THE EVOLUTION OF INCOMPATIBILITY
IN SPECIES OF *CENOTHERA*

LESLIE K. CROWE

John Innes Horticultural Institution, Bayfordbury, Hertford, Herts.

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I. INTRODUCTION

RELATED groups of plants often share a common type of incompatibility system. Of those systems which are not associated with differences in flower form, an incompatibility which is gametophytic appears to be widespread in the *Scrophulariaceae*, *Solanaceae* and *Rosaceae*. In these families all the eleven genera which have been studied in detail have this type of breeding system. Similarly, in the *Compositae*, a sporophytic incompatibility system has been found in all of the four self-sterile genera which have been studied and, in the *Cruciferae*, the same system is again present in five genera.

At the generic level the same likeness in the type of incompatibility is found in different species of *Nicotiana*, *Solanum*, *Petunia*, *Trifolium*, *Cenothera* and *Primula* (cf. table 1). Indeed, evidence from inter-specific hybrids shows that in the first three of these genera, the similarity is even closer, for not only is the type of incompatibility the same, but the S locus itself is sometimes the same in different species of the same genus.

Within diploid species of the genus *Solanum*, alleles of the same incompatibility gene are present in two distinct species of the *Tuberosa* group. Alleles of a different S gene are distributed amongst five species (or sub-species) in the *Commersoniana* series.

Hybrids between the two highly self-incompatible species *Nicotiana glauca* and *N. glauca*, are often pseudo-fertile (Brieger, 1930). Mather (1943) assumes that these two species have the same S locus, but different, highly selected, polygenic combinations on which the efficiency of the major gene depends. In the hybrids, the balance of the polygenic modifiers would be upset, and this would lead to a weakening of the incompatibility mechanism. Genes of the self-compatible species *Petunia axillaris* have similarly been found to weaken the incompatibility reaction of the alleles S₁ and S₂ from self-sterile *P. violacea* (Mather, 1943), cf. East (1932), Anderson and de Winton (1931) and Brieger (1930) on *Nicotiana*.

The present study is concerned with the evolution of the incompatibility system in different species of *Cenothera*. The genus includes many self-fertile species which preserve heterozygosity by means of ring chromosomes coupled with a system of balanced lethals. Apart

from these self-fertile types, there are also species which are self-sterile and five of these are confirmed examples of gametophytic incompatibility (*cf.* table 1).

TABLE 1

*The relation of the incompatibility of different species
within the same genus*

Within the six genera in the table all the species have the same type of incompatibility system. Some species, which are enclosed in boxes, have the same incompatibility locus. (*Nicotiana Langsdorffii* and *Petunia axillaris* are self-fertile, but the S locus is the same as that in related self-sterile species).

	Genus	Species	Authority
Heteromorphic Incompatibility	<i>Primula</i>	<i>sinensis</i> <i>obconica</i> <i>officinalis</i> <i>acaulis</i> <i>hortensis</i> <i>viscosa</i>	Bateson and Gregory, 1905 Lewis and Dowrick (unpub.) Dahlgren, 1916 Gregory, 1915 } Ernst, 1936
	<i>Trifolium</i>	<i>repens</i> <i>pratense</i> <i>hybridum</i>	Atwood, 1940 Williams and Silow, 1933 Williams, 1951
	<i>Oenothera</i>	<i>organensis</i> <i>rhombipetala</i> <i>deltoides</i> <i>pallida</i> <i>missouriensis</i>	Emerson, 1938 Hecht, 1944 } Crowe (unpub.)
Homomorphic Gametophytic Incompatibility	<i>Nicotiana</i>	<i>alata</i> <i>forgetiana</i> } <i>Sanderæ</i> <i>(Langsdorffii)</i>	East and Mangelsdorf, 1925 Anderson and de Winton, 1931 Brieger, 1930
	<i>Petunia</i>	<i>(axillaris)</i> <i>violacea</i>	Mather, 1943 Harland and Atteck, 1933
	<i>Solanum</i>	<i>Caldisii</i> <i>subtilis</i> <i>chacoense</i> <i>arac-papa</i> <i>ju-juense</i>	} Pal and Pushkar Nath, 1942 Pushkar Nath, 1953 Pushkar Nath, 1945
		<i>simplicifolium</i> <i>boyacense</i> = <i>Ribinii</i>	Black (unpub. see Lewis, 1949a) Carson and Howard, 1942

2. MATERIALS AND METHODS

The species of *Oenothera* used in these experiments are listed (table 2).

Our collections accord with the descriptions of the species given by Munz (1930, 1931, 1932, 1935).

Throughout this study, compatibility was determined by pollen-tube growth. At 25° C. there is a clear difference in the growth of compatible and incompatible pollen tubes from four hours onwards. The styles were normally examined whole

after one hour in iodine fixative (Lewis, 1948). When accurate measurements of the number and length of individual pollen tubes were required the styles were double stained in iodine and cotton blue, the iodine staining the starch granules and the cotton blue staining the cytoplasm.

Under certain conditions when it was necessary to limit the number of pollen grains used in a cross, the grains were counted on a slide under low magnification before being applied to the stigma.

In many crosses made for seed, the capsules were painted with an emulsion of lanoline and 5 per cent. Fulset, a commercial product containing beta naphthoxy-acetic acid. This procedure increased the yield of seed by stimulating the swelling of capsules containing only a small number of seeds which would otherwise not have developed.

TABLE 2

Subgenus	Species	Variety		Origin	Source
<i>Lavauxia</i>	<i>acaulis</i>	...	SF	...	Bot. Gdn., Adelaide
<i>Megapterium</i>	<i>missouriensis</i>	<i>Oklahomensis</i> (Norton)	SI	...	Bayfordbury
<i>Raimannia</i>	<i>rhombipetala</i> (Nutt.)	...	SI	...	"
<i>Euænothera</i>	<i>organensis</i>	...	SI	New Mexico	S. Emerson, Pasadena
<i>Hartmannia</i>	<i>speciosa</i>	<i>typica</i> (Nutt.)	SI	...	Bayfordbury
<i>Anogra</i>	<i>deltoides</i>	<i>typica</i> (Torr. and Frem.)	SI	California	P. A. Munz, Anaheim
	<i>runcinata</i>	<i>typica</i> (Engelm.)	SI	New Mexico	D. B. Dunn, New Mexico
	<i>latifolia</i> (Rydb.)	...	SI	Wyoming	A. Hecht, Pullman
	<i>pallida</i>	<i>typica</i> (Lindl.)	SI	Washington	" "
	<i>trichocalyx</i> (Nutt.)	...	SF	Washington	" "

SF = Self-fertile

SI = Self-sterile

The *Anogra* species used were from the Western States of America and had low interchange hybridity. Meiosis in the pollen mother cells of *O. pallida* and *O. latifolia* showed a chain of four chromosomes. Other species of the *Anogra* group had seven bivalents. The hybrids varied. Some had seven bivalents, others, a chain of four chromosomes. The cross *O. pallida* × *O. trichocalyx* gave two exceptional seedlings with a chain of six chromosomes.

3. GENERAL RESULTS

The ten species of *Ænothera* were selfed and crossed interspecifically in many combinations (cf. fig. 1). All the species were self-incompatible with the exception of *O. trichocalyx* and *O. acaulis*, both of which gave a full seed set after self-pollination.

All the crosses between species in different sub-genera were sterile. The failure to produce good seed was not due to lack of pollen-tube growth in any of the crosses. In many cases large numbers of seeds with aborted embryos were formed but even when no seed whatever was produced, observation of the pollen tubes 24 hours after pollination showed that they were at the base of the style.

The only crosses to produce viable seed were amongst the five species in the sub-genus *Anogra*. All the combinations were fertile with the exception of the cross *O. runcinata* $\times$ *O. deltoides*.

The *Anogra* group includes the self-compatible *O. trichocalyx* and the four self-incompatible species *O. deltoides*, *O. pallida*, *O. latifolia* and *O. runcinata*. A preliminary study of *O. pallida* and *O. deltoides* showed that the incompatibility system in these two species is of the multiple allelic gametophytic type. This conclusion is based on observations of F_1 and backcross families which gave the straightforward results expected with this system.

FIG. 1.—The fertility of interspecific crosses in 10 species of *Enothera*.

O. latifolia and *O. runcinata* were represented by single plants. It was therefore impossible to test their intra-specific behaviour, but for the purposes of this study they are assumed to have the same genetic mechanism as the related species. This was later confirmed by the behaviour of their hybrids.

The genetics of the *Enothera* hybrids is complicated by two factors. Firstly, the behaviour of self-sterility alleles in the cytoplasm of *O. trichocalyx* is abnormal. This point will be dealt with in detail in a later section. Secondly, the plant of *O. runcinata* carried a gametophytic lethal gene linked with one of the incompatibility alleles. This fact caused disturbed ratios and reciprocal differences in compatibility between plants. In describing the behaviour of hybrids between the four self-sterile species, anomalies due to the lethal gene will be mentioned briefly in passing leaving the detailed explanation until later.

4. INTERSPECIFIC HYBRIDS OF THE FOUR SELF-INCOMPATIBLE SPECIES

*F*₁ generations

The *F*₁ hybrids of the self-incompatible species were themselves self-incompatible. Plants within each family were intercrossed in all possible combinations. A summary of the results is given in table 3. The number of mating groups in the families varied. Large families contained four groups, while in smaller ones, only two or three groups were represented which can be attributed to the small samples available for testing.

TABLE 3
The distribution of mating types in F₁ hybrids of four self-incompatible species of Oenothera

Cross	No. of mating groups	Proportion of plants in each group
<i>O. pallida</i> × <i>O. latifolia</i>	4	3 : 2 : 2 : 2
Reciprocal	3	1 : 3 : 1 : 0
<i>O. pallida</i> × <i>O. deltoides</i>	4	3 : 3 : 2 : 3
Reciprocal	4	5 : 3 : 2 : 2
<i>O. pallida</i> × <i>O. runcinata</i>	3	12 : 8 : 1 : 0 *
Reciprocal	3	3 : 0 : 1 : 1
<i>O. latifolia</i> × <i>O. deltoides</i>	4	6 : 2 : 2 : 3
Reciprocal	3	3 : 2 : 2 : 0
<i>O. latifolia</i> × <i>O. runcinata</i>	2	3 : 1 : 0 : 0
Reciprocal	2	0 : 1 : 1 : 0
<i>O. deltoides</i> × <i>O. runcinata</i>	4	11 : 5 : 1 : 1 *
Reciprocal	...	...

* The disturbed ratio in these families is caused by a gametophytic lethal gene in *O. runcinata* pollen.

Plants within the same group were cross-incompatible. Plants in different groups were reciprocally compatible. These relationships were as clear cut as in intra-specific crosses. In no case was the result ambiguous. The growth of incompatible pollen tubes was confined to the stigma while compatible pollen was at the base of the style after eight hours.

The only remarkable thing about these *F*₁ families is that the mating types in crosses with *O. runcinata* as the male parent show a striking deviation from the expected ratios. This is one of the anomalies caused by the presence of the pollen lethal in *O. runcinata*.

Normally, in fully compatible crosses in which there is no allele in common between the parents, we expect to find the same mating groups in reciprocal progeny. For this reason reciprocal hybrids were intercrossed using single plants from each mating group. Only in crosses between *O. pallida* and *O. deltoides* were four groups present

in both families, and here the results fully confirmed that each class present in one cross was also represented in the reciprocal. Results from families involving other species conformed with this conclusion although the data were necessarily incomplete.

Backcross families

Single F_1 plants from six families were crossed reciprocally with at least one of their parents. (Refer to table 4 for details.) The families include all combinations of the four species.

All the progeny from these crosses were self-incompatible. Two cross-compatible sibs in each family were used as testers and the rest of the plants were classified on their ability to cross with them. In every family two intra-sterile inter-fertile mating groups were found, with the exceptions that, one family consisted of one plant only, and in the progeny from the crosses *O. runcinata* $\times$ (*O. runcinata* $\times$ *O. pallida*) and *O. runcinata* $\times$ (*O. deltoides* $\times$ *O. runcinata*) there was a reciprocal difference in compatibility between the two groups, caused by the lethal gene.

Reciprocal backcross families were intercrossed using single plants from each mating group. There was always incompatibility between one class in reciprocal families. This common genotype was compatible as pollen on both parents. The other genotypes were incompatible with their pollen parent, but compatible with the female (*cf.* table 4). There are four exceptions to this rule, all of which can be attributed to the lethal gene in *O. runcinata*.

These results are consistent with the regular behaviour of F_1 families and are to be expected if the *S* loci in the four species are homologous.

F_2 generations

It was impractical to raise large F_2 families because the seed from F_1 sib crosses gave a very low percentage of germination. This fact is important in so far as it confirms that the differences between the four *Oenotheras* from which the hybrids were produced are of specific rank. Three families of F_2 plants were obtained from crossing compatible sibs selected at random from the F_1 's (*O. pallida* $\times$ *O. latifolia*), (*O. deltoides* $\times$ *O. pallida*) and (*O. deltoides* $\times$ *O. runcinata*). Progeny from the first two crosses were of two genotypes; and in the third cross, four genotypes were present. All the plants were self-incompatible and reciprocally cross incompatible with other plants in the same group. Crosses between members of different groups gave only compatible results.

Thus, in hybrids of the four species *O. pallida*, *O. deltoides*, *O. latifolia* and *O. runcinata*, no evidence has been found in F_1 , F_2 or backcross families of segregation of different incompatibility loci. The regularity of the results leaves no doubt that the locus is homologous in these species.

The efficiency of the S alleles in the F_1 , and in particular in the F_2 and backcross hybrids, is equal to that within the species when

TABLE 4
The behaviour of progeny from reciprocal backcrosses between F_1 hybrids and the parent species

Parents	Progeny						
	No. of mating types	Group I			Group II		
		No.	$\times \delta^\dagger$	$\times \varphi$	No.	$\times \delta^\dagger$	$\times \varphi$
<i>(O. pallida</i> $\times$ <i>O. latifolia)</i>							
$\times$ <i>O. latifolia</i>	2	3	—	+	5	+	+
Reciprocal	2	4	—	+	4	+	+
<i>(O. runcinata</i> $\times$ <i>O. latifolia)</i> $\times$ <i>O. r</i>	0						
Reciprocal	2	5	—	+	3	—*	+
<i>(O. runcinata</i> $\times$ <i>O. latifolia)</i>							
$\times$ <i>O. latifolia</i>	2	3	—	+	1	+	+
Reciprocal	2	4	—	+	2	+	+
<i>(O. deltooides</i> $\times$ <i>O. pallida)</i>							
$\times$ <i>O. deltooides)</i>	2	4	—	+	6	+	+
Reciprocal	2	5	—	+	3	+	+
<i>(O. deltooides</i> $\times$ <i>O. latifolia)</i>							
$\times$ <i>O. deltooides</i>	2	2	—	+	5	+	+
Reciprocal	2	3	—	+	2	+	+
<i>(O. deltooides</i> $\times$ <i>O. latifolia)</i>							
$\times$ <i>O. latifolia</i>	2	4	—	+	3	+	+
Reciprocal	2	1	—	+	1	+	+
<i>(O. deltooides</i> $\times$ <i>O. runcinata)</i>							
$\times$ <i>O. deltooides</i>	2	3	—	+	7	+	+
Reciprocal	2	4	—	+	3	+	+
<i>(O. deltooides</i> $\times$ <i>O. runcinata)</i>							
$\times$ <i>O. runcinata</i>	1	1	—	—*	...	...	...
Reciprocal	2	3	—	+	4	—*	+
<i>(O. pallida</i> $\times$ <i>O. runcinata)</i>							
$\times$ <i>O. runcinata</i>	2	2	—	+	4	+	+
Reciprocal	2	2	—	+	1	—*	+

* The exceptional behaviour of these groups is caused by the gametophytic lethal gene in *O. runcinata*.

† Male ($\times \delta$) and female ($\times \varphi$) parents used as female testers.

judged on the criterion of pollen-tube growth. It must be concluded therefore that the non-allelic modifying genes in the species are also alike.

5. A GAMETOPHYTIC LETHAL GENE IN *O. RUNCINATA*

The progeny of crosses between *O. runcinata* and *O. pallida* are reciprocally different. Several characters are affected by the direction of the cross, and these are listed in table 5.

The variegation and male sterility occur only in crosses between *O. pallida* and *O. runcinata*. They are reminiscent of the differences in the two crosses between *O. hookeri* and *O. muricata* described by Renner.

The reciprocal differences in viability and in the frequency of the four mating groups are common to all *O. runcinata* hybrids. It is the cause of the uneven distribution of the mating groups in families

TABLE 5
The reciprocal differences in F_1 hybrids from
O. pallida and *O. runcinata*

	<i>O. pallida</i> $\times$ <i>O. runcinata</i>	<i>O. runcinata</i> $\times$ <i>O. pallida</i>
1	Green leaves	Variegated leaves
2	Fertile pollen	Pollen partially or completely sterile
3	High proportion of viable seed	Low proportion of viable seed
4	Uneven frequency of different mating groups	Normal frequency of different mating groups

with *O. runcinata* as a male parent which is of prime importance in this study.

Briefly recalling table 3, the situation is that the cross *O. pallida* $\times$ *O. runcinata* gave an uneven frequency of mating groups (12 : 8 : 1 : 0). This was also true of the cross *O. deltoidea* $\times$ *O. runcinata* (11 : 5 : 1 : 1). The reciprocal cross between *O. deltoidea* and *O. runcinata* was sterile, but five plants were obtained from the cross *O. runcinata* $\times$ *O. pallida* and amongst these the frequency of the different mating groups was normal (3 : 0 : 1 : 1) taking into account the small number of plants.

The obvious explanation for these facts is that one of the **S** alleles present in the pollen of *O. runcinata* does not get through the recipient styles except in rare cases. The presence of a common **S** allele does not account for the results, because of the reciprocal difference and the presence of a small proportion of the two deficient groups. But if one of the alleles of *O. runcinata* is linked with a gene which is lethal in the pollen but not in the ovule, this would give results similar to those that have been observed. Such a gene has been found in *O. organensis* by Emerson (1941). The pollen of *O. runcinata* was examined and about 50 per cent. of the pollen grains were obviously sterile being small and shrivelled without any starch granules. The actual figures were 658 sterile pollen grains to 603 normal ones.

If we denote the incompatibility alleles in *O. pallida* as $S_{1.2}$ and in *O. runcinata* as $S_{3^L.4}$, S_3^L being the allele to which the lethal gene is linked, then from the behaviour of the F_1 hybrids, which is summarised in fig. 2, we can deduce that the progeny from reciprocal crosses have the following genotypes :—

Parents		Progeny		
<i>O. pallida</i> × <i>O. runcinata</i>	$S_{1.4}$ $S_{2.4}$ $S_{1.3}$ (after crossing-over)			
($S_{1.2}$)	($S_{3^L.4}$)			
<i>O. runcinata</i> × <i>O. pallida</i>	$S_{1.4}$ $S_{1.3^L}$ $S_{2.3^L}$			
($S_{3^L.4}$)	($S_{1.2}$)

FIG. 2.—The effect of the pollen lethal gene in *O. runcinata* on the compatibility relations of F_1 hybrids from reciprocal crosses between *O. pallida* ($S_{1.2}$) and *O. runcinata* ($S_{3^L.4}$).

Note.—The occasional pollen tubes observed in the crosses $S_{1.4} \times S_{1.3^L}$, $S_{1.4} \times S_{3^L.4}$ and $S_{2.4} \times S_{3^L.4}$ can be explained by crossing over between S_3 and the lethal gene L .

Of the three mating types from the cross with *O. runcinata* as the male parent the $S_{1.3}$ type is the deficient group with only one plant.

The other genotype expected after crossing-over is $S_{2.3}$. This was absent in the progeny from the cross *O. pallida* × *O. runcinata* but as the sample tested was small this fact is without significance.

The absence of the lethal in this single $S_{1.3}$ plant is confirmed by the compatibility of the cross $S_{1.4} \times S_{1.3}$ and can be explained as the result of a cross-over between the lethal gene and the compatibility locus.

Pollen of an $S_{1.3^L}$ plant from the reciprocal cross is incompatible on $S_{1.4}$ styles and so, too, is pollen of an $S_{2.3^L}$ plant on $S_{2.4}$ styles. This conforms with the theory that the lethal gene can be transmitted by the female parent.

Seed from further crosses was obtained to confirm the validity of the theory. Details of these families will be found in table 6. The results given in the right-hand column are based on exhaustive tests

with the fifty-one mature plants obtained from the crosses. The plants were crossed amongst themselves and also with the F_1 genotypes $S_{1.4}$, $S_{2.4}$, $S_{1.3}$ and $S_{2.3}^L$.

Progeny suspected of being $S_{3.4}$ or $S_{1.2}$ could not be tested with the parent plants because these were no longer alive. It was anticipated that crosses 1 and 2 in table 6 would each give $S_{3.4}$ in their progeny and this genotype would be the only one common to both families.

TABLE 6
*The inheritance of a pollen lethal gene linked with
the S_3 allele in *O. runcinata**

Test cross		No. of pollinations	No. of seeds	No. of seeds per flower *	Genotypes of progeny
$F_1 \times O. runcinata$					
1.	$S_{1.4} \times S_{3^L.4}$	40	124	4.2	$S_{3.4}$ $S_{1.3}$ $S_{2.4}$ † 2 : 6 : 1
2.	$S_{2.4} \times S_{3^L.4}$	38	205		$S_{3.4}$ $S_{2.3}$ 2 : 4
$O. runcinata \times F_1$					
3.	$S_{3^L.4} \times S_{1.4}$	11	21	5.6	$S_{1.4}$ $S_{1.3^L}$ 2 : 1
4.	$S_{3^L.4} \times S_{2.4}$	7	79		...
$F_1 \times F_1$					
5.	$S_{1.3} \times S_{1.4}$	4	5	2.6	$S_{1.4}$
6.	$S_{1.3} \times S_{2.4}$	7	23		$S_{2.4}$
7.	$S_{1.4} \times S_{1.3}$	9	86	9.2	$S_{1.3}$
8.	$S_{2.4} \times S_{1.3}$	7	61		$S_{3.4}$
9.	$S_{1.4} \times S_{1.3^L}$	29	45	2.8	$S_{1.3}$
10.	$S_{2.4} \times S_{1.3^L}$	28	112		$S_{1.2}$ $S_{1.4}$ $S_{3.4}$ 4 : 3 : 2
11.	$S_{1.3^L} \times S_{1.4}$	7	17	5.3	$S_{3^L.4}$
12.	$S_{1.3^L} \times S_{2.4}$	6	38		...
13.	$S_{2.3^L} \times S_{1.4}$	16	95		$S_{1.2}$
14.	$S_{2.3^L} \times S_{2.4}$	17	94		$S_{2.4}$ $S_{3^L.4}$ 4 : 6

* The differences in the number of seeds set in crosses with the lethal gene present in the pollen as compared with others are slight. This is probably due to the masking effect of the overall sterility which is high.

† The $S_{2.4}$ genotype is a result of contamination.

It was, therefore, comparatively simple to pick out these plants and they were used as the $S_{3.4}$ testers throughout. Plants which were compatible with all the available testers were assumed to be $S_{1.2}$.

Although the poor germination has resulted in some gaps in the data, fifty out of the fifty-one plants gave results which are in agreement with theory. Families 3 and 11 in which the lethal gene was present in the female parent gave S_{3^L} types in the progeny. It was not possible to determine whether the lethal was present in the progeny in family 14 because all the plants were male sterile. The lethal gene was never transmitted by the male parent.

The one plant which did not fit in with the scheme was the S2.4 genotype in family 1. This can only be accounted for by contamination.

The existence of gametophytic lethal genes linked with the S locus in species of *Oenothera* which are self-incompatible and which do not form large rings of chromosomes, is a most interesting link with the self-fertile species in the genus which are permanent heterozygotes by the alternative mechanism of balanced lethals.

6. THE S LOCUS IN *O. TRICHOCALYX*

(i) Genetics

Reciprocal hybrids from crosses between the self-fertile species *O. trichocalyx* and the four self-incompatible species were self-fertile. Only the hybrids between *O. pallida* and *O. trichocalyx* were studied in detail.

If *O. trichocalyx* is represented as Sf.f, f being an allele with no incompatibility properties, and *O. pallida* as S1.2, then the F₁ hybrids should include equal numbers of the two self-fertile classes Sf.1 and Sf.2. We must expect these two classes to be compatible amongst themselves and reciprocally compatible with both parents because of the presence of the Sf allele. Assuming that the expression of S1 and S2 specificity is unaffected in the hybrids by genes introduced from *O. trichocalyx*, crosses between F₁ hybrids will be of two kinds. (1) Sf.1 × Sf.1 and Sf.2 × Sf.2, which will give only self-fertile plants, and (2) Sf.1 × Sf.2 and Sf.2 × Sf.1, which will give segregating families of three self-fertile classes, Sf.f, Sf.1 and Sf.2 to one self-sterile class S1.2. Crossed as male on to S1.2 both the F₁ genotypes will give self-fertile progeny Sf.1 and Sf.2. The reciprocal cross, however, should segregate to give families in which half the plants are self-sterile S1.2 types and the rest either Sf.1 or Sf.2.

O. pallida × *O. trichocalyx*. The F₁ generation in the cross *O. pallida* × *O. trichocalyx* consisted of eighteen self-fertile plants. Thirty-six sib crosses made at random were all compatible. All the F₁ plants were reciprocally compatible with *O. trichocalyx* and *O. pallida*.

Self-pollination of eight F₁ plants produced only self-fertile progeny (*cf.* table 7).

Of seven families raised from crosses between F₁ sibs, four consisted of self-fertile plants only, but the other three segregated into self-fertile and self-sterile classes (*cf.* table 7). The eight self-sterile plants obtained were cross-incompatible as was expected. These results make it possible to distinguish between the Sf.1 and Sf.2 types in the F₁. Plants 1, 5, 7 and 8 are of one class and 2, 3, 4 and 6 are of the other.

Four F₁ plants were crossed with pollen from the female parent S1.2. Progeny from these crosses segregated into self-fertile and self-sterile groups while the reciprocal crosses, as anticipated, gave only self-fertile plants.

From the behaviour of these hybrids there is no evidence that genes introduced by *O. trichocalyx* are having any effect on the efficiency of

the incompatibility reaction of S_1 and S_2 from *O. pallida*. There is, however, some deviation from the expected ratios of self-fertile and self-sterile plants.

TABLE 7
*The behaviour of families descended from hybrids
from the cross O. pallida × O. trichocalyx*

	Parents	Progeny	
		Self-fertile	Self-sterile
F_1 selfed	1 Selfed	7	...
	2 "	5	...
	3 "	9	...
	4 "	3	...
	5 "	12	...
	6 "	8	...
	7 "	10	...
	8 "	9	...
F_1 sib crosses	1 × 4	6	3
	2 × 3	7	...
	8 × 7	7	...
	5 × 6	4	3
	4 × 5	3	2
	7 × 1	6	...
	3 × 6	5	...
$F_1 \times O. pallida$	1 × <i>O. pallida</i>	6	5
	2 × "	5	3
	3 × "	9	7
	4 × "	7	4
<i>O. pallida</i> × F_1	<i>O. pallida</i> × 1	8	...
	" × 2	9	...
	" × 3	7	...
	" × 4	10	...
$F_1 \times O. trichocalyx$	3 × <i>O. trichocalyx</i>	8	...
	4 × "	6	...
	5 × "	11	...

These disturbed ratios may be due either to partial zygotic inviability or to selection of certain pollen genotypes. The back-crosses, $Sf.1 \times S_{1.2}$ and $Sf.2 \times S_{1.2}$, give a deficiency of $S_{1.2}$. This deficiency cannot be due to selection of pollen genotypes because in each of the crosses one class of pollen is incompatible and therefore only the one compatible type functions. The deficiency must therefore be due to partial zygotic inviability.

The sib crosses $Sf.1 \times Sf.2$ and the reciprocal, on the other hand, deviate in the opposite direction and give an excess of $S_{1.2}$ types. It must be presumed that the same partial lethality of $S_{1.2}$ is operating in these crosses, and thus, the excess of the $S_{1.2}$ class can only be due

to a very strong selection favouring S_1 and S_2 pollen in competition with S_f .

If the figures of the two classes of F_1 sib crosses are adjusted for the zygotic lethality estimated from the backcross families, then the deviations in the F_1 sib crosses due to selection against S_f pollen are highly significant (cf. fig. 3).

The full import of this selection against S_f pollen will be apparent later when the selection of certain pollen genotypes in F_1 plants which come from the reciprocal cross, *O. trichocalyx* $\times$ *O. pallida*, has been described.

O. trichocalyx $\times$ *O. pallida*. The behaviour of the twenty-six self-fertile, F_1 plants obtained from the cross $S_f.f \times S_{1.2}$ is summarised in table 8. The results are those expected and agree with the results obtained from the reciprocal cross with one exception. Eleven families produced by intercrossing eight different F_1 plants gave progeny consisting entirely of self-fertile plants.

$\left. \begin{array}{c} S_{f,1} \\ S_{f,2} \end{array} \right\} \times S_{1.2}$	OBSERVED RATIO		χ^2	LETHALITY OF $S_{1.2}$	
	SS	SF		30%	
	19	27	1.391		
$\left. \begin{array}{c} S_{f,1} \times S_{f,2} \\ S_{f,2} \times S_{f,1} \end{array} \right\}$	ADJUSTED RATIO		χ^2		
	SS	SF		11	13
	8	13	1.921	5.610	

FIG. 3.—The lethality of $S_{1.2}$ zygotes is calculated from the observed deviation from the expected 1 : 1 ratio in the backcross family. In the sib crosses the deviation from a 3 : 1 ratio is significant when the observed figures are adjusted for the lethality of $S_{1.2}$ zygotes.

There are several possible explanations for this fact.

- (1) The intercrossed F_1 plants may all have been of the same genotype, either $S_{f,1}$ or $S_{f,2}$.
- (2) S_1 or S_2 pollen of the F_1 may be inviable.
- (3) $S_{1.2}$ zygotes of the F_2 may be inviable.
- (4) S_f pollen may grow faster than S_1 and S_2 pollen and fertilise a disproportionate number of the available ovules.

First of all, if there are both $S_{f,1}$ and $S_{f,2}$ genotypes present amongst the eight F_1 plants then, by crossing them with a plant of *O. pallida* which is genotypically $S_{3.4}$, four different classes of self-sterile plants should be present in the progeny as opposed to two classes if only one of the genotypes is represented. $S_{f,1} \times S_{3.4}$ should give $S_{1.3}$ and $S_{1.4}$. $S_{f,2} \times S_{3.4}$ should give $S_{2.3}$ and $S_{2.4}$.

Secondly, if S_1 or S_2 pollen is sterile, it should be possible to detect the fact by counting the proportion of bad pollen grains in the hybrids. The pollen of the eight hybrids which were intercrossed was examined. Between 60 and 80 per cent. of the pollen grains appeared quite normal and the germination varied from 46-74 per

cent. If one type of pollen is sterile 50 per cent. or more of the pollen should be abnormal in appearance or fail to germinate.

TABLE 8
*The behaviour of families descended from hybrids
from the cross O. trichocalyx × O. pallida*

	Parents	Progeny	
		Self-fertile	Self-sterile
F ₁ selfed	12 Selfed	10	...
	13 "	7	...
	20 "	8	...
	25 "	12	...
	26 "	10	...
F ₁ sib crosses	26 × 25	8	...
	12 × 25	11	...
	13 × 25	9	...
	10 × 25	14	...
	14 × 25	6	...
	20 × 25	9	...
	23 × 25	10	...
	25 × 26	5	...
	25 × 13	12	...
	25 × 10	9	...
	25 × 23	7	...
F ₁ × <i>O. pallida</i>	12 × <i>O. pallida</i>	7	2
	25 × "	9	2
	10 × "	11	3
	14 × "	10	4
<i>O. pallida</i> × F ₁	<i>O. pallida</i> × 12	6	...
	" × 25	7	...
	" × 10	10	...
	" × 14	8	...
F ₁ × <i>O. trichocalyx</i>	12 × <i>O. trichocalyx</i>	4	...
	25 × "	9	...
	10 × "	7	...
	14 × "	6	...

Thirdly, it is unlikely that S_{1.2} zygotes in the F₂ are wholly inviable because they have occurred in backcross families with *O. pallida* as the male parent.

Fourthly, if Sf pollen achieves fertilisation more frequently than S₁ or S₂ because it grows more quickly, then by repeating the same sib crosses using a limited number of pollen grains, a proportion of self-sterile plants should be recovered. There are approximately two hundred ovules in the ovary of the hybrids, thus one hundred pollen grains of an Sf.₁ plant on an Sf.₂ stigma will leave enough ovules for slow growing pollen to fertilise.

Crossing the F_1 plants with an unrelated plant of *O. pallida* (S3.4) and the repetition of the sib crosses gave clear-cut results. Both Sf.1 and Sf.2 genotypes were identified. From the results of the crosses with S3.4, plants 10, 12, 13 and 20 are of one type and 14, 23, 25 and 26 are in the opposite class.

The repeated sib crosses from restricted pollination confirmed this classification, crosses between sibs in different groups giving a proportion of self-sterile progeny. This evidence strongly suggests that the abnormal behaviour of the intercrossed F_1 plants is due to the Sf pollen growing faster than either S1 or S2 pollen. The problem now is whether the slower growth of S1 and S2 is caused by the recipient style or the nature of the pollen.

The crosses between F_1 hybrids from the crosses *O. trichocalyx* $\times$ *O. pallida* and *O. pallida* $\times$ *O. trichocalyx* settle this point. If the cause is inherent in the pollen, then no self-sterile plants should occur in the crosses Sf.1 $\times$ Sf.2 and Sf.2 $\times$ Sf.1 when *O. trichocalyx* $\times$ *O. pallida* hybrids are used as pollen parents. On the other hand if the cause lies in the nature of the style, then no self-sterile plants should occur in similar crosses when *O. trichocalyx* $\times$ *O. pallida* hybrids are used as female parents. This would be particularly significant since as we have seen, pollen from Sf.1 and Sf.2 plants with *O. pallida* cytoplasm usually produces an excess of the self-sterile class.

The results in fig. 4 show that there is no effect produced by *O. trichocalyx* cytoplasm in the style, for while the proportion of self-sterile plants from these crosses appears low, this can be attributed to the lethality of S1.2 zygotes. This factor affects 70 per cent. of the potential S1.2 progeny when estimated from backcross data. But even allowing for this lethality, the absence of the S1.2 class in families where *O. trichocalyx* cytoplasm is present in the pollen parent is significant. Thus the cause of the absence of S1.2 types can be attributed solely to the pollen from Sf.1 and Sf.2 plants with *O. trichocalyx* cytoplasm.

There is therefore a cytoplasmic difference between the two species *O. trichocalyx* and *O. pallida*. This is manifest by the selection of different pollen genotypes in the reciprocal hybrids. With cytoplasm derived from *O. pallida* in the pollen grain of an F_1 hybrid, there is strong selection against Sf pollen tubes. With cytoplasm derived from *O. trichocalyx* in the pollen, there is even stronger selection against S1 and S2. Detailed observations of pollen-tube growth show that this selection can, as has been anticipated, be attributed to a difference in the growth rate of the various pollen genotypes.

(ii) Pollen-tube growth

As a preliminary step, the pollen-tube growth in compatible intra-specific crosses was plotted (cf. fig. 5). Compatible pollen tubes of both *O. pallida* and *O. trichocalyx* vary considerably in length. In both species the pollen tubes have a skew distribution with the peak

at the higher end of the scale. In *O. pallida* the mode is between 33 and 35 mm. and in *O. trichocalyx* between 42 and 46 mm.

In the inter-specific crosses $Sf.f \times S1.2$ and the reciprocal, the distribution of pollen-tube ends from the pollen of each species repeats its characteristic pattern (*cf.* fig. 5).

The distribution of pollen tubes in crosses between the *O. pallida* $\times$ *O. trichocalyx* F_1 hybrids, shows marked bimodality in the crosses

		O. PALLIDA	O. TRICHCALYX
THE CYTOPLASM
THE GENES		$S1.2$	$Sf1$
THE HYBRIDS			
PARENTS		PROGENY	
		self fertile	self sterile
$Sf1$ $\times$ $Sf1$		7	—
$Sf2$ $\times$ $Sf2$		18	—
$Sf1$ $\times$ $Sf2$		31	7
$Sf2$ $\times$ $Sf1$		14	4
$Sf1$ $\times$ $Sf1$		10	—
$Sf2$ $\times$ $Sf2$		9	—
$Sf1$ $\times$ $Sf2$		15	—
$Sf2$ $\times$ $Sf1$		19	—

FIG. 4.—The effects of *O. trichocalyx* cytoplasm in the pollen from $Sf.1$ and $Sf.2$ plants on the fertilising ability of $S1$ and $S2$ pollen.

$Sf.1 \times Sf.1$ and $Sf.2 \times Sf.2$ (fig. 6). Half the pollen tubes are less than 1 mm. long and these are presumed to come from the incompatible $S1$ and $S2$ pollen grains. The rest of the pollen, the Sf class, produces longer pollen tubes with a mode between 35 and 37 mm.

In crosses between the $Sf.1$ and the $Sf.2$ hybrids, a much smaller proportion of the pollen tubes are less than 1 mm. in length. These occasional short tubes are a regular feature in crosses known to be fully compatible. The bulk of the pollen tubes, which from the genotypes occurring in the progeny from these crosses must include $S1$ and $S2$ pollen as well as the Sf class, repeat the distribution curve of Sf pollen (fig. 6).

FIG. 5.—The growth of *O. trichocalyx* and *O. pallida* pollen in intra- and inter-specific crosses.

Note.—The small numbers of very short pollen tubes (less than 1 mm.) are observed in many fully compatible crosses.

FIG. 6.—The growth of pollen tubes in sib crosses between $S_{1,f}$ and $S_{2,f}$ plants from the cross *O. pallida* $\times$ *O. trichocalyx*.

Note.—In the crosses $S_{f,1} \times S_{f,1}$ and $S_{f,2} \times S_{f,2}$ about half of the pollen tubes are less than 1 mm. long and can, therefore, be classified as incompatible. In the cross $S_{f,1} \times S_{f,2}$ and the reciprocal there are only a few pollen tubes, no more than are normally found in fully compatible crosses (cf. fig. 4).

○ = *O. pallida* cytoplasm.

The results from $Sf.1 \times Sf.1$ and $Sf.2 \times Sf.2$ crosses in *O. trichocalyx* $\times$ *O. pallida* hybrids, where only Sf pollen will grow, show the same clear-cut bimodality as their counterparts from the reciprocal cross. The higher mode can again be attributed to the Sf pollen (cf. fig. 7).

In crosses between $Sf.1$ and $Sf.2$ where both pollen genotypes will grow there is, however, a marked difference between the reciprocal hybrids. With *O. trichocalyx* cytoplasm, apart from the few very short

FIG. 7.—The growth of pollen tubes in sib crosses between $Sf.1$ and $Sf.2$ plants from the cross *O. trichocalyx* $\times$ *O. pallida*.

Note.—As in fig. 5, approximately half the pollen tubes in crosses $Sf.1 \times Sf.1$ and $Sf.2 \times Sf.2$ are incompatible. In crosses between $Sf.1$ and $Sf.2$ the number of pollen tubes which are less than 1 mm. long is small but a very high proportion of the pollen tubes are less than 25 mm. in length.

□ = *O. trichocalyx* cytoplasm.

pollen tubes, there is one peak but it is in the region of 20 mm. instead of 37 mm. (fig. 7). The range of pollen-tube growth is the same as in the other crosses but there is a great increase in the numbers at the lower end of the scale. These crosses are the ones which failed to give any self-sterile progeny after normal pollination, and the distribution of pollen tubes conforms with the conclusion that this is caused by the difference in the rate of growth between Sf pollen on one hand and S_1 and S_2 pollen on the other, the lower peak being due to the retarded S_1 and S_2 pollen tubes.

It is interesting now to see how the self-sterile $S_{1.2}$ plants with *O. trichocalyx* cytoplasm obtained by restricted pollinations behave, for this will reveal whether the slow growth of S_1 and S_2 pollen tubes is entirely caused by the cytoplasm from *O. trichocalyx* or whether it also depends on the presence of S_f .

The amount of good pollen in the different $S_{1.2}$ plants varied from 40-79 per cent.

These self-sterile plants produce no long pollen tubes when crossed amongst themselves or when crossed with plants of *O. pallida* of the

FIG. 8.—The effect of the cytoplasm on the growth of S_1 pollen from $S_{1.2}$ hybrids.

Note.—With *O. trichocalyx* cytoplasm half the pollen (S_1) is incompatible in the cross $S_{f.1} \times S_{1.2}$ but S_2 grows normally. In the cross $S_{f.2} \times S_{1.2}$ in which S_1 pollen tubes should be fully compatible, a very small proportion are more than 1 mm. in length and many of these are less than 10 mm. long.

same genotype. When crossed as male on to $S_{f.1}$, half the pollen tubes are inhibited in the stigma and these are presumably the incompatible S_1 pollen tubes. S_2 pollen, however, grows at the normal rate producing a second peak at 35 mm. (cf. fig. 8). Thus the slow growth of S_2 pollen in F_1 sib crosses is caused by the joint action of *O. trichocalyx* cytoplasm and the S_f allele during the development of the pollen.

The cross $S_{f.2} \times S_{1.2}$ gave a different and totally unexpected result. With six different $S_{1.2}$ plants, 80-90 per cent. of the pollen tubes were less than 1 mm. in length and of the remainder about half were under 14 mm. long (see fig. 8). The same cross with two other

S1.2 individuals produced no long pollen tubes at all. S1 pollen is, therefore, retarded by the presence of *O. trichocalyx* cytoplasm when either Sf or S2 is present.

These crosses were repeated with a single S1.2 individual descended from the *O. pallida* × *O. trichocalyx* hybrids. In this plant only about 40 per cent. of the pollen was viable, but in both crosses half the good pollen grains produced long pollen tubes (fig. 8).

The incompatibility reaction of the S1 and S2 alleles in *O. trichocalyx* × *O. pallida* hybrids is not affected. Both pollen genotypes are strongly inhibited in incompatible styles. It is their ability to grow normally in compatible styles which is impaired. To confirm that the cause of this is not associated with incompatibility, a number of the critical crosses were repeated at two different temperatures.

The effect of temperature on the growth of compatible and incompatible pollen tubes has been described by Lewis (1942). In *O. organensis*, at low temperatures there is only a small difference between the rate of growth of compatible and incompatible pollen tubes. As the temperature increases incompatible pollen tubes make less growth and compatible pollen grows more rapidly up to a point between 25° and 30° C. when the maximum divergence in the growth rate of the two kinds of pollen is reached.

Observations on the pollen-tube growth of *O. pallida* at different temperatures shows exactly the same results. After fifteen hours at 25° C. incompatible pollen tubes are 1 mm. in length while compatible pollen tubes are 45 mm. long. After fifteen hours at 14° C. both incompatible and compatible pollen tubes are 20 mm. in length.

If the slow growth of S1 and S2 pollen from *O. trichocalyx* × *O. pallida* hybrids has any connection with the incompatibility reaction, then at low temperatures we would expect more rapid growth in relation to Sf pollen while at high temperatures, the difference in the rates of growth will increase.

After four hours at 30° C. instead of the 25° used in previous experiments, the relation between the growth rate of the two classes of pollen is unchanged, both from Sf.1 and S1.2 plants. The fact that all the pollen tubes are shorter than in the corresponding crosses at 25° C. is due to the reduction in the time between pollination and fixing (cf. fig. 9).

At 13° C. all the pollen tubes grow much more slowly and, therefore, 15 hours were allowed to elapse between making the pollinations and fixing the styles. At this temperature the difference in the pollen tube distribution between the crosses Sf.1 × Sf.1 and Sf.2 × Sf.1 is no longer apparent. The slow growing pollen S1 in the cross Sf.2 × Sf.1 is now less than 1 mm. in length judging from the increased number of pollen tubes in this group and the absence of the peak at 20 mm. Far from increasing the rate of growth of S1 pollen, low temperature seems to have exactly the opposite effect. It reduces still further the ability of S1 pollen to grow in compatible styles. This

fact is confirmed by the pollen tubes in the crosses $Sf.2 \times S1.2$ half of which were less than 1 mm. in length at $13^\circ C$. This was true of the pollen from all $S1.2$ hybrids with *O. trichocalyx* cytoplasm. The slow growth of $S1$ and $S2$ pollen is therefore not caused by an incompatibility reaction.

(iii) Hypothesis

To explain these facts, I propose that the S locus not only controls incompatibility but also the production of a substance necessary for pollen-tube growth. This is similar to the explanation of Straub (1947) for the results of physiological experiments on incompatibility in *Petunia*. On this hypothesis, retarded pollen-tube growth in the *Oenothera* hybrids would be caused by a shortage or absence of the growth substance.

FIG. 9.—The effect of temperature on the growth of $S1$ pollen from $Sf.1$ and $S1.2$ plants with *O. trichocalyx* cytoplasm, in compatible and incompatible styles.

Note.—At $30^\circ C$. there is no change in the relative growth of $S1$ and the other classes of pollen. At $13^\circ C$. the weak growth of $S1$ at higher temperatures is still further reduced. Thus half the pollen tubes ($S1$) in the cross $S2.f \times S1.2$ are less than 1 mm. in length, but the low temperature enables the incompatible $S2$ pollen tubes to grow. Similarly in the fully compatible cross $S2.f \times S1.f$ half the pollen tubes are less than 1 mm. long.

We know that $S2$ pollen from hybrids with cytoplasm derived from *O. trichocalyx* behaves differently, according to the S genotype of the plant on which it is borne. When it comes from a plant which also carries the Sf allele, then the growth of $S2$ is always retarded, but in the presence of $S1$ it grows normally. $S2$ and Sf must therefore interact, so that Sf deprives $S2$ of the hypothetical growth substance. Similarly the weak growth of $S1$ pollen tubes can be attributed to interaction of this allele with Sf and $S2$.

The superior efficiency of Sf in competition with $S1$ and $S2$, and of $S2$ with $S1$, is constant in all the hybrids from the cross *O. trichocalyx* $\times$ *O. pallida* which have been tested. It is concluded, therefore, that the ability to produce the growth substance is a property of the S alleles themselves.

Interaction between **S** alleles in the pollen occurs in sporophytic incompatibility systems, that is when gene action is before meiosis. It is also found in diploid pollen grains of autotetraploids with gametophytic incompatibility when gene action has been proved by X-ray experiments (Lewis, 1949*b*) to be after meiosis. Wherever interaction occurs in either of the systems, the two alleles are always present in the same cell. After meiosis in diploids, the **S** alleles are isolated from each other by the formation of the pollen grain walls. It is only before meiosis that they occur in the same cell. It is concluded, therefore, that it is at this stage that interaction takes place in the hybrids.

Interaction between alleles has never been found in hybrids with cytoplasm derived from *O. pallida*. All the evidence points to the conclusion that **S** gene action in *O. pallida* and in hybrids with cytoplasm derived from this species, takes place after meiosis. In *O. trichocalyx*, we do not know when **S** gene action takes place, but in hybrids with cytoplasm from this source, the interaction of **S** alleles can be explained if gene action is before meiosis. I propose, therefore, that the production of the pollen growth substance in the self-fertile species *O. trichocalyx*, takes place before meiosis, and that in hybrids, cytoplasm from *O. trichocalyx*, imposes this precocious, pre-meiotic activity on the alleles **S**₁ and **S**₂ and so creates the conditions for interaction between **S**₁, **S**₂ and **S**_f.

7. DISCUSSION

The genetic behaviour of the hybrids especially made in these experiments confirms that their parents are correctly classified as different species.

The homology of the **S** locus in five species of *Oenothera* has been established and is apparently matched by a corresponding similarity in the polygenes which affect the expression of the incompatibility alleles, for not a single example of weakened specificity was found in the hybrids. It is therefore probable that an efficient incompatibility system was already present in a common ancestral form from which these species have diverged.

It is particularly interesting that the incompatibility of **S** alleles from *O. pallida* is not weakened in hybrids by genes introduced by the self-fertile species *O. trichocalyx*. From this fact it is concluded that *O. trichocalyx* is a secondarily self-compatible type which has arisen by a change in the major incompatibility gene while the polygenic background of the incompatibility system has so far remained unaltered.

Not only in *Oenothera* but in all the genera in which a number of species have been studied, it seems that the breeding system must have been firmly established early in their evolution or perhaps even early in the evolution of whole families.

It is to be expected that selection would most favour out-breeding and its accompanying heterozygosity at just the time when speciation

is proceeding most rapidly. This is a stage in the history of a genus when variation is likely to occur. It is, therefore, not surprising to find that whole groups of allied plants share in common the same incompatibility locus or, if the relationship is very close that the polygenic balance of the breeding system is also the same.

Leaving the broader implications of the evidence and turning now to the actual mechanism of incompatibility, the behaviour of the hybrids of *O. pallida* and *O. trichocalyx* has been enlightening. The analysis is as yet incomplete but despite this fact, the hybrids have added to our knowledge in three directions which we will now consider.

(i) Functions of the *S* locus

Interaction between *S* alleles in the pollen has been shown to occur in hybrids with cytoplasm derived from *O. trichocalyx*. In F_1 hybrids which are *Sf*.1 and *Sf*.2, the effect of interaction is to slow down the growth of *S*1 and *S*2 pollen tubes in compatible styles. Recent tests with the pollen of seven F_2 plants with the same genotypes have shown the same retarded growth of *S*1 and *S*2 pollen tubes. Interaction in the F_2 generation also occurs between the alleles *S*1 and *S*2. *S*1 pollen is always slow growing when it comes from an *S*1.2 plant while *S*2 pollen from these plants grows normally. The competitive interaction between alleles in the pollen of F_1 and F_2 hybrids with cytoplasm derived from *O. trichocalyx* is therefore constant and characteristic for each of the genotypes *Sf*.1, *Sf*.2 and *S*1.2. It depends on the particular combination of *S* alleles present.

Interaction does not affect the incompatibility properties of the alleles in these hybrids and this fact together with the evidence on the effect of temperature on the growth of retarded pollen tubes confirms that the interaction does not concern the incompatibility antigen. It is competition between the alleles themselves for a substance on which the growth of the pollen depends.

The *S* locus therefore, is of great fundamental importance in the physiology of the pollen grain. It is concerned in the production of a substance which is essential for the pollen because it decides the fitness of the pollen tubes to grow. Another activity of the *S* locus confers the specific incompatibility reaction on the pollen and so determines the fitness of the pollen tubes to grow on particular styles. It seems that in the evolution of incompatibility, the specificity of the pollen has been built up in association with a fundamental growth requirement of the pollen.

(ii) The properties of the pollen-growth substance

It has been established (1) that the pollen-growth substance is distinct from the incompatibility antigen, and (2) that it is essential for normal pollen-tube growth. In formulating a working hypothesis we have also seen that interaction between the alleles in the production of the growth substance can best be explained in terms of sporophytic gene action.

Thinking of the growth substance in terms of cytoplasmic particles, these must presumably be in the pollen mother cells. If there is only one growth substance which can be used equally by all the **S** alleles, one pollen genotype must regularly capture a considerably larger number of the particles than the other genotype during meiosis and the concurrent division of the cytoplasm. Only in this way can the difference in the growth rate of pollen tubes of two compatible genotypes be explained. Since the pollen grains in the hybrids are very constant in size, this uneven distribution of the growth substance can be dismissed as most improbable.

The alternative is that each allele produces a different growth substance from a common precursor. Taking **Sf.1** pollen from plants with *O. trichocalyx* cytoplasm as an example, **Sf** must produce more of its own particular growth substance than **S1**. Random distribution of the two kinds of growth substance in the cytoplasm of the pollen mother cells would ensure that the great majority of **Sf** and **S1** pollen grains would have a preponderance of the **Sf** growth substance and a corresponding shortage of the **S1** growth substance (*cf.* fig. 10).

If this interpretation of the data is correct, to account for the observed handicap on **S1** pollen tubes, there must be discrimination by the nucleus in the pollen grain between the two different growth substances present in the cytoplasm. An **S1** nucleus must reject the **Sf** growth substance and utilize only the **S1** growth substance. The selection of the **Sf** nucleus must be exactly the opposite. Only when the right growth substance is present with the right nucleus is the growth of the pollen tubes assured. Consequently what has been referred to as the growth substance is not yet in an active state. It has still to obtain something from the appropriate **S** allele in the pollen grain nucleus before it is complete.

It will be clear from fig. 10 that with the earlier production of the growth substance in *O. trichocalyx* cytoplasm, in the heterozygote, neither **S1** nor **Sf** have as much of their own specific growth substance as when this is produced after meiosis. Thus not only does allelic specificity of the growth substance explain the differences observed in the growth rate of **S1** and **Sf** pollen, it also accounts for the fact that **Sf** pollen tubes from an **Sf.1** plant with *O. trichocalyx* cytoplasm do not grow quite as fast as **Sf** pollen tubes from homozygous **Sf.f** types (*cf.* figs. 5 and 7).

A further advantage of the proposed scheme is that it would provide a basis for the evolutionary beginning of an incompatibility mechanism. At first the activating substance contributed by the nucleus would be non-specific and the pollen would grow equally well on all styles. Later, as the **S** alleles evolved, each would superimpose its own specific hapten groups on the growth substance which would then react incompatibly with specific preformed antibodies in the style.

16. 10.—A diagram showing two stages in the synthesis of the hypothetical incompatibility substance in the pollen.

Stage 1.—In *O. pallida* cytoplasm, because the growth substances are produced after meiosis, each pollen grain in both homozygous and heterozygous plants has an equal amount of its own specific growth substance. In *O. trichocalyx* cytoplasm, when the growth substances are produced before meiosis, the situation in homogeneous pollen is unaltered. In heterogeneous pollen there is competition between the alleles, and the pollen grains receive a mixture of the two growth substances in which the type of the dominant allele is preponderant.

Stage 2.—In both types of cytoplasm this stage is postmeiotic. The S allele in the nucleus of the pollen grain contributes a substance which activates its own specific growth substance and no other. In the case of S_I this activating principle is the incompatibility antigen. In *O. pallida* cytoplasm, and in homozygous plants with *O. trichocalyx* cytoplasm, all the particles of the growth substance are activated, but in heterozygous plants with *O. trichocalyx* cytoplasm where the growth substances are mixed in the pollen grains, only a proportion of them are activated.

(iii) *Influence of the cytoplasm on gene action*

Interaction between **S** alleles in the production of the pollen growth substance cannot be attributed to the creation of new and untried combinations of alleles from different species. Firstly, interaction occurs between **S**₁ and **S**₂ both of which come from the self-sterile species *O. pallida*, and secondly, while interaction of alleles has never been observed in hybrids with cytoplasm derived from *O. pallida* it occurs regularly in both F₁ and F₂ hybrids with cytoplasm derived from *O. trichocalyx*. It is therefore the cytoplasm which decides whether or not the alleles will interact.

It is interesting that the cytoplasm of F₂ hybrids which has been derived exclusively from *O. trichocalyx* but has of course been in contact with genes from *O. pallida*, retains at least one and possibly all of the properties of pure *O. trichocalyx* cytoplasm.

The non-interaction of **S** alleles in *O. pallida* is normal in a species with gametophytic incompatibility when gene action is known to occur after meiosis. Since non-interaction is also the rule in hybrids with cytoplasm derived from *O. pallida*, it is concluded that there is no change in the time of gene action in these plants.

It is difficult to conceive how interaction of **S** alleles can occur after meiosis when they are separated from each other by the pollen grain walls, particularly when in other examples of interaction it depends on the alleles being present in the same cell. The immediate cause of interaction in the hybrids is therefore attributed to **S** gene action before meiosis. This condition is only found in the presence of *O. trichocalyx* cytoplasm. Thus the time of gene action must be under the control of the cytoplasm in the *Oenothera* hybrids.

Hybrids of *Nicotiana Sanderae* and *N. Langsdorffii* described by East (1932) also show sporophytic gene action of part of the **S** complex. Pollen from plants with two alleles from *N. Sanderae* is sterile in the presence of *N. Langsdorffii* cytoplasm but in sibs of these plants in which one of the *N. Sanderae* alleles is replaced by an **Sf** allele, both classes of pollen are viable. The **Sf** allele can shelter alleles from *N. Sanderae* from the deleterious effects of *N. Langsdorffii* cytoplasm.

The sterility of pollen from the genotypes referred to above also illustrates the importance of the **S** locus in the production of functional pollen grains. Thus there is corroborative evidence for both sporophytic gene action and the tripartite nature of the **S** locus in other species.

We have now discussed in some detail why, and how the competition between alleles in pollen from hybrid plants with *O. trichocalyx* cytoplasm takes place. Turning to the reciprocal hybrids with *O. pallida* cytoplasm we can compare the two.

In the F₁ hybrids with *O. trichocalyx* cytoplasm, **Sf** pollen tubes have an overwhelming advantage over **S**₁ and **S**₂ pollen tubes and consequently crosses between **Sf**.1 and **Sf**.2 give no self-sterile plants unless the pollination technique is adapted to favour the slow growing

pollen tubes. Crosses between the same genotypes with *O. pallida* cytoplasm on the other hand, give an excess of the self-sterile class and once again the cause lies in the behaviour of the pollen. There is strong selection favouring S₁ and S₂ pollen tubes compared with Sf in compatible styles; just the reverse of the situation in *O. trichocalyx* cytoplasm.

The discrimination between Sf pollen on one hand and S₁ and S₂ on the other is not so extreme in *O. pallida* cytoplasm, and it also acts at a later stage in pollen-tube growth. The slowing down in the growth rate of S₁ and S₂ pollen tubes in *O. trichocalyx* cytoplasm is very marked after seven hours growth in the style. The effect of *O. pallida* cytoplasm on the growth of Sf pollen is only just beginning after the first seven hours of pollen-tube growth. Comparing the growth of Sf pollen from Sff plants of *O. trichocalyx* with Sf pollen tubes from hybrids with *O. pallida* cytoplasm the latter are slightly shorter.

It is a requisite in the interpretation of competition of alleles in *O. trichocalyx* cytoplasm that the S alleles in the pollen should act after meiosis in *O. pallida* cytoplasm. Therefore the slowing down in the growth of Sf pollen in *O. pallida* cytoplasm cannot be attributed to sporophytic gene action and competition. We have already seen that the selection of pollen genotypes in *O. pallida* cytoplasm differs in degree and time and, in addition, the absence of competition between S₁ and S₂ in this cytoplasm also suggests that the cause of selection is different in the reciprocal hybrids. An alternative explanation is that Sf pollen in *O. pallida* cytoplasm is being weakly inhibited in the style.

It is more difficult to obtain seed from interspecific crosses with *O. trichocalyx* as the male than as the female parent. This reciprocal difference in fertility also applies to crosses between *Nicotiana alata* and *N. Langsdorffii* and again fertility is reduced when pollen of the self-fertile species is used. Weak inhibition of Sf pollen therefore becomes a more plausible explanation.

Lewis (1954) after reviewing the interspecific relations of self-fertile and self-sterile species concludes that (1) species which are self-fertile, and have pollen that is inhibited on the style of a self-sterile species, are species with primary self-fertility. (2) Self-fertile species with pollen which is not inhibited on a self-sterile style are secondarily self-fertile having reverted from a previous self-incompatible condition.

As we have already seen, the evidence of the polygenic background of *O. trichocalyx* suggests that it is a secondarily self-fertile species. Since it is only by careful scrutiny that the inhibition of the pollen can be detected, the species may be placed in Lewis's second category with the reservation that there is some inhibition of secondarily self-fertile pollen on the styles of self-sterile species.

As a result of his observations Lewis goes on to propose the following theory:—" . . . pollen contains a substance which is

probably necessary for the development and in self-sterile species and specificities have been imposed on this substance by hapten groups produced by the **S** alleles. In the style **S** alleles produce preformed antibodies to the pollen substance. In primary self-fertile species the pollen substance is entirely non-specific and reacts with all stylar antibodies ”.

Firstly, there is now evidence that the “ substance which is probably necessary for development ” does exist in the pollen and that it is essential for pollen-tube growth. Secondly, the attachment of specificities to this substance gives a workable scheme which fits all the known facts. But we can now elaborate this point because the growth substance takes on new properties. There is no longer one growth substance but probably as many variations of it as there are alleles. This change to a multiplicity of growth substances must have evolved with the development of the incompatibility mechanism.

Finally, while **Sf** pollen from secondarily self-fertile species does grow in styles of self-sterile species it does so with difficulty. The inhibition is, however, much less rigorous than that of primary self-fertile pollen. Secondary self-fertile pollen apparently retains some of its primary non-specific properties but the loss of the specific hapten groups does not completely restore it to the primary condition.

In the course of the discussion much emphasis has been laid on the cytoplasmic differences between the species *O. trichocalyx* and *O. pallida* and the possible effect of polygenic differences has been overlooked. Only by continued back-crossing can the complete role of the nucleus be determined. The gradual transfer of the genes of one species into the cytoplasm of the other will show to what extent the nature of the cytoplasm depends on the nucleus and whether any of the recognisable differences in the two kinds of cytoplasm are indefinitely self-perpetuating.

8. SUMMARY

1. Fertile hybrids were obtained from five species of *Oenothera* in the subgenus *Anogra* (see table 2).

2. F_1 and F_2 and backcross families show that the **S** locus is homologous in these species. The evolution of incompatibility in a common ancestor would account for this.

3. The efficiency of the incompatibility reaction of the **S** alleles in the hybrids is equal to that within the species from which it is concluded that the polygenic control of the incompatibility system is the same.

4. There is a cytoplasmic difference between self-sterile *O. pallida* and self-fertile *O. trichocalyx*. This affects the behaviour of pollen from F_1 and F_2 hybrids.

5. The facts are interpreted as follows :—

- (i) Apart from the two series of alleles at the **S** locus which determine the specificity of the pollen and the style there

is a third series which produces a substance necessary for pollen-tube growth.

- (ii) In hybrids with cytoplasm derived from *O. pallida*, the growth substances are produced after meiosis. In hybrids with cytoplasm derived from *O. trichocalyx*, the growth substances are produced before meiosis and because of this, different S alleles compete for a common precursor.
- (iii) The growth substances are transformed into an active state by the appropriate S complex in the pollen grain nucleus. In this final stage they carry the incompatibility antigens.

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