CYTOLOGICAL PHENOMENA CONNECTED WITH SELF-STERILITY IN THE FLOWERING PLANTS[1]

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GENERAL INTRODUCTION

GENETIC studies have established two explanations for self-sterility in the higher plants, the oppositional factor hypothesis (EAST and MANGELSDORF 1925, FILZER 1926) and the *Lythrum salicaria* scheme (VON UBISCH 1921, BARLOW 1923, EAST 1927). Most self-sterile plants so far investigated conform to the oppositional factor hypothesis; this group includes Nicotiana, *Veronica syriaca*, *Prunus avium*, *Trifolium pratense*, *Petunia violacea* and *Nemesia strumosa*. The Lythrum scheme has been applied only to the two trimorphic species, *Lythrum salicaria* and *Oxalis valdiviana*, and to *Capsella grandiflora*. Certain plants which appear not to conform to either of these two theories fall into line with the oppositional factor hypothesis when their polyploid nature is considered (LAWRENCE 1930). *Verbascum phoeniceum* and *Prunus domestica* are such polyploid plants.

In a few plants self-sterility seems not to be subject to simple explanation. *Brassica oleracea* var. *capitata* (KAKIZAKI 1930) and *Cardamine pratensis* (BEATUS 1934) are said to have oppositional factors of the Nicotiana-Veronica type, but in addition they are thought to possess genes stimulating to pollen-tube growth. In certain other self-sterile plants, no explanation has yet been found for the genetic behavior; in *Tolmiea Menziesii*, for example, CORRENS (1928) found no cross-sterility among sister plants or between parents and offspring, although all were self-sterile.

The present investigation consists mainly of cytological studies of self-sterility in species belonging to widely separated plant families. On some plants the cytological observations have been accompanied by physiological experiments and pedigree work.

The plants which were used will be taken up in three classes, according to the behavior found for incompatible pollen and pollen tubes, as follows:

1. Germination of pollen is decreased.
2. Germination is normal, but pollen tubes are inhibited in the style.
3. Tubes grow normally, and reach and fertilize the ovules, but no seed develops.

This grouping will also be used for plants whose pollen-tube behavior is reported in the literature, although certain of these plants are difficult to classify from the information available. Although it is believed that these groups are based on significant physiological differences, it is thought that the same fundamental process underlies all three types of behavior. Particular attention will be given to the suggestion of EAST (1929, 1934a) that inhibition of incompatible pollen tubes resembles an immune reaction, whereby the tubes secrete antigens which stimulate the style to produce antibodies.

CLASS I

Few cases are known in which germination of pollen is affected by self-incompatibility. MÜLLER (1868) mentioned that pollen of Notylia fails to germinate on the stigma of the same flower, and DARWIN (1877) came to the same conclusion regarding *Linum grandiflorum*. It was reported by STOUT (1931) that *Brassica pekinensis*, which undergoes cycles of self-sterility and self-fertility, may show no germination of selfed pollen at the height of its self-sterile period. According to AFIFY (1933), diploid cherries, in a cross of the $S_1S_3 \times S_3S_4$ type, show some incompatible grains which do not germinate and others which produce very short tubes with ends bent upward.

In compatibly pollinated plums and apples, AFIFY finds certain pollen grains which fail to germinate. These are thought to be the most highly incompatible grains from a mixture of groups of pollen whose compatibilities vary because of polyploidy. This suggestion is borne out by the observation of HALL and CRANE (1933) that in the most highly self-incompatible apples, pollen fails to germinate, or else produces tubes which go but a short distance into the style.

RILEY (1936) has shown that selfed grains of *Capsella grandiflora* do not germinate. According to BRINK (1934), *Melilotus officinalis* shows, when selfed, decreased germination of pollen. Many of the tubes produced remain short and fail to penetrate the stigma, while of those which do penetrate, few pass beyond the stigmatic region. In *Cardamine pratensis*, as reported by CORRENS (1912) and corroborated by ZOLLIKOFER (1932), incompatible pollen germinates, but pollen tubes are short and do not penetrate into the stigma. From the situation in other members of the Cruciferae, it seems likely that this early retardation of incompatible tubes is accompanied by some inhibition of pollen germination.

In the present study it was found that *Brassica oleracea* var. *italica* and *Raphanus sativus* show decreased germination of incompatible pollen and no penetration of tubes into the stigma. Incompatible pollen of *Pelargonium hortorum* rarely germinates; but when a tube is produced, it usually penetrates a short distance into the stigma. Germination of incompatible pollen of *Secale cereale* is frequently prevented, particularly on lower branches of the style, though tubes may enter the stigma and grow a short way.

Brassica oleracea L. var. *italica* Plenck

This study began on three plants grown from commercial seed. All the work to be reported was done either on these plants (designated 1, 2, and 3) or on offspring of two of them (1 and 3). All three original individuals were self-sterile. Certain cross combinations were fertile, others sterile.

In a typical series of pollinations made at the same time on these three plants, 55 selfings gave an average set of 20 percent with 2.5 seeds per fertile flower; 104 incompatible crosses resulted in 26 percent set and 3.7 seeds per fertile flower; and 27 compatible crosses gave 100 percent set with 18.2 seeds per fruit. This difference is considerably larger than that obtained by PEARSON (1932), whose fertile crosses gave but 74.6 to 94.3 percent set with 3.8 to 6.9 seeds per fruit.

Behavior of incompatible pollen

Cross-sterility and self-sterility involve decreased germination of pollen grains, and failure of those tubes which are produced to reach any considerable length. Although many incompatibly pollinated stigmas were examined, part after sectioning in paraffin and part after crushing in aceto-carmine, only one pollen grain was found which had emptied its contents into its tube. After compatible pollination, grains become empty soon after tubes have penetrated the stigma and have started down the style. Incompatible tubes usually cease to develop when they are about as long as the diameter of a pollen grain (plate 1, fig. 1), although occasionally they may attain twice that length. Some incompatible tubes flatten out on cells of the stigma, or bend around them, but none coil around projecting stigmatic cells as in *Brassica pekinensis* (STOUT 1931).

One series of stigmas was specially prepared to determine whether germination of pollen is less after incompatible pollination than after compatible pollination. Selfings, incompatible crosses, compatible crosses, and bud selfings were made on the same plant, pollinations being made with a brush so as to insure that all pollen grains on the stigma would have a nearly equal chance to germinate. After two days, from aceto-carmine preparations of the stigmas, counts were made of the number of germinated and non-germinated grains. Of 464 incompatible grains on mature stigmas (of opened flowers), 39.4 percent were germinated; of 610 compatible grains on mature stigmas, 92.8 percent were germinated; and of 358 selfed grains on immature stigmas (bud fertility will be discussed later), 85.5 percent were germinated.

Physiology of incompatibility

Germination of pollen does not depend on a specific stimulating substance, as is shown by the fact that it will germinate in sugar solutions or on stigmas of distantly related plants. Where germination of incompatible pollen is prevented, therefore, the stigma must possess or produce some substance which actively inhibits the pollen. Results of mutilation experiments (table 1) show that the inhibitory reaction occurs only in the stigma.

Control self-pollinations yielded no seed at all, so seed production in table 1 must be attributed to the rendering compatible of flowers which would otherwise have been incompatible with the pollen used. In every case where stigmatic material was removed, fertility ensued; and unless stigmatic material was removed, no fertility resulted. The flowers of which the stigmatic surface was macerated were probably subjected to a treatment equivalent to that given those from which a thin layer of the stigma was removed, except that the material was scraped off instead of being sliced off with a razor. The operation listed as "stigma much cut" consisted of slicing about half-way through the stigma with a razor in numerous planes parallel to the length of the style. The failure of flowers so treated to set seed shows that mere wounding is not sufficient to bring compatibility. Since there is no evidence that removal of more than a thin

TABLE 1

Broccoli. Yields of seed from mutilated flowers self-pollinated and kept in moist chambers.

DATE	PORTION OF PISTIL REMOVED	NO. SEEDS
1/ 1/36	Stigma+one-half style	12, 16, 15
1/16/36	Stigma+one-fourth style	7, 10
	Stigma	17, 13
	One-half stigma	15, 24
1/25/36	Thin layer of stigma	22, 19
	None; stigmatic surface macerated	20, 18
	None; stigma much cut	0, 1

layer of stigmatic tissue further increases fertility, it must be concluded that the inhibitory reaction is limited to the surface stigmatic cells. That the zone of interference for incompatible pollen of Capsella is located in the stigmatic hairs was suggested by RILEY (1936) to explain the very strong inhibition of pollen found there. The experiments of KIRK and STEVENSON (1931) and BRINK (1934), whereby the self-sterility of *Melilotus officinalis* plants was reduced by rubbing the stigma with a toothpick, suggest that most, if not all, of the inhibitory effect occurs in the stigma of that plant, and that the treatment removed part of the inhibiting region.

The fact that self-fertilization results when the stigma of a mature flower is removed demonstrates that self-sterility does not prevent the gametes from fusing when once they meet. This has generally been assumed for plants, though MORGAN (1923) pointed out that self-fertility of buds and of end-season flowers does not prove that self-fertilization would normally occur in self-sterile plants if pollen tubes could be induced to reach the ovules.

Failure to place the treated, pollinated flowers in moist chambers

lowered the average set of seed, but under moist greenhouse conditions, permitted a full set from some flowers.

Attempts to demonstrate the presence of an inhibitory substance in the stigma which would diffuse into agar and affect the growth of tubes thereon were unsuccessful. Similarly, no effect resulted from using parts of compatible pistils. CORRENS (1912) found germination of *Cardamine pratensis* pollen to be prevented by addition of either compatible or incompatible crushed stigmas to an agar medium.

Good pollen germination and tube growth were obtained on $\frac{1}{2}$ or 1 percent agar plus 15 percent commercial cane sugar. Boiled yeast extract and potato juice, used with success by BRINK (1924), decreased germination and growth. Tests for phototropism were without positive results, no effect

TABLE 2

Broccoli. Yields of seed from flowers pollinated at opening and at various stages in the bud.

PLANTS ♀	PLANTS ♂	DAYS POLLINATED BEFORE OPENING											
		0	1	2	3	4	5	6	7	8	9	10	11
1	1	0, 0	0, 0, 0		10, 18				18, 18				
2	2	0, 0, 1, 0				8			11		0	1	1
3	3	1, 1	8, 2	21	11, 9	16		22	17	13			4
1	2	0, 0, 0, 0		2, 2	21				15, 20				
2	1	7	0, 1	3	4		17, 18		18				
2	2	0, 0, 1, 3	2, 0	2, 9, 0	10, 14	16, 20	14, 20	18, 13	16				

of light being apparent on direction of growth of tubes. Attempts to induce negative phototropism by addition of eosin to the medium were unsuccessful, although tubes grew in concentrations of eosin strong enough to color their contents.

Bud fertility

The ability to set seed when self-pollinated before anthesis is possessed by numerous self-sterile plants. EAST (1923) observed it in Nicotiana, SIRKS (1926) in *Verbascum phoeniceum*, YASUDA (1930) in *Petunia violacea*, KAKIZAKI and KASAI (1933) in *Brassica pekinensis* and *Raphanus sativus*, and ALAM (1936) in *Eruca sativa*. PEARSON (1929) reported bud fertility for *Brassica oleracea* var. *capitata*, and KAKIZAKI (1930) confirmed this finding.

Experiments conducted on broccoli showed it to be highly fertile in the bud. Table 2 gives the results of bud pollinations. All buds of reasonable size on a flowering branch were pollinated at one time and the branch covered with a bag. On succeeding days each flower was tagged as it opened.

Good sets of seed resulted from some buds pollinated two or three days

before opening, and from practically all flowers pollinated four to seven days before opening. Had younger buds been pollinated, it is believed that many of them would have set seed. Selfed buds of plant 2 which opened on the ninth, tenth, and eleventh days probably failed to produce more seed because of the weakened condition of the plant and the stunted and abnormal condition of the buds. Tests the following year on a plant of another generation—1-(1)—showed that three flowers which opened on the tenth day after being pollinated had 4, 10, and 11 seeds, respectively.

Behavior of compatible pollen

The rapidity of germination of pollen after compatible, incompatible, and bud pollinations was found to differ. Several buds were pollinated by sister flowers, and open flowers on the same plant were pollinated at the same time, some compatibly crossed and some selfed. Cytological observations of stigmas at intervals showed that activity of pollen on the stigma is more rapid after bud pollination than after pollination of open flowers. Germination occurs sooner (in less than two hours), and tubes are more rapid in their growth into the stigma. Compatible pollen on open flowers, while slower than pollen on buds, germinates sooner than incompatible pollen on open flowers. The latter pollen, as pointed out earlier, never reaches the normal percentage of germination.

No striking differences occur between growth rates of compatible tubes in mature styles and of tubes in immature styles. Tubes could be found in the ovary in about 24 hours after pollination in each case. PEARSON (1933) reported that tubes reached the ovary in approximately the same amount of time after bud pollination as after compatible pollination at anthesis.

The fact that immature stigmas were more receptive than mature ones indicates that pollen is inhibited to some extent on the mature stigma even though it is compatible. Since this inhibitory effect is confined to the stigma, as is the inhibition of incompatible pollen, it seems very likely that incompatibility results from what is only a stronger expression of an effect also exerted on compatible pollen. In other words, an inhibitory reaction, of the type which would cause incompatibility if it were stronger, occurs between compatible pollen and the stigma.

Further evidence that compatibility in broccoli may involve an inhibitory reaction similar to that of incompatibility was furnished by the behavior of certain plants when changed to an unfavorable environment. In some instances the effect of moving a plant from the greenhouse to the garden was to destroy its fertility to pollen from certain plants, but not to lower its fertility to pollen from certain other individuals. This may be explained satisfactorily as due to pollen from certain compatible plants being nor-

mally somewhat inhibited, so that raising the general inhibitory ability of the plant by unfavorable environment increases the inhibition of this pollen until the dividing line between compatibility and incompatibility is passed. Pollen from other compatible plants is less inhibited, or is not inhibited at all, and the general increase in inhibitory effect does not force this pollen across the line into the incompatible class. A sharp dividing line between fertility and sterility is indicated by the rarity of partially fertile combinations.

Genetics of incompatibility

The majority of self-sterile plants studied have been shown to follow the oppositional factor hypothesis. According to this theory there exists an allelic series of factors, S_1, $S_2 \cdots S_n$, which affect pollen-tube growth. A tube is inhibited in a style which has in homozygous or heterozygous condition the same S factor as carried by the tube.

PEARSON (1932) concluded from a preliminary analysis that broccoli follows the oppositional factor scheme. Pollinations of offspring of plants 1 and 3 show, however, that a single series of oppositional factors will not account for the results obtained (table 3). In this study each fruit was classified as to whether it contained 0, 1–3, 4–9, or more than 9 seeds. In most cases this method gave a clear distinction between compatible and incompatible combinations. Usually all or nearly all the fruits from a particular mating fell either in the first two classes or in the last one. In two cases, half or more were in the 4–9 group, and these have been indicated in table 3 as $\pm$.

The first 18 plants fall into four sharply defined groups. The next two differ only in their reactions with the two plants which immediately follow them. The most important observations to be made from the table are:

1. Self-fertile plants resulted from this cross between self-sterile plants.
2. Reciprocal crosses between groups do not always give the same result.

Differences in behavior of reciprocal crosses show that reactions of the style are toward the gametophytic constitution of pollen rather than toward the sporophytic constitution. More direct proof of this was obtained from cytological studies, which showed that in certain compatible combinations more than half the pollen, though obviously viable, was failing to function.

Although RILEY (1936) has shown that the self-sterility of *Capsella grandiflora*, another member of the Cruciferae, can be explained on the Lythrum scheme, the fact that broccoli pollen reacts according to its gametophytic nature eliminates the possibility that this hypothesis fits broccoli also. The Lythrum theory, which involves two pairs of factors,

Aa and *Mm*, of which *A* is epistatic to *M*, calls for pollen to react in accordance with the diploid constitution of the plant from which it came.

In two other Crucifers a situation exists which is similar to that in broccoli. The common cabbage (*Brassica oleracea* var. *capitata*) and *Cardamine pratensis* both show differences in reciprocal crosses, and both throw self-fertile individuals from crosses between self-sterile plants.

TABLE 3

Broccoli. Fertility relationships in 3 families. Family 1-1=plant 1 selfed; family 1-3=1 ♀ ×3 ♂; family 3-1=3 ♀ ×1 ♂. +=fertile, −=sterile, ±=incompletely fertile, as shown by 6 or more pollinations. ?=less than 6 pollinations, probably −.

♀ \ ♂	1-1(1)	1-1(2)	1-1(3)	1-1(4)	1-1(5)	1-1(6)	1-3(1)	1-3(2)	1-3(5)	3-1(1)	3-1(3)	3-1(5)	3-1(6)	3-1(2)	1-3(7)	1-3(10)	1-3(8)	1-3(9)	1-3(3)	1-3(4)	1-3(11)	1-3(6)	3-1(4)
1-1(1)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-1(2)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-1(3)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-1(4)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-1(5)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-1(6)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-3(1)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-3(2)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
1-3(5)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+	+	+	+
3-1(1)	−	−	−	−	−	−	−	−	−	−	?	−	−	+	+	+	+	+	+	+	+	+	+
3-1(3)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+		+	+	+	+
3-1(5)	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+	+	+		+	+
3-1(6)	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	−	+	+	+	+	+
3-1(2)	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+
1-3(7)	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+
1-3(10)	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	+	+	+	+	+
1-3(8)	+	+	+	+	+	+		+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	±
1-3(9)	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	−	−	−	−	−	−	−	±
1-3(3)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	+	−	−
1-3(4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	−	−	−	+	−
1-3(11)	+	+	+	+		+	+	+	+	+	+		+	+	+	+	+	+	+	+	−	?	+
1-3(6)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+
3-1(4)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

KAKIZAKI (1930) proposed a theory for cabbage involving one allelic series of inhibiting factors ($S_1, S_2 \cdots S_n$) and another series of stimulating factors ($T_1, T_2 \cdots T_n$). A pollen tube carrying an *S* factor which was present in the style in heterozygous condition was compatible if it carried a *T* factor for which the style was homozygous. BEATUS (1934) explains self-sterility in *Cardamine pratensis* by a hypothesis similar to that of KAKIZAKI except that he uses two series of inhibiting factors and two of stimulating factors.

Stimulating factors were proposed by KAKIZAKI and BEATUS from analysis of genetic data. For explaining the genetic facts, such factors are satisfactory, but they present serious difficulties in physiological interpretation. The same difficulties arise from LAWRENCE'S (1930) assumption that unlike *S* factors positively promote pollen-tube growth. Growth of pollen tubes in the style is presumably a simple, nutritive reaction not involving special stimulating substances, since tubes will grow in styles of plants of other families or orders. Any effect of the style on the tube, therefore, must be one of inhibition rather than of stimulation. This conclusion is even more necessary in the case of *Brassica oleracea* and *Cardamine pratensis*, where incompatible pollen either does not germinate, or where tube growth is stopped at such an early stage that lack of a specific stimulation is very improbable.

The conclusion that actual stimulating factors cannot exist leaves two possible explanations for the "stimulating" factors of KAKIZAKI and BEATUS: Either they are factors which neutralize the inhibiting action of the *S* genes, or they are non-inhibitory members of an allelic series, of which some genes inhibit pollen and others have no inhibitory effect. Although the second explanation requires modification of the theories of KAKIZAKI and BEATUS, it is the one which seems more plausible, because of its greater physiological simplicity and its closer analogy to the situation in other self-sterile plants.

The allelic series of inhibiting factors in Nicotiana contains genes which inhibit tubes greatly, others which inhibit them little, and others which presumably inhibit them not at all (EAST and YARNELL 1929, EAST 1929, EAST 1934a). Since *Brassica oleracea* and *Cardamine pratensis* are tetraploid types (LAWRENCE 1930), they may be assumed to have two such series of factors, each made up of genes whose inhibiting power varies from none to great. These series may be designated $S_1, S_2 \cdots S_{f-1}, S_f$; and $Z_1, Z_2 \cdots Z_{f-1}, Z_f$, with S_1 and Z_1 the strongest inhibitors and S_f and Z_f non-inhibitory. S_1 may then be assumed to inhibit pollen successfully, no matter which *Z* factor is present. The same may be assumed for Z_1, S_2, Z_2, and possibly others low in the series. S_{f-1} and other intermediate factors of themselves do not inhibit pollen sufficiently to prevent it from functioning; only when one of these is aided by one of the intermediate *Z* factors does the inhibition become strong enough to give incompatibility to pollen carrying the two of them. Possibly the combined effect of an *S* and a *Z* factor is not always a simple addition of the two separate inhibitory effects. Further complications can result from certain factors in the *S* series being identical with certain factors in the *Z* series.

Such a theory would be difficult to substantiate genetically, but evidence for it is provided from another source. Although several types might

be genetically self-compatible, only S_fZ_f plants would exhibit no inhibition of their own pollen; and numerous cross-combinations should occur in which pollen is somewhat inhibited but not enough to result in incompatibility. In broccoli it has been demonstrated that pollen which is genetically compatible is somewhat inhibited.

Raphanus sativus L.

Four plants of the common radish proved to be highly self-sterile, thus confirming earlier observations of STOUT (1920). Certain cross-combinations were fertile and certain others sterile.

Pollen behavior was found to be similar to that of broccoli. Some tubes were produced after sterile pollination, but these were short, and none penetrated the stigma.

Bud fertility was less in evidence among the Raphanus plants than in *Brassica oleracea* var. *italica*, only one of the four individuals showing it. KAKIZAKI and KASAI (1933) found all four of the *R. sativus* plants they tested to be bud fertile.

Pelargonium hortorum

Incompatible pollen of the geranium is usually prevented from germinating, although tubes may be produced which swell and burst soon after penetrating the stigma.

According to BAILEY (1933), the common garden geranium, *Pelargonium hortorum*, is a horticultural species which probably originated from crosses between *P. zonale* Willd. and *P. inquinans* Ait.

Self-pollination of several varieties showed three to be self-sterile. Numerous cytological preparations were made of self-pollinated styles from plants of these varieties, and it was found that usually no pollen germinated. Rarely were more than one or two germinated grains found to a style. The tubes from these grains were short and usually swollen at the end or burst (plate I, fig. 6). Of all the styles examined, only one had a grain which had emptied its contents into its tube, and that tube had a burst end.

Preparations were made by boiling the upper part of the style for three minutes in sodium sulfite solution, staining in acid fuchsin, and mounting, after some dissection, in lactic acid.

Secale cereale L.

It has been noted by LOWIG (1928) that in self-pollinated rye the pollen tubes seldom grow long enough for the pollen grains to become empty. The present investigation confirmed this observation and showed that inhibition of incompatible pollen is strongest on lower branches of the style, where germination is frequently prevented.

Plants were grown in the greenhouse from commercial seed. All four used as females were self-sterile.

Each pistil of the rye flower has two styles, which are joined for but a short distance at their base. As shown in plate I, figures 4 and 5, each style gives off numerous stigmatic branches. A pollen grain anywhere on a branch may form a tube, and this tube enters the branch and grows down its center into the style.

The styles were stained a few minutes in aceto-carmine on a glass slide and then crushed with a cover glass. In the stigmatic branches, tubes are easy to find and follow, and can even be detected in the lower, thicker portion of the style (plate I, fig. 5).

Observations

Few incompatible tubes grew long enough to cause their pollen grains to become empty. That practically all pollen germination and tube growth took place within 24 hours or less was shown by the fact that no increase in the percentage of emptied pollen grains could be noted after that time.

Only a very small percentage of the incompatible pollen grains, even in the more favorable pollinations, became empty. Many grains did not germinate, and those that did seldom produced other than very short tubes, which frequently failed to reach the inside of a stigmatic branch. Near the top of the style it was often possible to follow the longest tubes even after they had entered the stylar tissue. Seldom did these tubes grow more than a short distance after leaving the stigmatic branches, none of those observed growing more than half way to the ovary. Such incompatible tubes were usually swollen and abnormal.

There was considerable variation among the plants as to the amount of germination. Styles with the best germination were the most likely to have emptied grains.

In every style studied, stigmatic branches were more likely to have emptied grains and long tubes the farther they originated from the ovary (plate I, fig. 4). If there were only a few emptied grains, those were always on top branches. If but few grains had germinated, those were on the uppermost branches.

Compatible pollinations made on plant 4, a member of a different population, showed that emptied pollen grains were more likely to occur on the lower stigmatic branches of the style (fig. 5) than on branches originating farther from the ovary. At 50 hours after pollination the emptied grains tended to be more numerous on lower branches, with a more or less regular reduction toward the top in number of grains emptied. The observations in the following table are typical for six styles studied quantitatively.

EXPLANATION OF PLATE I

FIGURE 1.—Pollen grain of *Brassica oleracea* var. *italica* on stigma one day after incompatible pollination, showing typical length and shape of tube. ×1100.

FIGURE 2.—Burst pollen tubes of Abutilon 5 days after self-pollination, at a distance of 7.5 mm from the stigma. ×50.

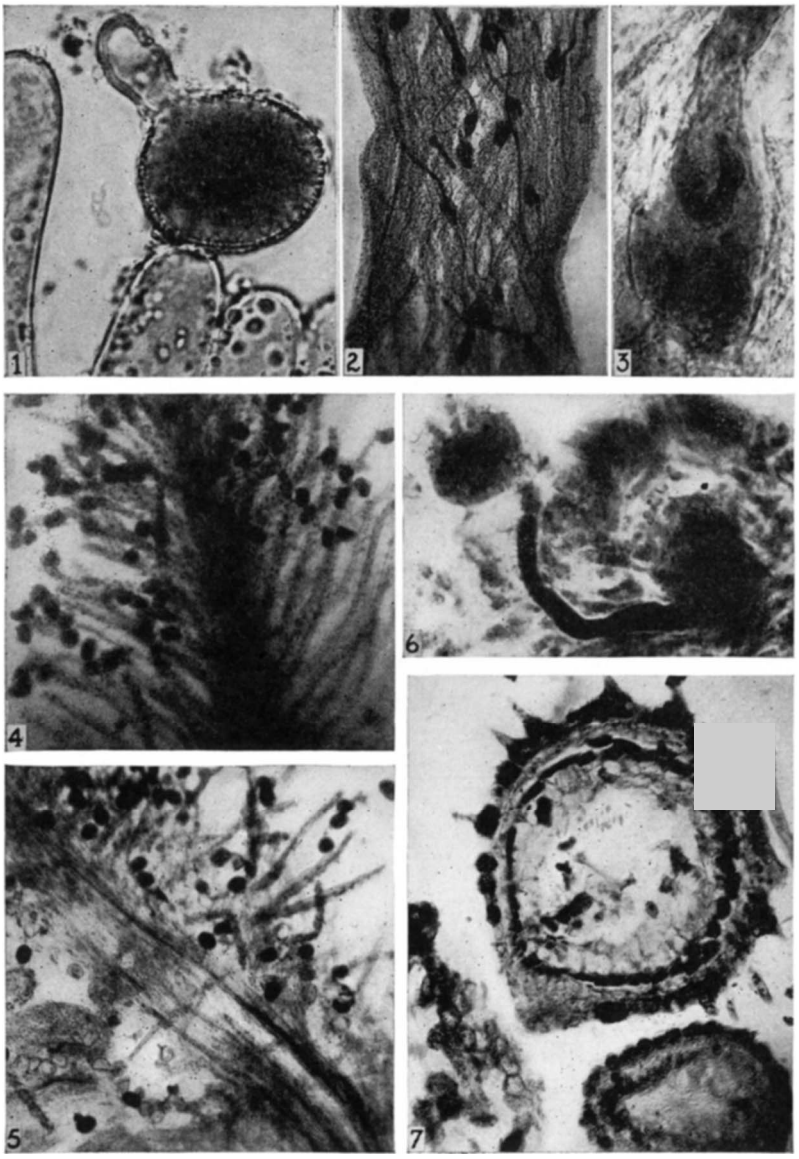
FIGURE 3.—Tube end in same style, 6 mm from stigma. Extruded protoplasm surrounds the tip of the tube. ×650.

FIGURE 4.—Part of style of *Secale cereale* 50 hours after self-pollination. Most ungerminated pollen grains have washed off. Fewer grains are left toward the bottom of the style, showing that less germination occurred there. No grains emptied of contents. ×60.

FIGURE 5.—Lower portion of *Secale cereale* style 50 hours after cross-pollination. Most grains emptied on bottom stigmatic branches. Pollen tubes visible in style. ×50.

FIGURE 6.—Pollen grain (upper left) and burst tube of *Pelargonium hortorum* two days after self-pollination. ×260.

FIGURE 7.—Ovules of *Tolmiea Menziesii* four days after self-pollination. Ovule at bottom right not fertilized. The other ovule, which has been fertilized, has an embryo of several cells, and many endosperm nuclei, of which a few are visible in this section. Surface cells of this ovule beginning to develop hair-like appendages. ×145.


1
2
3
4
6
5
7

Regions represent fields of view of the microscope, at levels of the style progressing from the base to the top.

REGION OF STYLE	NUMBER OF GRAINS	PER CENT EMPTIED
1	12	66.7
2	11	54.5
3	9	66.7
4	24	41.7
5	55	18.2
6	53	5.7
7	46	6.5
8	27	7.4
9	32	3.1
10	13	0.0

Inasmuch as a pollen grain must be emptied of its contents in order for its tube to reach considerable length, few grains on upper branches of these compatible styles can have had tubes long enough to reach the ovary. It is possible that entrance of tubes into the lower part of the style occasions some process that affects unfavorably tubes farther up in the style and results in their ceasing to grow. This idea is supported by a test which consisted of pollinating only the top half of the style of three flowers. The only flower which showed the compatible reaction had a high proportion of emptied grains on the upper stigmatic branches. Failure of the compatible reaction to occur when expected was not unusual in this material.

Since both incompatible and compatible pollinations were made by shaking the spike in a closed bag containing the desired pollen, the amount and distribution of pollen grains must have been similar for the two kinds of pollination. It is clear, then, that the specific inhibition of incompatible pollen is strongest on the lower branches of the style. This suggests that an inhibitory substance is produced in the ovary or lower style and diffuses up the style and out the stigmatic branches; but an equally acceptable explanation is that a local inhibitory reaction occurs which is stronger in regions near the ovary.

CLASS II

In most self-sterile plants pollen germination is not affected by incompatibility, but incompatible tubes fail to reach the ovary. In the most intensively investigated case of this kind, Nicotiana, EAST (1934b) has shown that incompatible pollen tubes undergo a reduction in growth rate in passing through an interference zone in the stigmatic tissue, and that after passing this zone they assume a uniform but slower rate. Studies of EAST and PARK (1918) showed that the incompatible tubes maintain this fairly steady rate until the flower falls. This rate, and hence the length

reached by the tubes, depends on which of the several *S* factors is concerned (EAST 1929).

Less detailed investigations on other plants have shown that the amount and manner of tube growth varies greatly. In Escholtzia (MÜLLER 1868), *Corydalis cava* (JOST 1907), and Tradescantia (MOORE 1917, ANDERSON and SAX 1934), incompatible tubes penetrate but a short distance into the style. *Brassica pekinensis* (STOUT 1931) shows growth to varying distances in the style, depending on the strength of the incompatibility reaction at the time, and ends of tubes are characteristically coiled when they stop growing. KOSTOFF (1927) reported for *Lythrum salicaria* a growth rate which decelerates rapidly at first, but which later gives indication of slight acceleration. In *Trifolium pratense* (SILOW 1931) tubes proceed at approximately the normal rate a little over half way down the style, but are then rapidly retarded. ASAMI and HAYAMI (1934) believe that incompatible pollen tubes of the Japanese pear grow almost as rapidly as compatible tubes to the base of the style, but that they stop there. *Eruca sativa* is reported by AKHTAR (1932) to have incompatible tubes requiring 48 hours to reach the ovary instead of five, but no information is supplied as to rate of growth.

In plums and apples AFIFY (1933) found that although completely incompatible pollen fails to germinate, partially compatible grains produce tubes which progress part way through the style. DORSEY (1919) reported a very slow growth rate of incompatible tubes of plums. In Bömischer Rosenapfel, OSTERWALDER (1910) found club-shaped swellings on the ends of the tubes, which grew to a length of but 2 to 4 mm. COOPER (1928) observed in apples an average growth rate slower than the compatible but found a few tubes growing at the normal rate and accomplishing fertilization. It seems likely that COOPER was not dealing with completely self-sterile varieties.

To the plants in Group II may be added Oncidium (SCOTT 1865); Gomeza, Stigmatostalix, and Burlingtonia (MÜLLER 1868); and *Linum perenne* (DARWIN 1877). These are said to produce pollen tubes of considerable length after self-pollination, and probably belong here.

The present investigation shows that in *Petunia violacea* and *Abutilon hybridum*, inhibition of incompatible tubes is strongest just below the stigma. Usually this inhibition results in abnormalities of the tubes, although some may progress nearly to the bottom of the style before becoming abnormal. Inhibition in *Nicotiana Sanderae* is occasionally so strong that all tubes stop after penetrating but a few millimeters into the stigma. In *Nemesia strumosa* and *Linaria reticulata*, incompatible pollen tubes grow more than half way down the style before being inhibited. A similar

condition apparently exists in *Tolmiea Menziesii*, but in this plant a few ovules may be reached and fertilized.

Petunia violacea Lindl.

Self-sterility in *Petunia violacea* has been shown by HARLAND and ATTECK (1933) to conform to the oppositional factor hypothesis. YASUDA (1929) reports that whereas compatible tubes show an accelerating growth rate and reach the ovary in 36 hours, incompatible tubes exhibit a slower and decelerating growth rate and reach no more than about one-fifth of the way down the style. Incompatible tubes are further characterized by being irregular and swollen at their tips.

The present investigation shows that although a few incompatible pollen tubes may grow nearly to the bottom of the style, the large majority stop within a distance of a few millimeters of the surface of the stigma. These short tubes usually have abnormally thick-walled ends.

All the investigations to be reported were made on first generation descendants of two self-sterile plants of *Petunia violacea* (possibly *P. hybrida* Hort.), discovered in a population of thirteen grown from commercial seed. The genetic constitution of these two plants corresponded to the types S_1S_2 and S_2S_3, as shown by the fact that progeny of a cross between them fell into only two intrasterile, inter-fertile groups. A bud selfing of one of them resulted in four plants homozygous for one S factor, five homozygous for the other, and only three heterozygous, showing that homozygous sterility factors are not always detrimental or lethal to the Petunia plant, as the work of HARLAND and ATTECK (1933) indicated. No difference in vigor was noted in favor of either heterozygotes or homozygotes.

Although in some experiments no self-pollinated flowers were left on the plant as controls, occasional selfings throughout the flowering period gave no indication that the plants were undergoing periods of self-fertility. It was only at the very end of the flowering season that even bud pollinations were made to yield seed, and then only a poor set was obtained.

Methods

Studies were made from whole mounts prepared as follows: Styles were boiled for about 10 minutes in 4 percent sodium sulfite solution, washed in water, and transferred to a drop of water on a glass slide. By pressure from one side with a flat needle, the opposite side of the epidermis was split open and the soft inside portion of the style forced out. The style was then put successively for a half day or more each in absolute alcohol, water, 50 percent saturated aqueous solution of chloral hydrate, and saturated aqueous solution of chloral hydrate (MASSART'S method—1894). To the latter fluid was added enough acid fuchsin to stain the material lightly.

Mounts were made in MASSART'S medium (100 cc water, 16 cc glycerine, 100 gm chloral hydrate, 50 gm gum arabic), to which about as much stain had been added as to the previous solution. Sufficient pressure was applied to the cover glass to squeeze the style into a thin layer only two or three cells in thickness.

With this treatment the tubes themselves did not become stained, except the protoplasm near their tips, but the high refractiveness of the callose plugs in the tubes caused them to be quite conspicuous at low magnification. Careful study at higher powers was necessary to determine the exact number of tubes at a particular level in the style, and especially to find ends of tubes. In the stigmatic region of the style, tubes were frequently inconspicuous.

Observations

The distribution of ends of pollen tubes in several styles (table 4) was calculated from counts of the number of tubes at intervals of a millimeter.

TABLE 4

Petunia. Distribution of ends of pollen tubes in styles of plant 13—1(12) as influenced by type of pollination (compatible or incompatible) and by amount of pollen applied. The abundant pollinations were made on a different day from the sparse pollinations. Compatible matings were of the $S_1S_2 \times S_1S_3$ type. Incompatible pollinations were selfings.

TUBE LENGTHS IN MM	SPARSE POLLINATIONS 38 HRS.				ABUNDANT POLLINATIONS 41 HRS.		
	COMPAT	COMPAT.	INCOMPAT.	INCOMPAT.	COMPAT.	INCOMPAT.	INCOMPAT.
3—	6	14	32	32			
4—	4	7	12	14			
5—	2	5	12	7			
6—	3	5	9	5			
7—		1	1	3			
8—	2	4	3	3		7	
9—		1		4		18	
10—	2	1		3		2	
11—	1	1		2		10	
12—	4	1	1	2	96	1	16
13—	3	2	1	2	59	9	8
14—	8	4			76	6	16
15—	9	18	1	2	111	1	11
16—	20	22	1		100	1	1
17—	17	34			71		1
18—	10	30			40		1
19—	7	11			11		1
20—		13			7		
21—		5			2		
Style L.	21.3	21.3	23.3		21.7	21.6	22.6

In the sparsely pollinated compatible styles, ends of shorter tubes were located by direct observation, since tubes were many and ends few.

Table 4 shows that in compatible styles after sparse pollination (columns 2 and 3), tube ends approach a normal distribution, if only the longer tubes are considered. Tubes shorter than 13 mm are doubtless nearly all incompatible ones in this $S_1S_2 \times S_1S_3$-type mating, as shown by the fact that very few of these shorter tubes are ordinarily found in completely compatible combinations, as $S_1S_1 \times S_2S_2$.

Ends of incompatible tubes show no such tendency toward normal distribution (columns 4 and 5). They are most abundant near the stigma, with a gradual diminution in number down the style. This type of distribution is similar to that found by EAST and PARK (1918) for incompatible tubes of Nicotiana, although their distribution curve was not so strongly skewed. The negative skewness of their curve they largely attributed to differences in time of germination of pollen. These differences are not the factors that upset normal distribution in Petunia, unless incompatible pollen shows more variability in time of germination than compatible pollen does. Although YASUDA (1929) believes that an inhibiting substance is produced in the ovary and diffuses up the style, this skewed distribution of tube ends indicates that inhibition is strongest in the region of the stigma. Possibly all the inhibition occurs in this region, and tubes which succeed in passing through the area are so affected that they eventually come to a stop.

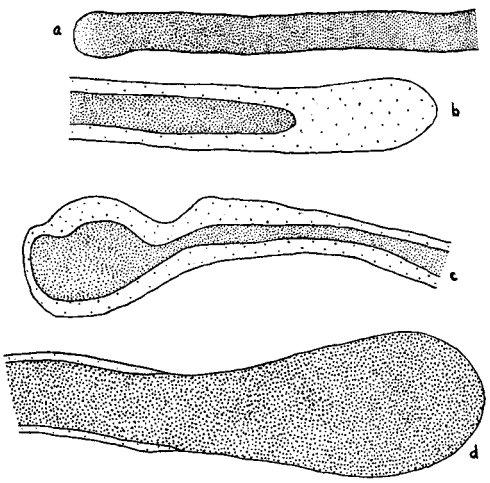
Application of an abundance of compatible pollen upsets the normal distribution of tube ends. After preliminary studies of several styles so pollinated, a typical one was selected for detailed observation of tube-end distribution. In this style (table 4, column 6), the mode came farther behind the longest tube than in the sparsely pollinated styles, and the number of tube ends fell off more slowly toward the stigma. Although complete counts were not made in the upper 12 millimeters of the style, many tubes ended in that region.

Most of the short tubes in the sparsely pollinated compatible styles are probably incompatible S_1 tubes in these S_1S_2 styles. Not nearly so many of these were found as were of the compatible S_3 tubes in the same styles, although equal amounts of S_1 and S_3 pollen must have been applied. However, the preparations did not permit an accurate determination of the number of tube ends in the upper two millimeters of the style, where many of the incompatible tubes probably ended. The two incompatible styles which received approximately as much pollen as these compatible ones showed far fewer total tubes than the compatible styles. In most trials more than half the incompatible tubes could be classified as abnormal. These were mostly ones in which the walls had become so thick and irregular that the tubes appeared capable of little or no further growth. Often the end wall of the tube, which remains very thin in a normal tube (text

fig. 1a) was as thick as the side walls or thicker (text fig. 1b, c). Occasional tubes, particularly the shorter ones, had swollen, thin-walled ends, as (d) in the figure; but the wall back from the tip was thick.

In some styles considerably less than half the incompatible tubes could be classified as abnormal. This reduction in abnormality was accompanied by greater length of tubes, and apparently represented a tendency toward pseudo-fertility.

For studies on rate of growth of compatible tubes, only the longest tube in each style was measured, since this measurement is shown by table 4 to be a fairly reliable index of growth in compatible styles. No support was



TEXT FIGURE 1.—Ends of pollen tubes of Petunia. a is compatible; b, c, and d incompatible. b is the most common type of abnormal tube. All ×1180.

obtained for YASUDA'S (1929) statement that compatible tubes of Petunia have an accelerating growth rate. In table 5 the July data (column 3) indicate a slight deceleration, but much or all of the decrease in growth rate is probably due to cooler conditions during the 12 to 24 hour period which came at night. The slow growth of the tubes in the February experiment (column 5) probably was also due to low temperature, since the plant was then in a cool greenhouse. The February data cannot be used to calculate growth rates, since pollinations were not made on the same day for the 24-hour and 49½-hour observations. It is likely that compatible tubes of Petunia have a nearly steady growth rate. Such a rate was reported by BUCHHOLZ and BLAKESLEE (1927) for Datura, and by HUMPHREY (1934) for *Lilium regale*. EAST and PARK (1918) found an accelerating rate in Nicotiana.

Growth rate of tubes in incompatible styles cannot be compared with

that in compatible styles, since the type of tube distribution differs greatly in the two kinds of matings. The fact that an incompatible style has as long a tube as any in a compatible style does not mean that equal amounts of growth have occurred in the two styles. The lengths attained by the longest incompatible tubes are worth considering, however, for these tubes may occasionally reach the ovary and effect fertilization. Table 5 shows

TABLE 5

Petunia. Length in mm of longest tube in styles of plant 13—1(1) at intervals after compatible and incompatible pollination. Style length=20.0 to 22.8 mm.

HOURS AFTER POLLINATION	JULY, 1934		FEBRUARY, 1935	
	SELFED	×13-1(2)	SELFED	×13-1(7)
6½	4.1	2.5		
	3.3	2.9		
12	9.1	7.9		
	9.9	9.2		
24	18.6	17.3	4.9	9.5
	17.0	20.4	6.0	8.5
			7.0	
32	22.6	through		
	18.4	through		
48	21.4	through		
	19.2	through		
49½			13.8	20.5
			15.5	19.5

that all incompatible tubes may be inhibited almost from the beginning of growth (column 4), or that a few may grow as rapidly as the fastest compatible tubes until nearly the bottom of the style is reached (column 2). In this and other experiments, growth of incompatible tubes ceased in from one to four days, although the style remained fresh for two weeks or more. In some studies tubes reached a maximum length of less than 10 mm in certain styles after incompatible pollination. At other times a few tubes grew 20 mm or farther.

No significant difference was found in growth of tubes after self-pollination of two plants homozygous for different factors.

In spite of the close relationship between Petunia and Nicotiana, and in spite of KOSTOFF'S (1930) success in obtaining fertilization of Nicotiana with Petunia pollen, Nicotiana pollen did not function normally in Petunia styles. Germination was normal, but tubes were very short, and apparently completed most of their growth within 24 hours.

Abutilon hybridum Hort.

Practically all incompatible pollen tubes of *Abutilon hybridum* swell at their ends and burst. Although a few may grow nearly to the bottom of the style, most tubes burst within a few millimeters of the stigma.

Most of the pollen-tube studies were made on a plant obtained from Mr. JUDD of the Arnold Arboretum. This plant, designated hereafter as plant 1, probably belongs in the highly variable horticultural species, *A. hybridum*, although its leaves had five lobes, and BAILEY (1933) describes no more than three-lobed leaves for this species. A second plant, plant 2, originated as the only self-sterile individual in a population of nine raised from commercial seed of *A. hybridum*. It was reciprocally fertile with plant 1. Two other plants were obtained later for genetic work. One of these, a plant with variegated, five-lobed leaves, will be listed as *A. striatum* Dicks., var. *Thompsonii spurium* Lynch, although BAILEY points out that many specimens cultivated as such belong in the *A. hybridum* group, most members of which have many features of *A. striatum*. The remaining plant, one with white flowers instead of orange or reddish-orange, and with unlobed leaves, was the variety of *A. hybridum* known as Boule de Neige.

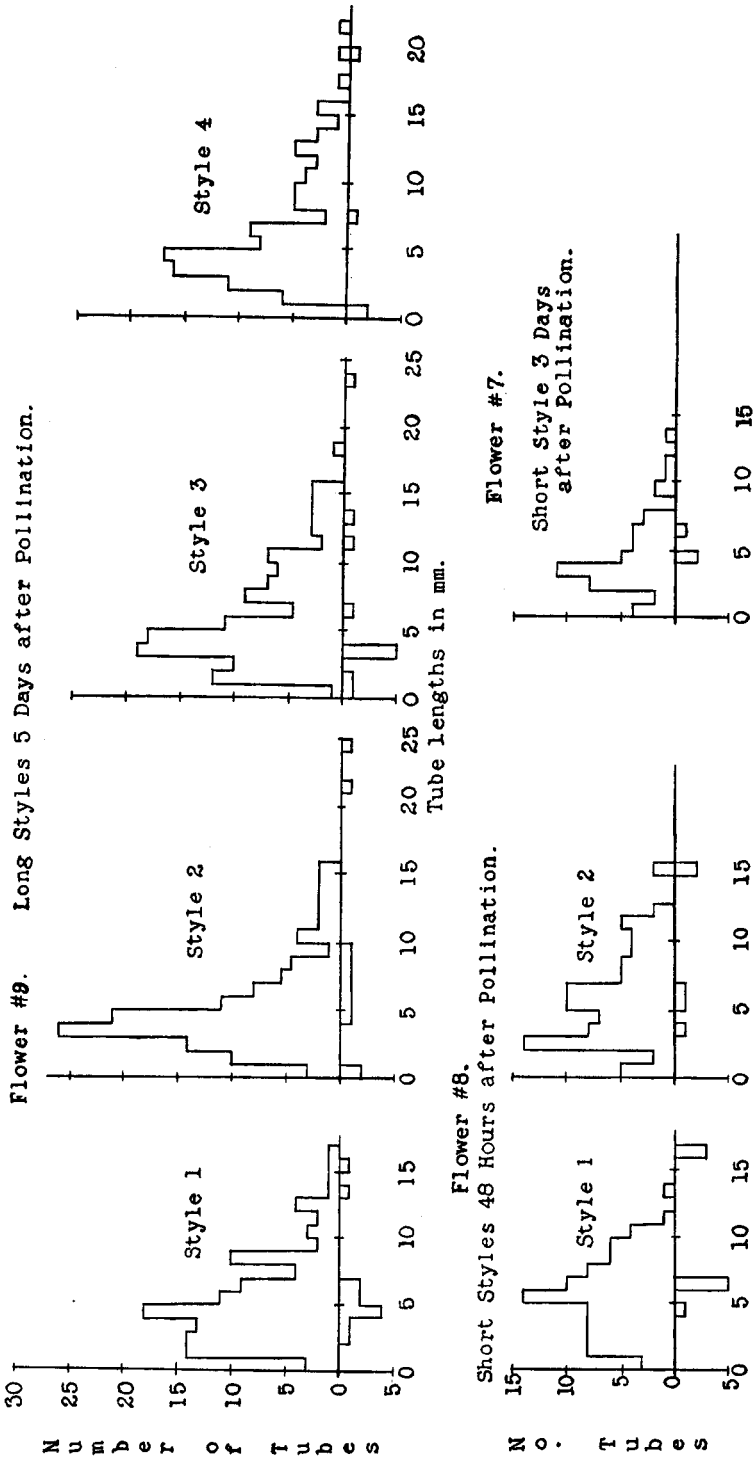
Since flowering maple has several styles to the flower, typically ten, it is possible to pollinate a single flower and determine from sister styles the progress of the tubes at intervals after pollination. This procedure was followed in nearly all the experiments.

Studies of pollen tubes were made for the most part from whole mounts prepared in the way described for Petunia, except that styles were left for only four or five minutes in the hot sodium sulfite solution, and that no stain was used. Also, more manipulation was necessary in removing the epidermis, pressure alone not being sufficient to break it loose. The preparations thus obtained showed very little color except in the protoplasm in the pollen tubes, which was a bright red. This red color was probably due to an anthocyanin transferred by the sodium sulfite solution from the epidermis of the styles to the contents of the pollen tubes. Plant 2, whose styles had none of this red color in their epidermis, gave a much poorer result with this method.

Satisfactory results were obtained by staining the sulfite-treated styles in aqueous solution of acid fuchsin and mounting in lactic acid, or by staining with acid fuchsin in chloral hydrate as described for Petunia.

Cytological observations

Although studies were made on plant 1 at various intervals during a 14-month period, no important variations were noted in behavior of incompatible pollen tubes. In brief the situation was this: Germination was as good as after compatible pollinations, but almost all the incompatible



TEXT FIGURE 2.—Distribution of tube ends in selfed styles of Abutilon. Burst tubes plotted above line, unburst ones below line.

tubes swelled in the style and burst (plate I, figs. 2 and 3), none ever reaching the ovary. While the longest tube in one style stopped after growing only 7 mm, in other styles tubes reached lengths of up to nearly 25 mm. Most of the growth occurred in the first 24 hours, and was probably all finished within 48 hours.

Styles of plant 1 averaged about 25 to 26 mm in length during one period, and about 31 mm at a later time. There was a tendency for tubes to attain a greater maximum length in the long styles. Whereas the longest tube found in the 25–26 mm styles was 19.0 mm, tubes as long as 24.5 mm occurred in the 31.0 mm styles.

To illustrate the type of tube distribution that occurs, graphs are included (text fig. 2) showing the location of the ends of tubes of plant 1 in both short and long styles. Burst tube ends are plotted above the abscissa and unburst tubes below. Unburst tubes were nearly always swollen and abnormal. It is probable that many listed as unburst were actually burst, but at a considerable distance from the end, where the extruded protoplasm may have been interpreted as coming from the nearby end of another tube.

The graphs show that the mode for burst tubes comes between 3 and 6 mm from the stigma, with a gradual diminution in the number of tubes from there down the style. Although most of the very long tubes (19 mm or over) were unburst, they were swollen and abnormal and had probably ceased growing.

The two short styles from flower 8 had fewer tubes than the longer styles, particularly in the 3 to 5 mm region, and no tubes were as long as in the longer styles. The type of distribution is the same, however. The one style from flower 7 illustrates that the same type of distribution occurs when still fewer tubes are present.

Distribution of incompatible tubes of Abutilon is similar to that found in Petunia. The same tendency occurs for tube ends to be grouped near the stigma, although the mode for Abutilon appears to be farther down the style than does the mode for Petunia. There is, further, the common tendency for a few tubes to exceed greatly the modal length. This similarity is probably due to both plants having the inhibitory reaction partially or completely localized in or near the stigma.

While incompatible pollen tubes of Petunia tend to develop an abnormally thick wall, Abutilon tubes burst in the style, a behavior which is correlated with thinness of wall, possibly with abnormal thinness. This dissimilarity in tube behavior is probably due to a difference between tubes of the two species rather than to a difference in the type of incompatibility reaction. When compatible tubes of either Abutilon or Petunia are sub-

jected to crowding in the style, the characteristic abnormality of each occurs.

It is possible that the self-incompatibility reaction in Abutilon and Petunia results only in making tubes abnormal, in that way retarding or stopping them. It is also possible that visible abnormality is merely the end result of an inhibitory process which first acts to slow down the growth of tubes. In Abutilon it is doubtful that tubes stop growing without becoming abnormal in appearance, but there is indication that some Petunia tubes are retarded or stopped before developing visible abnormality.

As with Petunia, the type of tube distribution in Abutilon and the variability from style to style in the same flower prevented the determination of growth rate of incompatible pollen tubes. It was observed, however, that practically all elongation occurs within the first 48 hours. Studies of styles more than two days after pollination indicated no increased tube length over the two-day measurement on styles from the same flower.

Plant 2 showed the same type of distribution of tube ends as plant 1. In four styles (31 mm in length) the longest tube after two days was 16.5, 15.5, 15.0 and 13.0 mm, respectively.

After cross-pollination the tubes traverse the style in less than a day, with little swelling or bursting.

Effect of temperature

There is some evidence in the literature that temperature may have an effect on total amount of tube growth as well as on rate of growth. DORSEY (1919) found that low temperature decreased the set of fruit in plums, and presented evidence that even compatible tubes may be retarded so much by low temperature that they cannot reach the ovary before the stigma falls off. PASKEVITCH and PETROV (1925) reported a variety of apple as being self-sterile in southern Russia but self-fertile in the cooler region around Moscow; another variety, however, was self-sterile in the north and slightly self-fertile in the south.

Abutilon plants were tested at constant temperatures of 30°C (86°F) and 9°C (48°F). In neither case did self tubes grow longer than at ordinary greenhouse temperatures averaging about 20°C. Heat, in fact, decreased the length reached by tubes; the average after 41 hours for 7 styles (longest tube) was only 8.8 mm. Growth at the lower temperature had about the same maximum as normal; two styles had longest tubes at 14.4 mm and 16.4 mm, respectively, after 8 days. As was to be expected (BUCHHOLZ and BLAKESLEE 1927), temperature affected greatly the rate of tube growth. Tubes in the hot chamber had neared their maximum length in $7\frac{1}{2}$ hours, while cold-treated tubes apparently underwent some elongation

after 4 days. At both temperatures flowers remained fresh for some time after growth of tubes had ceased.

The fact that hot conditions decrease tube growth suggests the possibility that the optimum temperature range for some species of plants may be too low to include temperatures ordinarily occurring at flowering time, and that therefore cooler conditions might increase self-fertility in those plants.

Genetic observations

Although the low chromosome number of these plants ($n=8$) gives little indication of polyploidy, results from pedigree studies are best explained on the assumption that two or more series of oppositional factors are present. Two families were raised, one from a cross of plant 1 female by plant 2 male, and the other from the reciprocal cross. These plants were tested as both male and female with both the parent plants. In addition, each plant was tested for self-fertility, and numerous pollinations were made between sister plants and with the two non-related plants mentioned under "material." This work was begun out-of-doors and completed in the greenhouse in the fall and winter. Data are presented in table 6.

Although many of the relationships in the table were determined by a single observation, most are thought to be reliable. Doubtful and borderline cases were usually repeated. There was no sharp distinction between the + and the ± class, the line being drawn arbitrarily at 30 seeds per capsule. Actually the sets ranged from 10 seeds to over 100. If a combination was tried more than once with widely varying results, the highest yield was ordinarily used, since the possibility of pseudo-fertility was not borne out by the occurrence ever of self-fertility. Sets of less than 10 would doubtless have been common also, were it not for the fact that 10 is approximately the minimum number of ovules which must be fertilized for the flower to hang on and form a capsule. Abscissed flowers were found which had 10 to 15 ovules in apparently normal process of seed formation.

Comparison of table 6 with SIRKS's (1926) table 1 of his first generation in *Verbascum phoeniceum* reveals striking similarities: (1) In neither case does self-fertility occur; (2) neither population appears to contain two plants which behave exactly alike; (3) in both, the occurrence of plants reciprocally sterile with both parents is rare, SIRKS finding but one such case in 48 offspring, and none appearing in the Abutilon population of 36; (4) in Verbascum 13 of the 16 possible relationships with parents occur in 30 individuals completely tested, compared to 11 out of 16 with 23 Abutilon plants; and (5) the largest class in both cases is that of plants reciprocally fertile with both parents.

The resemblance of the behavior of Abutilon to that of Verbascum is

close enough to indicate that a similar factor scheme is operating in both genera. For Verbascum, LAWRENCE (1930, 1931) has presented good evidence that the complications in SIRKS's results were due to allotetraploidy, which increased the number of sterility factors from one allelic series to

TABLE 6

Abutilon. Fertility relations between two families (1–2 and 2–1), the parent plants (1 and 2), A. hybridum var. "Boule de Neige," and A. striatum var. Thompsonii spurium. + = fertile; − = sterile; ± = partially or doubtfully fertile. Plants arranged according to their fertility with parents.

♀ \ ♂	2-1(2)	2-1(14)	1-2(17)	2-1(1)	1-2(18)	2-1(9)	1-2(21)	1-2(23)	1-2(24)	1-2(16)	2-1(8)	1-2(22)	1-2(25)	2-1(3)	1-2(6)	2-1(7)	2-1(15)	1-2(11)	2-1(2)	1-2(19)	1-2(12)	1-2(9)	1-2(1)	1-2(4)	1-2(3)	1-2(5)	2-1(6)	1-2(26)	2-1(10)	1-2(10)	1-2(13)	1-2(15)	1-2(8)	2-1(4)	1-2(7)	1-2(20)	1	2	B. de N.	Th. spu.
1-2(2)	−			+	±	+	+			−				−			+		+									+									+	+	+
1-2(14)		−																												+	+					+	+	+
1-2(17)			−	−	+									−	+				−																	+	+	+
2-1(1)	+			−	+		−		−	±		+	−				+		−	−										+		+					+	−
1-2(18)	+		+	+	−		−																															±	+	+
2-1(9)							−											+											+	+								±	+	−
1-2(21)	+			±	+		−			+							+	+	+		+																+	+	+
1-2(23)								−																														±	±	−
1-2(24)									−																													±	±	
1-2(16)	−			+			+			−				−			+		−	+																	+	+	−
2-1(8)						−					−																			+								+		
1-2(22)	−		+	−		−						−					−																					+	+	−
1-2(25)	+		−	−		−			+			−	−	−			+		+				−															+	+	+
2-1(3)	+	+	+						+			+	−	−	+							+	−		+	+					+							+	+	−
1-2(6)				+											−																								+	+	±
2-1(7)	+			+		+				+			+	−	+	−		+	+						+		−	−			+					−		+	+	−
2-1(5)						−											−		+	−																		−	+	+
1-2(11)	+			−			+			−				−	−		+		−	+									−									+	−	−
2-1(2)	+	+	−	−		−	+	−	−			−	−	−	−	−	+	+	+	−	−	+	−	±		−		−	+	−		−				+	−	±	+	−
1-2(19)																			+	−		−				+												+	±	+
1-2(12)																			+		−																	+	−	+
1-2(9)							+								−					−		−				+													+	+
1-2(1)						−								−	+				+				−							−									+	+	±
1-2(4)																								−															−	−	
1-2(3)															−										−		+												−	+	±
1-2(5)						−								±	−	±			+	+		+				−													−	+	+
2-1(6)				−															−						+		−												−	+	+
1-2(26)						+													+									−											+	−	+
2-1(10)						−					−			−			−												−									+	−	
1-2(10)						+																									+	−							−	+	−
1-2(13)		+																														−	−				+	−	−	−	−
1-2(15)																																	−					−	−	−	−
1-2(8)																																		−				+	−		
2-1(4)																			+																−		+	±	±	−	−
1-2(7)																																				−		−	−	−	−	−
1-2(20)																																	+				−	−	−	−	−
1	+	+	+	+	+	+	±	+	+		+	−	−	−	+	+	+	+	+	+	+	+	−	+	+	+	+	−	+	−	−	+	−	−	+	+	−	+	±	+
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B. de N.																							+														±	+	−	
Th. spu.																																					−	−	+	−

two. As LAWRENCE points out, the existence of two series of factors does not explain the fact that of 26 F_1 plants sufficiently tested, no two were the same. Only 16 genotypes could be produced from segregation of two pairs of factors. LAWRENCE is inclined to believe that only two main series of factors were present in SIRKS's F_1 of Verbascum, but that there may have been other factors which were responsible for the incompatibility of cer-

tain combinations. Successive generations brought more and more regularity, until in F_6 and F_7 there was fair agreement with a two-series scheme. Inbreeding and selection may have eliminated the subsidiary factors or resulted in a masking of their effect.

It is probable that such minor factors affecting self-sterility occur in many plants. Selection has frequently been necessary to bring genetic regularity into self-sterility. Even in Nicotiana, one of the plants for which the oppositional factor hypothesis was developed, early generations gave little indication of cross-sterile groups (EAST and PARK 1917), and ANDERSON and DE WINTON (1931) and EAST (1932) found independent factors which modify the action of the *S* genes.

No data being available for later generations of Abutilon, it cannot be said with certainty that further breeding would show two main series of factors as in Verbascum. Such a result seems probable, however, because of the close similarity of F_1 behavior to that in Verbascum. The evidence is not as clear for Abutilon as for Verbascum that the second series of oppositional factors has arisen through tetraploidy. The diploid chromosome number of *Abutilon hybridum* is only sixteen, and no number lower than fourteen is reported for the genus (SKOVSTED 1935). *Sphaeralcea miniata* of the same family has been shown by SKOVSTED, though, to have a haploid number of five, thus heightening the possibility of chromosome duplication in *A. hybridum*.

Two loci for self-sterility in one plant could arise, in such complex hybrids as these Abutilons are thought to be, through hybridity without chromosome duplication. Hybridization of two species, each with a self-sterility factor, but with this factor on different chromosomes, would establish in the offspring two loci for sterility. Since each incompatibility factor would be accompanied by its normal allele, this plant would be self-fertile, but inter-breeding with other F_1 plants might produce a small proportion of self-sterile individuals.

Nicotiana Sanderae Hort.

Observations were made on plants of two families, using the same method of preparing styles as with Petunia. These families came from seed whose genetic constitution as regards self-sterility was known from pedigree work at the Bussey Institution (EAST and YARNELL 1929). One family, 846, was from a bud selfing of an S_1S_9 plant, and the other, 848, was from $S_1S_4 \times S_1S_9$.

Studies of tube-end distribution after sparse, incompatible pollination showed that in nearly all cases the longest tube was not much longer than others; therefore the length of only the longest tube in each style was recorded (table 7). In this experiment incompatible tubes were subject to

much stronger inhibition than was occurring when EAST and PARK (1918) and EAST (1934b) made their studies. During a period in which compatible tubes would have neared the bottoms of the 40–50 mm styles, these incompatible ones progressed only 1.6 to 5.0 mm. Further evidence of extreme

TABLE 7

Nicotiana. Length of longest tube in styles 50–51 hours after pollination.

FEMALE PLANT		MALE PLANT		LENGTH OF LONGEST TUBE, MM	AVERAGES
NUMBER	CONSTITUTION	NUMBER	CONSTITUTION		
848-2	S_4S_9	846–18	S_9S_9	5.0	
				3.2*	
				3.9	
				3.5	3.9
846–1	S_1S_9	846–9	S_1S_1	1.6	
				3.9	
				1.8	
				1.8	2.3
846–1	S_1S_9	846–18	S_9S_9	1.9	
				2.5	2.2
846–6	S_1S_9	846–18	S_9S_9	2.9	
				2.8	2.85
846–9	S_1S_1	Selfed		3.2	
				3.0	
				3.9	
				2.1	3.05
846–17	S_1S_1	Selfed		2.1	
				3.5	2.8
846–18	S_9S_9	Selfed		2.2	
				2.3	
				2.0	2.2

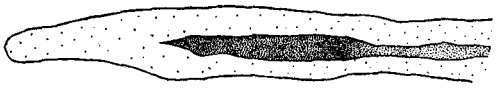
* One tube, probably from accidentally placed, compatible pollen, was 21.6 mm in length.

inhibition is given by the fact that the ends of nearly all tubes were abnormally thick-walled (text fig. 3), presumably capable of little, if any, further growth. Also, very few bud pollinations were effective in producing seed, although EAST (1934a) has shown that S_9 pollen normally gives good yields of seed from incompatible pollinations made in the bud.

There is no indication in the data that a tube grows farther in a style heterozygous for the factor carried by the tube than in a style homozygous for that factor. The significance of the data in table 7 may be questioned because of the high degree to which tubes were inhibited, but in EAST'S

(1934a) experiments, where rate of tube growth was determined by the amount and constitution of seed set by homozygous and heterozygous plants after pollination of open and bud flowers, heterozygous factors usually did not permit faster tube growth than homozygous ones. One of the few apparent exceptions was S_1S_9, in styles of which S_1 tubes grew more rapidly than in S_1S_1, but he interpreted this as being due to the rapid growth of S_9 tubes and their stimulating influence on S_1 tubes.

The failure of S_9 pollen to grow appreciably faster than S_1 may perhaps best be ascribed to the extreme conditions of inhibition prevalent at the time of this experiment. Perhaps strength of inhibition of tubes, while differing normally for different factors (EAST 1934a), under certain conditions may reach a maximum which is much the same for all the factors. Particularly does this seem possible in the light of EAST'S (1934b) demon-



TEXT FIGURE 3.—Abnormal end of incompatible pollen tube of Nicotiana. ×1180.

stration that inhibition is strongest in the upper style; when the reaction is very strong, it may be expressed in completely preventing tubes from passing this zone.

Linaria reticulata Desf.

Self-sterility in *Linaria reticulata* is due to differential pollen-tube growth. Whereas in compatible cross-combinations tubes reached the bottom of the style in less than 25 hours, incompatible tubes grew only 2.8 mm in 4 days, a distance still about .7 mm from the bottom of the styles.

During the first 6 hours after self-pollination, growth of tubes was fairly rapid, a length of about 1 mm being attained. This was approximately equal to the growth of tubes in the same time after compatible cross-pollination. But after 25 hours incompatible tubes were only an average of 1.75 mm long, compared to more than 3.5 for compatible tubes. In 48 hours a length of about 2.5 mm was reached. Further growth in the next 48 hours was only .3 mm, indicating that tubes had ceased elongating by the end of that period.

The number of observations is scarcely sufficient to establish an accurate growth curve. It is evident, however, that incompatible tubes grow at a diminishing rate. Although the 25-hour compatible tubes had completely traversed the style, indications were that they had not yet proceeded far into the ovary. It is thus unlikely that compatible tubes have an accelerating growth rate.

In this study, mounts of whole styles were used. Styles were macerated for 10–15 minutes in hot sodium sulfite, stained in acid fuchsin, and mounted in lactic acid.

Nemesia strumosa Benth.

RILEY (1935) has shown that *Nemesia strumosa* fits the simple oppositional factor scheme. From material provided by DR. RILEY, it was found that incompatible pollen tubes grow nearly to the bottom of the style at a rate approximating that of compatible tubes, but that they then undergo rapid deceleration and come to a stop at the top of the ovary.

Observations based on whole preparations in aceto-carmine indicated that tubes produced after selfing traverse the entire style at a rate equal to that of compatible tubes. Both kinds of tubes reached the bottom of the style (average length, 1 mm) in about three hours.

Determination of the further behavior of the tubes was made from entire pistils fixed at various intervals after self- and cross-pollination, and sectioned in paraffin. Four pistils five hours after selfing showed the longest tubes between .26 and .32 mm (.28 average) from the ovary, or about .90 mm from the stigma. Ten hours after selfing, tubes were .065 to .195 mm from the ovary in four styles, an average of .114 mm, and a growth of only .166 mm in five hours. At $24\frac{1}{2}$ hours the tubes had completed their growth, two pistils showing tubes projecting into the ovary for distances up to .03 mm, one having tubes just ready to enter the ovary, and a fourth showing tubes .065 mm from the top of the ovary. In four 48-hour preparations tubes were still barely at the point of entering the ovary. Preparations after 74 and 99 hours showed the same situation.

After fertile crosses, tubes in two styles were .065 mm and .09 mm from the top of the ovary (approximately 1.1 mm from the stigma) in 5 hours. This is about the length reached by incompatible tubes in 10 hours. Compatible tubes in 10 hours had entered the ovary and could be found halfway to its bottom, a total distance from the stigma of 1.85 mm in one pistil and 1.98 mm in another.

These results show that incompatible pollen tubes of *Nemesia strumosa* grow approximately as fast as compatible ones through the first three-fourths or four-fifths of the style, but then undergo a rather sudden deceleration in rate such that they grow more and more slowly, and finally come to a stop at the top of the ovary. The period of rapid growth lasted in this experiment for about three hours. The tubes were still growing after ten hours but had stopped in $24\frac{1}{2}$ hours.

The incompatibility reaction is either partially or completely lacking in flowers pollinated a sufficient time before anthesis. Good sets of seed were obtained from certain flowers pollinated in the bud. It is possible that the

failure of buds pollinated by RILEY (1935) to set seed was due to the flowers not being young enough. No records were kept in the present investigation as to the number of days required for successfully self-pollinated buds to open.

Tolmiea Menziesii Torr. & Gray

CORRENS'S (1928) observations on *Tolmiea Menziesii* make this plant of particular interest for pollen-tube studies. He found that all the offspring of a cross were reciprocally fertile with each other and with the parents, but that no plants were self-fertile. This relationship differs from any observed in other plants. The present investigation indicates that incompatible pollen tubes are inhibited, particularly in the lower style, to such an extent that too few ovules are fertilized to prevent abscission of the flower.

Only one plant was available for the investigation. This was obtained from MR. F. J. MACGREGOR, who had succeeded in getting it to flower. Plants grown from seed had failed to flower in the Bussey greenhouses. Since lack of other plants made it impossible to study normal, compatible behavior of pollen tubes, inferences regarding the compatible situation are drawn from analogies with other species. These inferences permit certain conclusions regarding incompatible behavior.

Some of the cytological preparations consisted of whole mounts of carpels, made in lacmoid-martius-yellow (NEBEL 1931) after some dissection. For post-fertilization studies, paraffin sections stained in haematoxylin were used.

Pollen tubes are apparently inhibited in their growth, particularly in the lower part of the style. One day after pollination, tubes were almost down to the first ovules, but preparations made on subsequent days showed that only a few tubes had progressed much farther. Only a small percentage of the tubes which grow nearly to the bottom of the style proceed into the ovary. Of these few tubes, still fewer reach the ovules.

As a rule, fertilized ovules are limited to the upper half of the ovary, but in two cases some were found toward the bottom. From the evidence at hand it seems likely that although tubes are rather strongly inhibited in the lower part of the style, those which are able to proceed into the ovary suffer little, if any, further inhibition. The conclusion that incompatible tubes are inhibited is based on the observation that they have a decelerating rate of growth. No case is known of compatible tubes having a decelerating growth rate.

From a study of sectioned material it was found that fertilization very probably occurs in all the ovules entered by pollen tubes. In all but one of six ovaries pollinated in the first few days of the study and fixed for sec-

tioning after three days or more, four or more ovules (4, 4, 6, 5, and 4, respectively) were in more or less advanced states of development. In one four-day ovary, the five developing ovules had embryos of 4 to 9 cells (plate I, fig. 7) and endosperms of about 20 to 60 nuclei. One six-day flower showed still more advanced embryos. Integuments of fertilized ovules evidenced conspicuous development (fig. 7).

Although no fertile material was available for comparison, it is believed that embryo and endosperm development was proceeding normally in the fertilized ovules. In no case, however, did seed form. In six or seven days flowers fell off. In ovaries examined at the time of abscission, all ovules had dried up. Some flowers studied before abscission also showed degenerated ovules. In no case were unfertilized ovules shown to degenerate before fertilized ones, although few observations were made at the critical time.

From the present evidence one is inclined to conclude that not enough ovules are fertilized in Tolmiea to stimulate the ovary sufficiently to cause it to hang on, and that if more ovules were fertilized, seed would develop normally. The most fertilization observed was six ovules, a small proportion of the more than one hundred present in an ovary. In other self-sterile plants where incompatible pollen tubes are known to be inhibited in the style, seed develops normally if enough ovules are fertilized.

CLASS III

It will be shown in this section that incompatible pollen tubes of Gasteria grow normally to the ovules and fertilize them, but that the integuments of the ovules degenerate as if fertilization had not occurred.

For no other self-sterile plant has it been shown definitely that incompatible pollen tubes grow to the ovary at the same rate as compatible tubes and fertilize the ovules. KRAUS (1915) states that the most important and inclusive type of self-sterility among orchard fruits is one in which all processes up to and including fertilization are apparently normal; but the work of CRANE (1927), AFIFY (1933), and HALL and CRANE (1933) points to slow or abnormal pollen-tube growth as the principal cause of self-sterility in these species. Since KRAUS's conclusions were based largely on apples, which are seldom completely self-sterile (HALL and CRANE 1933), it is possible that the pollen tubes he and COOPER (1928) traced into the ovules were actually compatible tubes, and that failure to set seed was due to a cause other than self-sterility.

STOUT (1923) says, "There is evidence that in many grades of self-incompatibility the injurious effects may be exhibited after what is apparently a successful fertilization." The work of STOUT and CHANDLER (1933) on Hemerocallis suggests that self-incompatibility there may not have its effect until after fertilization, for they find it to be the rule that the

reactions of incompatibility are expressed at the entrance to the ovary or within the ovary. STOUT and CHANDLER do not show, however, that self-fertilization actually takes place in these self-sterile day-lilies.

Gasteria

Most of the data to be presented were derived from a single self-sterile plant obtained from the Harvard Botanic Garden, under the name *G. verrucosa* (Mill.) Haw., var. *intermedia* Hort. This plant was reciprocally fertile with a self-fertile *G. Lingua* (Thunb.) Berger from the same source and with an unclassified, self-sterile plant. A less detailed study of the unclassified plant confirmed the general observations on *G. verrucosa intermedia*. In the related genus Haworthia the same general cytological phenomena of self-sterility were found.

Self-incompatibility was complete where it occurred. Not one seed was obtained from scores of self-pollinations of the *G. verrucosa intermedia* plant during four flowering periods.

MARSHAK (1934) classes five out of eight Gasteria species as self-fertile, suggesting, however, that these five may be merely pseudo-fertile. In view of the results obtained in the present investigation, the term pseudo-fertile, as used by EAST and PARK (1917), hardly seems applicable to Gasteria species, As defined by them, pseudo-fertility refers to the phenomenon of pollen tubes which are ordinarily unable to reach the ovules being enabled to do so by a combination of environmental conditions promoting their growth.

Gasteria flowers are proterandrous, so that it is necessary to wait a day or more after the pollen of a flower is shed before pollinating that flower. Just before the stigmatic cells enlarge and the stigma becomes receptive, the style undergoes a considerable elongation. Pollen will not germinate if placed on a stigma not yet ready to receive it, nor will this prematurely placed pollen germinate when that stigma later becomes receptive. Fresh pollen will function, however, if placed on a prematurely pollinated stigma after it has become receptive.

No genetic studies were attempted, because seedlings require several years to reach a flowering stage.

For observations on the growth of pollen tubes, the hollow style was split up one side with a razor blade and flattened out in aceto-carmine.

For study of fertilization, paraffin sections were used. Ovary walls were first trimmed with a razor to expose the ovules to the fixative (NAWASCHIN'S). Iron-alum-haematoxylin was used for staining. Most sections were cut at a thickness of 15 microns.

Tube growth

Two methods were used in determining whether behavior of incompatible pollen tubes differs from that of compatible tubes. The first procedure

was direct observation of tube growth in different styles compatibly and incompatibly pollinated for the same length of time. Discrepancies between tube length in styles pollinated with identical pollen at the same time were not infrequent, presumably because germination is slower on some stigmas than on others. Tube growth is so rapid that differences in time of germination are emphasized; the entire style (length about 12 mm) is traversed in less than six hours. Percentage of pollen germinating is as high after incompatible as after compatible pollination, practically all grains germinating in either case. No consistent difference could be found in the length of time required by compatible and incompatible tubes to reach the ovary. Different flowers pollinated compatibly and incompatibly at the same time and fixed at the same time showed, on sectioning, no difference in the time when sperms were discharged into the embryo sac, nor when fusions of male and female nuclei were completed.

The second method used to obtain evidence of the comparative growth rates of compatible and incompatible tubes was to pollinate flowers with various mixtures of compatible and incompatible pollen and count the number of seeds set per capsule. If incompatible tubes grow as rapidly as compatible ones, presence of incompatible grains amongst the pollen applied should reduce the number of seeds produced per capsule. Such a reduction did result, and it was in general proportionate to the percentage of compatible pollen used. During one period, however, the set of seed from 100 percent compatible pollination was approximately as low as that from 50 percent compatible pollen. Since the set from 50 percent compatible pollination was no lower than usual during this period, it may be concluded that if a sufficient number of incompatibly fertilized ovules are present, no compatibly fertilized ovules abort. If this abortion of some of the ovules is due to a limitation of the amount of some necessary substance, then incompatibly fertilized ovules do not compete for this substance.

The consistent reduction from diluting compatible pollen with incompatible pollen is probably not due entirely to incompatible tubes crowding out compatible tubes. A count of the emptied pollen grains on one stigma showed about 230, which is approximately two and one-half times the number of ovules. Even if all tubes from the emptied grains reached the ovary, there would be too few compatible tubes in mixtures less than 40 percent compatible to fertilize all the ovules. This suggests the possibility that reduction of seed setting after use of pollen mixtures is due to a cutting down of the number of compatible tubes, and not to presence of incompatible tubes which grow as rapidly as compatible ones. If this were true, though, the faster-growing compatible tubes would be expected to fertilize the top ovules in the ovary, as faster tubes have been shown to do in Da-

tura (BUCHHOLZ and BLAKESLEE 1930). No such tendency was apparent here.

Fertilization

Incompatible fertilization corresponds in all details to compatible fertilization. The following description applies equally well to both types.

Tubes may be found entering micropyles six hours after pollination. Division of the generative nucleus occurs in the lower part of the style or the upper part of the ovary. This division, as shown in plate II, figure 8, is characterized by complete absence of any kind of metaphase plate. The two daughter nuclei round up into dense, highly chromatic bodies only slightly elongated (plate II, fig. 9). The entry of the tip of the pollen tube into the embryo sac (plate II, fig. 10) through the micropyle results in the degeneration of the two synergids, although remains of them can be seen for some time.

When the two generative nuclei are discharged into the embryo sac, they assume the elongated, somewhat curled shape illustrated in plate II, figure 11. One proceeds to the endosperm fusion nucleus, while the other remains near the micropyle with the egg nucleus. The elongated sperm, on coming in contact with a female nucleus, rounds up (fig. 13) and then flattens out (fig. 14) on the surface of the nucleus with which it is to fuse. Within $13\frac{1}{2}$ hours after pollination some sperms were already flattening out on the female nuclei. The flattened male mass spreads out into a thin, irregular layer covering approximately an eighth of the area of the haploid female nucleus and about a twentieth of the diploid (the diploid nucleus having from two to three times the surface of the haploid). During this time there is no appreciable increase in volume of the sperm or change in its staining properties. Eventually the male nucleus comes to lie beneath the surface of the female nucleus. For a time the remains of the sperm may be distinguished as a darker area beneath the surface of the other nucleus (plate II, fig. 15). Soon not a trace of the sperm can be found; at least it cannot be distinguished from female nuclear material. The female nucleus undergoes no apparent change during the fusion process, remaining in an interphase condition throughout. When fertilization is completed, fusion nuclei cannot be distinguished from unfertilized female nuclei until time for the first division. There is no apparent precedence of either female nucleus over the other in speed of fusion; either may precede the other, or both may complete the fusion at approximately the same time.

The process of fertilization in Gasteria is quite similar to that described by SAX (1918) for Triticum, except that in Gasteria the two polar nuclei have already fused before the sperm reaches them. Also, there is little indication in Gasteria that the chromosomes from the sperm form a sepa-

EXPLANATION OF PLATE II

All figures are from ovaries of self-pollinated flowers of *Gasteria verrucosa intermedia*.

FIGURE 8.—Metaphase of division of generative nucleus in pollen tube. ×1100.

FIGURE 9.—Daughter nuclei soon after division of generative nucleus. Upper one slightly dislodged in sectioning. ×975.

FIGURE 10.—Pollen tube entering micropyle. Vegetative nucleus (near tip of tube) and one generative nucleus. ×440.

FIGURE 11.—Sperm lying next to endosperm nucleus soon after entering embryo sac. ×920.

FIGURE 12.—Much later stage, from prematurely fertilized flower. Fusion has been completed, and the endosperm has reached a stage where it consists of six nuclei, of which four are shown, one slightly out of focus. The embryo, still one-celled, is at the left, with remains of synergids between it and the micropylar end of the embryo sac. ×140.

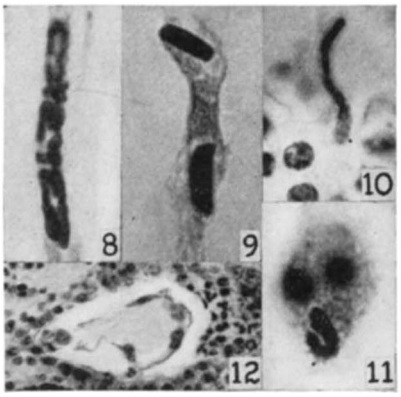
FIGURE 13.—Early stage in fertilization. The sperms have rounded up on the surfaces of the large endosperm nucleus and the small egg nucleus. ×400.

FIGURE 14.—Later stage in fertilization. The sperms have flattened out on the surfaces of the female nuclei. Egg nucleus slightly out of focus. ×465.

FIGURE 15.—Still later stage in fertilization. The male nucleus is beneath the surface of the egg nucleus, only showing as darker places to the left and top in the photograph. ×975.

FIGURE 16.—Telophase of first division of endosperm. Degeneration of integuments particularly apparent at top, where some nuclei have disappeared. ×300.

FIGURE 17.—Endosperm soon after first division. ×650.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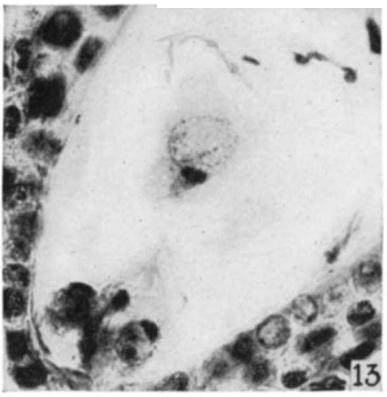
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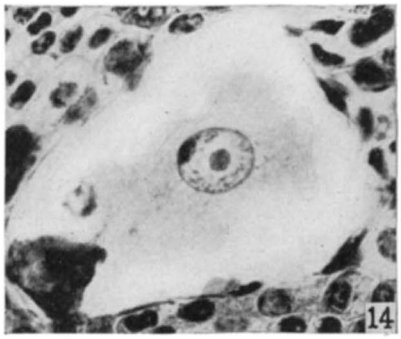
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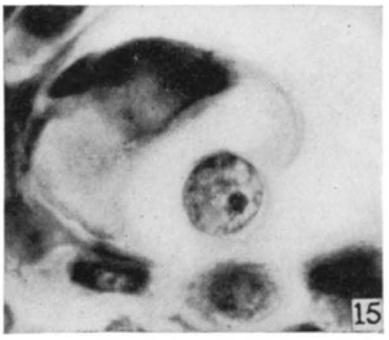
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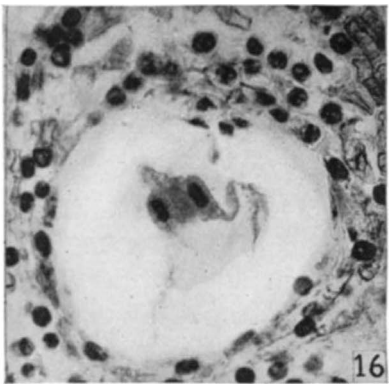
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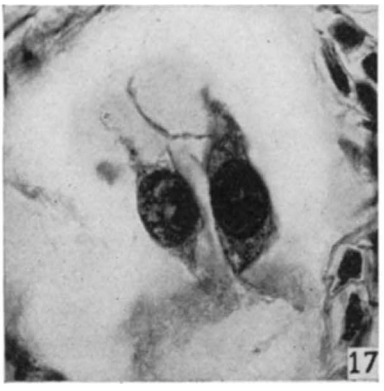
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rate group from the female chromosomes in the first division of the endosperm.

Post-fertilization phenomena

The fusion process is usually completed within the first 30 hours after pollination; then there ensues a resting stage. The first division of the endosperm fusion nucleus usually occurs within 48 hours after pollination, sometimes in less than 36 hours. Text figure 4 and plate II, figures 16 and 17, illustrate the anaphase of the first endosperm division, the telophase of the same division, and the two-nucleate stage of the endosperm, respectively. Development of incompatibly fertilized ovules ceases at this point or earlier. Text figure 4 shows that fusion actually occurs after incompatible fertiliza-

TEXT FIGURE 4.—Side view of anaphase of first division of endosperm, showing the triploid number of chromosomes going to each pole. The chromosome at the top just left of the middle has apparently been displaced by the sectioning knife. Not all the short chromosomes are shown, but twelve long chromosomes can be seen at each pole. The haploid complement of Gasteria is four long and three short. ×2600.

tion and gives the triploid number of chromosomes to the endosperm nucleus.

After compatible fertilization, the endosperm reaches an approximate 16-nucleate stage in 150 to 200 hours. After the 4-nucleate stage, there is little tendency for divisions to occur at the same time throughout the embryo sac. The embryo does not reach a 2-celled stage until the endosperm has 8 to 16 or more nuclei.

Incompatibily fertilized ovules usually begin to degenerate within 48 to 96 hours after pollination. If pollination is made as soon as the stigma becomes receptive, the endosperm of most ovules reaches the two-nucleate stage before degeneration occurs. Counts were made of the numbers of two-nucleate endosperms in ovaries in which the ovules were showing obvious signs of degeneration. Of two ovaries self-pollinated $72\frac{1}{2}$ hours, one showed 35 endosperms divided or dividing in 56 ovules in which the condition could be definitely determined, and the other had 42 divided or dividing endosperms in 53 ovules. The control compatible pollination

showed 43 divided or dividing endosperms in 50 ovules. Since the value for the second incompatibly pollinated ovary so closely approaches that of the compatibly pollinated control, it appears that incompatible pollination may result in as high a proportion of fertilized ovules as does compatible pollination.

There were evidences of degeneration of the integuments of the ovule before the contents of the embryo sac showed any sign of degeneration. Counts were made of the number of dividing cells in the integuments of compatibly and incompatibly fertilized ovules fixed at approximately the same time after pollination. 78 hours after a compatible pollination, there were 201 dividing cells in one 10-micron section of 40 ovules; and 70 hours after another compatible pollination, 128 dividing cells in 21 ovules. Only 43 dividing cells in one section of 31 ovules and 87 dividing cells in 35 ovules were obtained for incompatible pollinations of $77\frac{1}{2}$ and $71\frac{1}{2}$ hours, respectively. This slowing up of division in the integuments of the ovule before any abnormality can be detected in the embryo sac indicates that degeneration starts in the integuments of the ovule rather than in the ovule's contents.

Ovules degenerate at approximately the same time whether fertilized incompatibly or not at all. This fact, coupled with the observation that degeneration starts in the integuments, suggests that the fusion nuclei are quite capable of further division, but that this division is prevented by degeneration of the rest of the ovule, which for some reason is unable to develop after incompatible fertilization. Support for this idea is provided by a study of flowers pollinated with mixtures of compatible and incompatible pollen. At the time degeneration starts in the integuments, endosperm of incompatibly fertilized ovules is as advanced in development as that of compatibly fertilized ovules in the same ovary. At this stage the embryo has not yet undergone its first division in either type of ovule. Presence of compatibly fertilized ovules amongst the incompatibly fertilized ones has no effect on time of onset of degeneration, this occurring before or soon after the first endosperm division.

Premature fertilization

By means of splicing styles, it was demonstrated that the endosperm of incompatibly fertilized ovules is capable of more than a single division. By grafting the upper half of a style whose stigma had become receptive onto the lower half of the style of a younger flower, it was possible to get pollen tubes inside the ovary four days or more sooner than would normally occur. After such a premature pollination, those ovules which were fertilized did not degenerate when the endosperm reached the two-nucleate stage, but remained normal until division had gone further (plate II, fig.

12). In one ovary pollinated 176 hours, four ovules were found with endosperm in an eight-nucleate stage and eleven in a four- to seven-nucleate condition. All the rest which could be accurately determined had not divided at all, indicating that they were not ready for fertilization when the pollen tubes reached them.

The method of style grafting was adapted from that used by BUCHHOLZ, DOAK, and BLAKESLEE (1932) for Datura styles.

After the grafting experiments had been completed, it was found that similar premature fertilization can be obtained by cutting off the upper part of the style and pollinating the cut surface of the remaining part. The one flower operated on, which was compatibly pollinated approximately one day before the stigma would have become receptive, yielded 76 seeds. This flower was put in a moist chamber after the operation.

As stated earlier, ovules are susceptible to fertilization four or five days before the stigma becomes receptive; but pollination previous to that time is ineffective. Development beyond the eight-nucleate-endosperm stage probably cannot be obtained by the premature-pollination method, since the above-mentioned 176-hour flower was pollinated nearly as early as possible; younger flowers on the stalk, pollinated at the same time, showed no sign of having been fertilized.

There was no positive correlation between the amount of development that had proceeded within an ovule and the extent to which it had degenerated. In fact, ovules with developing endosperm tended less to be degenerated than those whose endosperm was not developing, although numbers were too small to make this tendency significant.

Ovule culture

By removal of self-fertilized ovules and culture of them in nutrient solution, development of endosperm was induced to proceed further than after premature fertilization. Two types of nutrient medium were used in a preliminary trial with compatibly (self-)fertilized ovules of *Gasteria Lingua* put in culture $5\frac{1}{2}$ days after pollination. TUKEY'S (1933) nutrient agar permitted some growth of the ovule as a whole, and the outer integument still contained non-degenerated cells 140 hours after the beginning of the experiment; but no certain cases appeared, in the small number tried, of development within the embryo sac. WHITE'S (1934) nutrient medium gave several instances of further development of embryo and endosperm, so it was used for subsequent experiments.

Trials with compatibly fertilized ovules of *Gasteria verrucosa intermedia* showed them undergoing some development when placed in the medium two days after pollination, the earliest test made. At this time the endosperm of approximately half the ovules had divided once, the other half

not at all. Development did not ordinarily proceed much beyond the 2-celled embryo stage (approximately 20 endosperm nuclei), although the outer integument of the ovule continued to grow for some time after the embryo and endosperm had stopped developing and had degenerated. In one instance an embryo reached a 15-celled stage. This ovule was put in the nutrient medium three days after pollination and left there for seven days.

Of the many incompatibly fertilized ovules which were cultured, only one showed good evidence of development subsequent to its removal from the ovary. This one, taken from the ovary 54 hours after pollination, had a 17-nucleate endosperm after three days in culture. Although the embryo had not yet reached a 2-celled stage, this delay is not abnormal. The reason that all other ovules of this type failed to develop in nutrient solution is thought to be that these had already started to degenerate when taken. Ovules are frequently dried up two days after pollination, and even though the ones used in these experiments appeared normal, they may actually have already started to degenerate.

Discussion

Endosperm development proceeds normally after incompatible fertilization in Gasteria, and presumably the embryo is also capable of development, since normal fusion appears to take place. No 2-celled embryos were found, but no ovules had gone beyond the stage where the first division of the embryo normally occurs.

Apparently the only process occurring at compatible fertilization which is upset by incompatible fertilization is stimulation of the integuments of the ovule. After incompatible fertilization the integuments degenerate as if no fertilization had occurred.

From the work of KOSTOFF (1930) it is known that the entrance of a pollen tube into the micropyle of an ovule may stimulate the integuments of the ovule to development, even though fertilization does not occur. This stimulation is not specific, since it may be given by tubes from plants of another genus; therefore, the failure of incompatible tubes of Gasteria to stimulate the ovules is presumably not due to a specific lack of the power to stimulate. Some reaction between the incompatible pollen tube and the ovule must occur at or near the time when the stimulus is ordinarily given, which either upsets the stimulating reaction or else exerts a separate, inhibiting effect which offsets the stimulation. The specificity of this reaction indicates that it has analogies with immune reactions. The necessity for it to coincide in time with the stimulating action makes it likely that it is a reaction between the pollen tube and the integuments of the ovule.

The simplest explanation for the failure of ovule-stimulation at incompatible fertilization is that some reaction of the immune type occurs between the pollen tube and the integuments of the ovule which involves the substance that would otherwise stimulate the ovule. The same failure of the tube to stimulate the ovule could arise through a separate, inhibiting effect which offsets the stimulating action; but no indication of such a separate effect can be obtained, even though fertilization may be made to occur up to a week before the ovule starts to degenerate. This demands a perfect balance between the inhibiting and the stimulating effects as to strength, and a perfect coincidence between the two as to the time when they become effective.

Although it has been assumed that the incompatibly fertilized egg of Gasteria is capable of division, this has not been demonstrated. It does not seem probable, however, that inability of the egg to divide could be responsible for degeneration of the ovule. Since Kostoff has shown that stimulation of the ovule is not dependent on fertilization, the effect of the incapacity for division would have to be inhibitory. Such an effect could not be due to a reaction between the fusing nuclei, since on any genetic scheme explaining self-sterility, the gametes would frequently carry non-oppositional factors (in half the cases on the Nicotiana-Veronica scheme) and therefore be compatible. Consequently, the inhibitory effect would be due to a reaction between diploid ovule tissue and the sperm, and would be subject to the difficulties pointed out in the preceding paragraph. Also, if the sperm is responsible for degeneration of the ovule, it is probable that its reaction with the diploid ovule occurs before fusion rather than after.

Although there is little support for the idea that the egg fusion nucleus is responsible for degeneration of the ovule, it can still be argued that this nucleus is incapable of division. It is possible that the incompatible gametes undergo apparent fusion without actually fusing completely; that the two gametes co-exist, unfused, under a single membrane. This could occur, on the oppositional factor scheme, if one or the other gamete retained some influence of diploid tissue. But it seems scarcely possible that the gametes should fuse completely and that the zygote should be incapable of dividing. The only satisfactory means by which such a phenomenon could be explained genetically would be for the cytoplasm of the egg nucleus to retain the diploid influence of the megaspore mother cell, and then to prevent proper functioning of a zygote which possessed no factor different from either of the corresponding pair of factors in the cytoplasm. Sameness of factors could not be the cause of the upset, since even compatibly fertilized eggs would carry one factor identical with one of those in the egg cytoplasm. This sort of reaction would be of a fundamentally different type from any that has been demonstrated for self-sterile plants; it

would be a stimulation by unlike factors rather than an inhibition by like factors.

GENERAL DISCUSSION

Physiology of self-sterility

The only satisfactory explanation thus far advanced for the physiology of self-sterility is that of EAST (1929) that the reactions of self-incompatibility resemble those of immunity. EAST'S suggestion was based on the following considerations:

1. The effect of the style on incompatible pollen tubes is probably an inhibition of them rather than a failure to stimulate them. Since pollen tubes will grow on artificial media containing no protein, growth of compatible tubes in the style must be of a simple, nutritional nature, not involving special stimulating substances.

2. The reaction between stylar tissue and incompatible tubes is extremely specific, for only S_1 tubes are inhibited by an S_1 factor in the style.

Although the antigen-antibody hypothesis was applied by EAST only to cases where the pollen tube was inhibited in the style, it fits even better the several instances now known where pollen germination is affected. It might be argued that tubes in the style do receive specific stimulation, since the tube growth obtainable in culture is usually much less than the distance from stigma to ovules. It can scarcely be said, however, that germination of incompatible pollen is prevented by a lack of specific stimulation, for germination may occur in moist air alone.

In every case where pollen germination is affected by incompatibility (Brassica, Raphanus, Capsella, Pelargonium and Secale) the stigmatic cells do not secrete appreciable amounts of fluid. In apples, where incompatible pollen may be prevented from germinating, certain varieties have considerable stigmatic fluid; but others have very little, and these are perhaps the varieties where incompatible pollen does not germinate. It thus appears that germination cannot be affected if much fluid is present on the stigma. This phenomenon has two possible explanations: (1) The inhibiting effect is based on a mutual reaction between pollen grain and stigmatic cell, for which closer contact is necessary between the pollen and the cells of the stigma than occurs on stigmas with large amounts of the more purely nutrient fluid. (2) An inhibiting substance is present in cells of the stigma, but this substance is so labile that it is rendered ineffective when released into the stigmatic fluid. Either of these explanations would account for the fact that germination is normal in such plants as Nicotiana and Petunia, which have a copious stigmatic fluid, but that inhibition begins soon after the tube penetrates into the stigma. Either explanation would also account for the numerous instances where it has not been possible to

extract any inhibiting substance from incompatible stigmas or styles and make it affect pollen tubes in culture.

It is perhaps a general rule that stigmas which prevent germination of incompatible pollen have no stigmatic fluid, but the converse is not true, that incompatible stigmas without fluid always prevent germination. The inhibiting reaction may be localized in another part of the pistil, and even if it occurs on the stigma, it may not be strong enough to prevent germination.

It is conceivable that a pollen tube in the style might be affected by a substance produced by a cell with which the tube did not come in contact. An inhibitory substance might be stable within the tissues of the style, where it is not directly exposed to the air. The substance might not be capable of much diffusion in the style, however, since the antigens concerned in immune reactions, which are the reactions presumably analogous to those of self-incompatibility, are protein molecules which would probably have difficulty in passing through cell membranes. Postulates demanding considerable diffusion of proteins, nevertheless, are more readily acceptable today than they were earlier, because of the recent rapid growth of knowledge regarding allergic manifestations.

When the zone of action of the inhibiting substance is sharply localized, as in broccoli, the cause of this localization may be, (1) non-diffusibility of the inhibiting substance, (2) lability of this substance such that it loses its effectiveness soon after passing from the cell where it was produced, or (3) lack of any such substance except as produced by direct interaction of the pollen tube and stylar cells. Where the zone of inhibition passes gradually into the zone of non-inhibition, as in Secale, the situation can be explained by assuming that a diffusible inhibiting substance is present. But it can be explained without postulating diffusion by supposing that the capacity for an inhibitory reaction varies in different parts of the pistil.

For Gasteria, where neither pollen nor pollen tubes are inhibited, but where the integuments of the ovule undergo no development after incompatible fertilization, it is assumed that a substance carried by the tube stimulates the integuments of compatible ovules, but is involved in a reaction with integuments of incompatible ovules which prevents it from stimulating them. All cases of self-sterility are thus given a similar explanation, in that the incompatible male gametophyte reacts with certain parts of the pistil in a manner analogous to immune reactions in animals. Differences in behavior of pollen and pollen tubes thus depend on what part or parts of the pistil develop the power of reacting in this way, and on what time this power of reaction develops. Bud fertility is due to the inability of the pistil to react against the male gametophyte until near the time of anthesis.

Correlation between taxonomic groupings and cytological phenomena

If self-sterility in all higher plants involves the same type of reaction between male gametophyte and diploid female tissue, as has been assumed in this investigation, then the only differences that can occur are due to differences in the location or strength of the reaction. Such differences might or might not correspond to taxonomic groupings. From the fact that of all plants thus far examined, in only two close relatives, Gasteria and Haworthia, does the reaction occur between pollen tube and ovule, it may be concluded that this type is rare in the plant kingdom but that it is probably the predominant kind in the Aloinae.

In several members of the Cruciferae (*Brassica oleracea, Raphanus sativus, Capsella grandiflora* and *Cardamine pratensis*) pollen is inhibited on the stigma. This may be true of the family in general. Although incompatible tubes of *Brassica pekinensis* grow part or all the way to the ovary at certain times in the flowering season, possibly pollen is slightly inhibited on the stigma even during these periods.

Self-sterility in animals

Self-sterility has been described for two animals, *Ciona intestinalis* (CASTLE 1896) and *Styela partita* (PLOUGH 1933), both tunicates. In Ciona, MORGAN (1923) finds that the block to fertilization is in the test cells, which are diploid cells beneath the egg membrane not derived from the egg. The sperm is unable to penetrate the test cells and reach the true surface of the egg. When these cells are removed, normal fertilization occurs. While the self-sterility of Ciona has not been subjected to complete genetic analysis, the physiological situation appears to be very similar to that in plants: A haploid male element is inhibited by diploid female tissue. This inhibition of the sperm of Ciona can be explained as due to the same antigen-antibody type of reaction as was assumed for plants.

Styela appears at first not to be subject to the same interpretation as Ciona, for PLOUGH found that the sperm reaches the true surface of the egg without being able to enter. However, the cytoplasm of an animal egg arises entirely under diploid influence; and the first maturation division is not completed in Styela until the sperm has entered. The cytoplasm is therefore functionally diploid, and the situation in Styela does not differ fundamentally from that in Ciona.

Self-sterility in fungi

The Ascomycetes *Sclerotinia Gladioli* and *Pleurage anserina* are hermaphroditic and self-sterile, as shown by DRAYTON (1934) and AMES (1934), respectively. The incompatibility occurs between two haploid tissues instead of involving at least one diploid tissue as in the higher plants and

in animals, but the genetic situation conforms to the oppositional factor theory, wherein an *S* factor in female tissue inhibits a male element which possesses the same *S* factor. No reason is apparent why this reaction of *S* factors may not be of the immune type. At the same time, it is realized that other interpretations are possible. Neither AMES nor DRAYTON found more than two incompatibility factors in material from several different localities. If S_1 is fertile only to S_2, as is indicated, it is possible that S_1 stimulates S_2 and that the incompatibility of S_1 and S_2 is due to an absence of this stimulation. Not enough cytological details are known of the processes involved to aid in settling the question.

From an evolutionary standpoint, self-sterility should be of more importance to fungi than to the higher plants or to animals. Whereas self-fertilization of diploid individuals results in homozygosity only after several generations, in fungi it gives complete homozygosity immediately. Thus recombination of factors is prevented at once, and variation thereby limited. Homozygosity of the zygote has no advantage to fungi, since it cannot aid in eliminating deleterious recessives, as it does in the higher plants. Haploidy of the dominant generation produces in the fungi the equivalent of complete homozygosis at all times.

GENERAL SUMMARY

Self-sterility in all higher plants thus far investigated bears interpretation on the basis of a reaction of the immune type between the male gametophyte and diploid female tissue. Differences in behavior of the male gametophyte depend on localization of this reaction in different parts of the pistil.

The present investigation permits the following classification according to when the incompatibility reaction occurs:

I. Before the pollen germinates.
II. While the pollen tube is growing in the style.
III. When the tube reaches the ovule.

Group I represents a tendency toward localization of the incompatibility reaction in the stigma, accompanied by a lack of stigmatic fluid. In group II, the reaction occurs in the stigma or in various parts of the style. In group III the integuments of the ovule are concerned in the reaction, which prevents the incompatible tube from stimulating them to development.

Investigation of plants in these groups has provided the following information:

GROUP I

A. *Brassica oleracea* var. *italica*

1. Incompatible pollen either does not germinate or else produces but very short tubes. Compatible pollen is inhibited slightly.

2. The inhibitory reaction is confined to the surface layer or layers of the stigma. Removal of this region permits self-fertilization.
3. Two series of oppositional factors are probably present, each of which is composed of factors of varying inhibitory potency.

B. *Raphanus sativus*

1. Incompatible pollen is affected in the same way as in *Brassica oleracea* var. *italica*.

C. *Pelargonium hortorum*

1. Germination of incompatible pollen may be completely suppressed, or a few tubes may be produced. These swell and frequently burst soon after penetrating the stigma.

D. *Secale cereale*

1. Germination of incompatible pollen is poor, and few tubes grow to sufficient length for their pollen grains to become empty. Inhibition is strongest on lower branches of the style.

GROUP II

A. *Petunia violacea*

1. Most incompatible tubes stop shortly below the stigma, but a few may grow nearly to the bottom of the style.
2. Incompatible tubes usually become abnormally thick-walled.

B. *Abutilon hybridum*

1. Inhibition is strongest in the stigma and upper style. Incompatible tubes swell and burst.
2. High temperature (30°C) decreases total growth of incompatible tubes.
3. The genetic situation can be explained as due to two main series of oppositional factors and several other factors of similar nature but less activity.

C. *Nicotiana Sanderae*

1. Environment favoring extreme incompatibility results in similar inhibition of tubes which would normally be inhibited to different degrees. Tubes are very short, and most are abnormally thick-walled.

D. *Linaria reticulata*

1. Incompatible tubes are inhibited in the bottom half of the style. About four-fifths of the style is traversed before growth ceases.

E. *Nemesia strumosa*

1. Inhibition occurs in the lower style. A few tubes may reach the ovary and their tips protrude into its cavity.

F. *Tolmiea Menziesii*

1. Growth of incompatible tubes appears to be retarded, but a few ovules are reached and fertilized. In these, embryo and endosperm development proceeds rapidly, but the number of fertilized ovules is probably too small to prevent abscission of the ovary.

GROUP III

A. *Gasteria*

1. Incompatible tubes grow as fast as compatible ones and effect fertilization.
2. Incompatibly fertilized ovules degenerate at the same time as unfertilized ovules. This occurs before development has progressed beyond the binucleate-endosperm stage, but premature fertilization, obtained by grafting styles, resulted in endosperm reaching an 8-nucleate stage. In nutrient solution one incompatibly fertilized ovule developed a 17-nucleate endosperm.
3. Incompatibly fertilized ovules are not influenced by the presence of compatibly fertilized ovules in the same ovary.
4. It appears that an incompatible pollen tube fails to stimulate the integuments of the ovule; that the substance which would otherwise provide this stimulus is prevented from doing so by a reaction of the immune type between the pollen tube and the integuments.

The following general conclusions may be drawn:

1. Self-incompatibility of the Gasteria type is rare in the plant kingdom.
2. Incompatible pollen of Crucifers tends to be inhibited on the stigma.
3. Self-sterility in animals has a physiological basis similar to that in the higher plants.
4. Self-sterility in fungi perhaps depends on a reaction of the same immune type as assumed for animals and the higher plants.

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