollen. A modified form of Straub's hypothesis has been adopted by Lewis (1954) and Crowe (1955) to explain interspecific relationship in *Œnothera.*

The (SI ×SC) inhibition can be readily accommodated into this hypothesis if we assume that (1) the S complex is necessary for the production of a pollen enzyme : (2) that by a further activity of the S complex this enzyme is bound to a highly specific protein : (3) that the **S** gene produces two types of antibodies in the style, a primitive one which is able to combine with the unbound enzyme and a more recent one which combines with the protein of the enzyme-protein complex.

We assume that (1) SC species have only the enzyme without the protein antigen and without the two antibodies in the style. (2) The SI species have the full array of antigens and enzymes. (3) The exceptional self-compatible species, the pollen of which is not inhibited in SI styles, have two stylar antibodies and an altered protein antigen in the pollen. This class (Sc) receives detailed consideration in the following section.

5. THE ORIGIN OF SELF-COMPATIBLE SPECIES

From the data presented in this paper and from arguments which will be developed later, it is assumed with Whitehouse (1950) that self-incompatibility has been established in the angiosperms almost from the time of their origin. The question to ask at this stage is how the self-compatible species existing to-day have originated, and not how self-incompatibility has evolved.

The present results, and the published data in *Nicotiana* species (Anderson and de Winton, 1931) ; *Petunia* species (Mather, 1943) ; *Lycopersicon* species (McGuire and Rick, 1954) and *Antirrhinum* species (Harrison and Darby, 1955) allow the following rule to be formulated :

> *In the four possible combinations in crossing self-incompatible (SI) and self-compatible (SC) species, pollen-tube growth is uninhibited except in the cross SI style ×SC pollen, where it is inhibited similarly to SI pollination.*

Naturally where the crosses are very wide there may be other factors obscuring the relationships outlined in the rule. For example these are present sometimes between different families ; as we have shown, the pollen of the Solanaceæ does not germinate on stigmas of *Antirrhinum*. The situation in the Onagraceæ (fig. 3) does not conflict with our thesis, though this family requires special treatment. But even in crosses between closely related species there are several exceptions to the rule which prove to be of great significance.

In the Solanaceæ (fig. 1) our specimens of *Lycium chinense* are self-compatible, and yet its pollen is not inhibited in styles of self-incompatible species. Furthermore its styles inhibit the pollen of the self-compatible species. In *Antirrhinum*, Harrison and Darby (1955) found that the self-compatible *A. majus* was not inhibited in SI species, and its own style rejects pollen of the SC *A. orontium*. Similarly in the Papaveraceæ, the self-compatible *Papaver alpinum* pollen is not inhibited in the styles of the SI, *Papaver nudicaule* (Fabergé, 1944). The pollen of Sc *Nicotiana langsdorfii* is not inhibited in SI *Nicotiana alata* (East, 1919).

We are faced with the four exceptions and others will, no doubt, be found. There is good evidence that all the four species have been SI in the past, and that the change to self-compatibility has occurred recently. *Lycium chinense* has close relatives which are reported to be self-incompatible (East, 1940). It has been grown as an ornamental plant for its berries and is propagated by cuttings. A self-compatible form by mutation would be of great horticultural value and would be selected. *Antirrhinum majus* has been cultivated and selected for self-compatibility. All its wild races are SI (Baur, 1932). Darwin (1876), states that *Papaver alpinum* is self-incompatible, indicating that there are two forms of this species, the Sc presumably being a recent mutant of the self-incompatible form. *Nicotiana langsdorfii* also has a self-incompatible race (Goodspeed, quoted by East, 1940). Thus all these four species have SI races and there is evidence that the Sc condition is a recent acquisition.

Turning to the SC species which conform to the rule we have no evidence that self-incompatible races exist, and there is good evidence in two of the species that they have been self-fertilising for a long time. For example, in the Solanaceæ, *Lycopersicon esculentum* is self-compatible in wild populations (Rick, 1950). This species also shows little heterosis and no inbreeding depression. In the Scrophulariaceæ *Antirrhinum orontium* has a small flower which enforces self-pollination. These are differences from other SI species of *Antirrhinum* which could have accumulated only after long self-pollination.*

Our data for the sporophytic system in the Cruciferæ show that pollen tubes are not inhibited in crosses between SI and Sc species whichever way the cross is made, if the Sc parent is from a genus which contains both SI and Sc species. Two genera *Æthionema* and *Draba* in which only SC species have been found (Bateman, 1955) show the (SI × SC) inhibition. Thus there is a parallel with the behaviour found in the gametophytic system. Only those species show the inhibition which have been SC for a long time, as inferred by the occurrence of only SC species in the genus.

The facts indicate the following sequence of events leading to a self-compatible species in families which are primarily and predominantly self-incompatible. A mutation in a self-incompatible species, SI——>Sc : this is the condition in a recent self-compatible species like *Antirrhinum majus*. A further mutation occurs to give Sc——>SC : this is found in a long established self-compatible species like *A. orontium* and *Lycopersicon esculentum*.

The genetic evidence for this sequence of mutations is obtained from mutation studies of the **S** gene in *Œnothera*, *Prunus* and *Trifolium* (Lewis, 1951 ; Pandey, 1956). Spontaneous and induced mutations in pollen-mother-cells give **S** alleles that are Sc in their action, so that the pollen carrying these alleles is not inhibited in its own style or in styles carrying any of the many normal SI alleles. These mutations occur spontaneously at a frequency of 1×10^{-7}, and if self-pollination occurs they will be rigorously selected. An **SISc** heterozygote is produced, which on further selfing produces homozygotes **ScSc**.

The stylar activity of the **Sc** mutants is unimpaired (Lewis, 1954). A homozygote **ScSc** produced experimentally in *Œnothera organensis* behaves like the Sc *Antirrhinum majus*. It is self-compatible, its pollen will grow down the style of SI species and its style has the same power of inhibition as the SI styles. Once such an **ScSc** homozygote became thoroughly established as a self-pollinating species, isolated from its ancestor and near relatives, other changes would occur. Not only will the flower be reduced in size and attraction, having no longer to cater for the pollinating insect, but the self-incompatibility gene will undergo further mutation. The activity of the **S** gene controlling the style reactions is now redundant. Mutations affecting this activity are bound to occur, and without the force of selection against them,

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* Information in the literature is in accord with the rule and its exceptions. Kostoff (1941) reports that the pollen tubes of *Lycopersicon esculentum* (SC) are arrested in the styles of *Nicotiana tabacum* (probably Sc) whereas the pollen of *Petunia violacea* SI reaches the ovaries of *Nicotiana glauca*, *N. langsdorfii* (SI and Sc) and *N. suaveolens*. Cooper and Brink, (1940) report pollen tubes of *Petunia violacea* SI and *Lycopersicon esculentum* in the ovaries of *Nicotiana rustica* (SC). *Cf.* Bellartz, *Planta*, *47*, 1956.

the mutants will spread in the population. This should give an intermediate stage of a self-compatible species (Sc′) whose style does not inhibit pollen of SC species and whose pollen is not inhibited in SI styles. Such an intermediate species has not been found.

So far the mutations postulated have been observed in experiment ; the remainder is conjecture.

The final stage as found in the old SC species would be the result of a third mutation of the **S** gene, leaving the pollen unprotected from the inhibitory effect of the SI style. The second mutation, Sc——>Sc′, would have to be well established in homozygous individuals before this last mutant could survive. This three-stage sequence of events, two steps of which are known from experiment, is somewhat elaborate. But after it has been postulated it is obvious that these are the minimum steps required. Clearly a mutation in an SI species in one step to SC could not become established. A pollen grain carrying such a mutant would be barred from fertilisation because there would be no style in which it could grow. Even if such a mutant occurred in an embryo-sac, there would be no selection for it, and furthermore a plant arising from it would be a heterozygote, **SI.SC.** This would be self-incompatible (McGuire and Rick, 1954 ; Harrison and Darby, 1955) with the result that homozygotes could not be formed. There is a small chance of it arising in an embryo-sac followed by non-reduction and the production of an apomictic homozygote **SCSC**. This would have little chance of survival because, as an isolated individual, it would be deprived of the interchange of genes by outcrossing at the crucial early stages of evolution to SC. There is good evidence that one-step mutations from SI to SC do occur but are lethal. For example in the tetraploid species *Trifolium repens*, Pandey (1956) finds that mutations which lack all detectable activities, stylar and pollen, of the **S** gene occur frequently. But these are not recovered in diploid species. It is presumed that a locus, no longer directly concerned with incompatibility, is able to mask the lethal effect of a deletion at the S locus in this tetraploid.

Once a species has self-pollination enforced upon it by lack of a pollinating insect then there would be a great selective pressure to follow the sequence of events SI——>Sc——>Sc′——>SC.

6. BALANCED LETHALS AND INCOMPATIBILITY IN *ŒNOTHERA*

The great similarity of the incompatibility systems in the Solanaceæ, Scrophulariaceæ, and Onagraceæ gives us some grounds for applying the conclusions derived from the data in the Solanaceæ and Scrophulariaceæ to the Onagraceæ.

From the data in the Solanaceæ and Scrophulariaceæ we have concluded that Sc species which are not inhibited as pollen in SI styles have only recently become Sc. The results of all interspecific pollinations in *Œnothera* show no inhibition of Sc pollen in SI styles. These pollinations include the ring-forming species *O. biennis* and *O. lamarckiana*, so we conclude that these species have been SI in the past and have not progressed very fàr towards a full SC species. But the elaborate system of balanced lethals and ring chromosomes accompanied by cleistogamy would suggest that self-pollination has been established over a long period (Cleland, 1950 ; Darlington, 1931). This seeming paradox, an old self-pollinating species retaining the vestiges of self-incompatibility which allow its pollen to grow down an SI style, can readily be resolved if we assume that the SI system has played a key role in the evolution of the balanced lethal system.

Before doing this we should point out the main weakness of our data in the Onagraceæ. There is no clear-cut (SI ×SC) inhibition in any crosses even when *Epilobium* and *Godetia* pollen are used. These are genera without a regular ring-chromosome mechanism and without incompatibility. The data would be more compelling if some of these had shown (SI ×SC) inhibition.

The ring-forming, self-pollinating species with balanced lethals have evolved from ancestors with seven bivalents and cross-pollination. Steiner (1956) presents good evidence that the alpha complex of *O. biennis* contains an incompatibility allele while the beta complex does not carry such an allele. In the ring-forming species there may be balanced lethals in other chromosomes, but the key pair is the one which has the pollen lethal. Catcheside's (1936) observations from trisomics indicate that only one or two chromosomes contain lethals. Our hypothesis is that the pollen lethal was the first to arise and was present as the incompatibility gene in all plants of the cross-pollinating ancestor. This is similar to Crosby's (1954) and Steiner's (1956) hypotheses.

There are two known ways in which a balanced lethal system could start from self-incompatibility and both these ways have their counterpart in modern ring-forming *Œnotheras*.

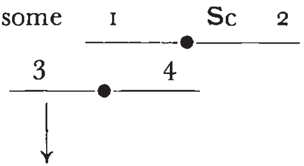
(i) *Sc mutation*

First a mutation of S1 ⟶ Sc similar to the mutation found in experiments with *Œnothera organensis* would be constantly occurring in an SI ancestor. If cross-pollination was reduced by the lack of a pollinating insect then this mutant would be selected rigorously. The homozygote ScSc would be viable at first, and the population would consist of groups of plants, ScSc, ScS1 : ScSc, ScS2, etc. All that is required to complete the first stage is a reciprocal interchange in one arm of the Sc chromosome with another non-homologous chromosome, accompanied by either a position effect, gene mutation or minute deletion that is lethal in the homozygote or in the egg.

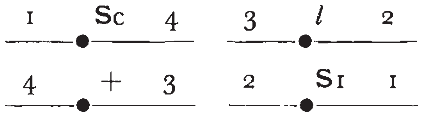
The steps in the evolution of the system would be :

1. $S1S2 \xrightarrow{\text{mutation}} S1Sc \xrightarrow{\text{population}} S1Sc, ScSc, S2Sc$
2. Interchange of chromosome

with chromosome



A chain of four



transmitted on selfing only in pollen

transmitted only in egg

3. Further interchanges may occur in other chromosomes ending in a ring of 14.

If the lethal, *l*, is a zygotic lethal we could have complexes :

These would segregate regularly to give functional pollen and eggs of both complexes, but only the heterozygotes survive as in *Œnothera lamarckiana* (Renner, 1914).

The pollen-egg lethal system would be :

This is found in many species including *O. biennis* (*cf.* Cleland, 1950).

One essential feature of this origin is that the first interchanged chromosome should involve the mutant Sc allele and not the S1 allele. Cleland (1950) in his detailed analysis of the interchange in N. American *Œnotheras* states that " none of the alpha (pollen lethal, *i.e.* S1) have suffered the interchange 1.2 3.4 → 1·4 3·2 whereas all the beta (egg lethal, *i.e.* Sc) complexes have done so ".

Cleland (*loc. cit.*) is also impressed by the narrow range of interchanges found in a wide sample of material. He states " The presence of so many complexes with similar segmental arrangements constitutes, therefore, a fact of real phylogenetic significance. It seems to indicate that these complexes are more or less closely related and that a considerable proportion of the complexes existing throughout the range of *Œnothera* have not departed very far from the ancestral segmental arrangement from which they have been derived." If, as we believe, the first important interchange in all cases involved the Sc locus then this narrow range of complexes is not unexpected.

One puzzling feature of the original balanced lethal system of the ring-forming *Œnotheras* in which the lethals are gametophytic is that these " lethal " pollen grains are often viable in outcrosses. The balanced lethal system working on pollen gametophytes cannot satisfactorily explain how these gametophytes are viable when they are on another female. The explanation for this is to assume, as Steiner (1956) has done for *O. biennis* that different heterozygotes (species) have different SI alleles. For example in the cross between S1Sc × S2Sc, the S2 pollen, which is " lethal " on selfing, can function normally.

An unexplained but extremely important phenomenon was found in the autotetraploid of *O. pictorubata* by Renner (1939). The diploid is a self-pollinating ring-forming heterozygote : the tetraploid is self-incompatible, setting no seeds. The tetraploid is fertile when used as a female or a male with other tetraploid heterozygotes and as a female with its own diploid. It was sterile when used as a pollen parent on to its diploid form. Clearly the cause of the self-incompatibility was not because of sterile pollen or eggs, but because the pollen would not function when selfed, and the cause was actually found to be due to inhibition of pollen tubes. It was also found that the viable pollen grains of the tetraploid contained both the egg and pollen complexes.

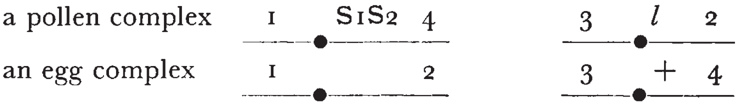
This unusual phenomenon of self-incompatibility in an autotetraploid of a diploid Sc species is in agreement with our hypothesis. If the pollen " lethal " in the diploid were a recessive lethal causing a deficiency in the pollen, as the balanced lethal theory implies, then the diploid pollen of the tetraploid with both complexes should be able to function in selfing because there would be the dominant wild-type gene to cover the deficiency. But the results with the diploid pollen show that the lack of pollen-tube growth is not due to a deficiency but to a positive inhibition which cannot be rectified by the gene which allows pollen-tube growth in the haploid grain. Clearly, if we assume the diploid *O. pictorubata* is ScS1, then diploid pollen grains of the tetraploid, which are viable, will be ScS1 and these will be inhibited in ScSc S1S1 styles of the tetraploid or ScS1 styles of the diploid but will not be inhibited in ScSc S2S2 styles of another tetraploid heterozygote.

(ii) *S_1S_2 duplication*

The second way of starting a pollen lethal system from the S gene is by unequal crossing-over to give a duplication of the S locus in one chromosome and a deficiency in the other. This has consequences different to those of the first method of Sc mutation. Catcheside (1947) has described such an interchromosomal duplication and deficiency that arises frequently in *Œnothera blandina.* Indeed, Darlington (1931) postulated an interchromosomal duplication in two chromosomes and a deficiency of a small non-lethal interstitial segment in the two homologous chromosomes as a means of obtaining viable segmental interchanges in *Œnothera.* Duplication to give an adjacent repetition of the same chromosomal material is common in *Drosophila* (Lewis, E. B., 1951).

In an SI species two S genes in one chromosome and none in the other would have two effects. The chromosome with the two S genes in an SI species would be S1S2. In diploid pollen grains of tetraploid *Œnothera organensis* and in several other genera (*cf.* Lewis, 1954) competition between two different S alleles occurs so that neither allele can operate. Hence by analogy we would expect a similar situation to occur in haploid pollen grains carrying different S alleles in the same chromosome, in which event S1.2 pollen would be compatible on styles carrying S1 and S2 and on all other styles. The S1S2 duplication would then be the starting point of the pollen transmissible complex. The S deficiency, on the other hand, would be lethal in the pollen. That S gene deficiencies are lethal in pollen has been found from mutation experiments in *Œnothera organensis*, *Prunus avium* and *Trifolium* species (Lewis, 1954 ; Pandey, 1956).

Thus, with the necessary translocation, we have :



The classical example of this type of complex is found in *Œnothera muricata* in which half the pollen is inviable (Renner, 1919). Professor Cleland has drawn our attention to *O. chicagnensis* which has normal pollen but one complex is never transmitted through the style.

Test of the hypothesis—general evidence

There are several tests for proving or disproving these two ways of origin of the balanced lethal complexes. A detailed study of pollen-tube growth in controlled self- and cross-pollinations of well chosen genotypes would be informative. The growth of incompatible pollen tubes in SI species of *Œnothera* has a characteristic temperature curve (Lewis, 1942 ; Crowe, 1955). If a similar temperature curve was found in the pollen carrying the lethal complex, then there could be little doubt that the same incompatibility reaction and probably the same S gene is operating.

The behaviour of autotetraploids could be a valuable indication. In species such as *O. lamarckiana*, with zygotic lethals and a supposed ScSc constitution, the autotetraploid should be self-compatible. In fact the autotetraploid *O. lamarckiana gigas*, which has zygotic lethals, is self-compatible. In species which have gametophytic lethals and in which the pollen can function in some outcrosses such as *O. biennis*, *O. pictorubata*, etc. then we might expect self-incompatibility of the autotetraploid. However, if the Sc chromosome in the duplex state, ScSc, is viable in the pollen then the tetraploid would be self-compatible. In such species as *Œnothera muricata* with a supposed S1S2 duplication with competitive interaction of the S alleles, then the pollen from the tetraploid should remain self-compatible.

Darlington and Gairdner (1937) have made a comprehensive comparison of the ring-forming species in plant genera and note that "the self-sterility of *Campanula* while promoting the occurrence of interchange heterozygotes at the beginning, is not likely to favour the further development of a multiple ring system for this reason : self-sterility has already given the species a high hybridity equilibrium which a multiple ring system can do little to increase ". But if self-pollination became a necessity because of difficulty of ensuring cross-pollination then there would be a great advantage of initial self-incompatibility not only in forwarding the interchange but in preserving it later when the self-incompatibility had broken down by the Sc mutation or by the S1S2 duplication. It is significant that the three species *Zea mays*, *Pisum sativum* and *Datura stramonium* which have interchange ring chromosomes in experiment but not in the wild are from exclusively SC genera.

It is also suggestive that the two other examples of ring-forming heterozygotes species are *Rhœo discolor*, which has the SI genus, *Tradescantia*, as a near relative, and *Hypericum punctatum* (Hoare, 1931) which has species in the same genus which are SI East, 1940).

The important feature of the Sc origin of the balanced lethals is that a ready-made pollen lethal was there in the cross-pollinated ancestor, and only the simplest structural change with the assistance of enforced self-pollination was necessary to get the system launched. Clearly the final success of the change depends upon all the factors which have been expounded by Darlington (1936), such as terminal chiasmata and regular orientation of centromeres.

The paradox, referred to earlier, that the ring-forming *Œnothera* have been self-pollinated for a long time and yet our results on interspecific pollination indicate that they are behaving like species which have only recently mutated from self-incompatibility, is now resolved. Clearly if the **SI** gene is the key to the balanced lethal system, then it must retain the activity revealed by the interspecific pollinations.

7. ADAPTATION AND THE BREEDING SYSTEM

Darwin proved the advantages of outbreeding, and it has often been demonstrated since in a wide variety of organisms. We do not intend to add to this list but wish to make two points.

1. The evolution of many complex outbreeding mechanisms in flowering plants, and also in bacteria, fungi, algæ and animals, emphasises their extreme importance in the evolution of organic life. The recent discoveries of sporophytic systems in the angiosperms are only more examples of the lengths an organism will go to in its pursuit of hybridity (*cf.* Darlington, 1939).

2. In the flowering plants and fungi, and possibly in other groups, where great diversity of outbreeding devices has been found, a high

proportion of highly successful species exist which have abandoned outbreeding and have had sexual self-reproduction for a long time.

Whitehouse (1950) has actually attributed the explosive flowering of the angiosperms in the Cretaceous period to the evolution of the style and self-incompatibility. Undoubtedly self-incompatibility, particularly of the multiple-allelic type, is one of the most efficient outbreeding mechanisms that can be envisaged. Our evidence presented in this paper proves that self-incompatibility systems are not recent innovations in the angiosperms. There is only one conclusion to be drawn from the results of crosses within and between the two families, Solanaceæ and Scrophulariaceæ. The incompatibility system was fully evolved in the common ancestor of these two large families.

One other conclusion which seems highly probable from our data is that the self-compatible species in families which have self-incompatibility have been derived by a degradation process from self-incompatible ancestors.

The paradox of evolution is the prime importance of outbreeding on the one hand and the presence of a high proportion of long established and successful species that are inbreeders. It has frequently been said that inbreeding may lead to an immediate advantage at the expense of long-term fitness. This is true, but it is not the whole answer to the paradox, because our evidence shows that many plants have been inbreeders for a long time and have yet maintained their fitness. The answer to this may be found in what we may call the *critical stage in evolution*. In the life of an organism, whether it is a self-reproducing macromolecule in a primitive sea or a higher plant or animal, there is a period in its history when it is competing in a common environment with other organisms. This is the period when an outbreeding mechanism is absolutely essential for survival. It is only by an outbreeding system that the immense advantage of gene recombination can be obtained. This period is probably short as implied by Gause's (1934) principle, that two or more forms with identical ecological requirements cannot co-exist indefinitely in the same environment. After the species has captured its environment and the critical stage is over it may still retain its outbreeding system, or because of some advantage or because it is forced to by causes outside its own control, such as the lack of a pollinator, it may abandon the very means of its success. Only after a cataclysmic change in the environment which might not occur for a million years will the inbreeder be at a disadvantage.

8. SUMMARY

1. It is argued that the distribution of the five self-incompatibility systems in the families of the angiosperms is evidence that the systems were perfected at an early stage in the evolution of the group.

2. The value and shortcomings of interspecific crosses to reveal similarities and differences in breeding systems are outlined.

3. In the gametophytic system the literature reveals four examples of pollen inhibition of a self-compatible (SC) species in the styles of a self-incompatible (SI) species. This is the (SI ×SC) inhibition. But at the same time four examples are quoted where such interspecific crosses do not show the inhibition.

4. Pollinations between different SI and SC species and genera in the Solanaceæ and between this family and the Scrophulariaceæ prove that the (SI ×SC) inhibition rule is a general phenomenon.

5. The exceptions to the rule are varieties and species (Sc), which have only recently mutated from an SI species. The species which obey the rule have also originated from an SI ancestor but a long time ago.

6. A probable sequence of mutations, some of which have been produced experimentally, is postulated to explain the occurrence and behaviour of the three types of species SI, Sc (recent), SC (old).

7. Pollinations between species and genera in the Cruciferæ with a sporophytic system, and examples culled from the literature, indicate that the (SI ×SC) rule may hold in sporophytic systems.

8. Interspecific and intergeneric pollinations in the Onagraceæ lead to the conclusion that the ring-forming, self-pollinating *Œnotheras* behave like recent Sc species. A theory of the origin of the permanent heterozygote and balanced lethals from the incompatibility gene is given : (1) by mutation SI——>Sc or (2) by a duplication and deficiency to give an S_1S_2 duplication and an S deficient chromosome.

9. The (SI ×SC) inhibition is discussed in relation to the biochemistry of incompatibility and the " critical stage in evolution ".

9. APPENDIX, SI . SC . Sc

The symbol SI is used for a self-incompatible species.

The symbol SC is used for an old self-compatible species which shows (SI× SC) inhibition.

The symbol Sc is used for a self-compatible species which does not show the (SI× SC) inhibition. In most cases such species have a recent history of self-incompatibility.

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