Incompatibility in the genus campanula (campanulaceae)

F. May Block

How to cite:

Block, F. May (1964) Incompatibility in the genus campanula (campanulaceae). Doctoral thesis, Durham University.

Incompatibility in the genus Campanula (Campanulaceae)

by

F. May Block, née Watt, B.Sc.,

(St. Mary's College).

Being a thesis presented in candidature for
the degree of Doctor of Philosophy of the
University of Durham, September 1964.

ACKNOWLEDGMENTS.

The writer wishes to thank Dr. J.L. Crosby under whose guidance this work was carried out, and Professor D.H. Valentine in whose department this study was made.

Thanks are due to the laboratory staff of the Botany Department, especially Mr. Redhead, for technical help, and to Mr. Rose of the Botany Garden for continual advice.

The writer is further indebted to Dr. M.A. Keay (Department of Agricultural Biology, Makerere University College, Kampala, Uganda) for her help, and kind provision of a room in her department in which to complete this thesis. Thanks are also due to the staff of Makerere library for duplicating the figures, and to Mrs. W. Gleeson for typing the final draft.

Grateful acknowledgment is made to my husband for reading the manuscript.

This study was carried out whilst in receipt of a Durham Colleges Research Studentship, for which the author expresses her sincere thanks.

CONTENTS.

II. THE BACKGROUND.

A) Incompatibility

B) The genus _Campanula_

page

III. CAMPANULA PERSICIFOLIA

A) Breeding patterns

B) Tetraploidy

IV. SOME OTHER SPECIES OF CAMPANULA

I. INTRODUCTION.

The numerous methods by which outbreeding is maintained in the plant kingdom suggest that the resultant vigour and stored viability produced by heterozygosity is of great value to the species. There are many methods by which inbreeding is prevented and the consequent heterozygosity is ensured, both in a precise and an imprecise manner. One example is dichogamy, the plant showing either protandry or protogyny. This phenomenon applies to each flower on a plant and does not prevent self-pollination, for one flower may easily be pollinated by another on the same plant. Outbreeding is also maintained by elaboration of the floral mechanism, thus reducing the chance of self-pollination, e.g. Orchidaceae and Apocynaceae. More precise methods of maintaining heterozygosity are by the phenomena of dioecism and incompatibility.

Extremely specialised methods are found in some members of the Onagraceae, where heterozygosity is enforced even in the absence of outbreeding. The breeding systems of the Onagraceae have been studied in great detail, for its species show considerable diversity in this respect. The most common breeding system is that typified by _Oenothera_ _lamarckiana_, in which survival of the homozygous form is prevented by disjunction of interchange complexes containing balanced lethals. Both complexes are carried through the pollen and ovule, and the lethals act at the zygotic stage.

In _O.muricata_ selection of the complex takes place at an earlier stage, in the megaspore and microspore. _O.muricata_ is an interchange heterozygote between the _rigens_ and _curvans_ complexes. Almost all the functional eggs carry the _rigens_ complex, while only curvans pollen succeeds in reaching the ovary. In Oenothera it is the megaspore at the micropyle end which usually develops into the embryo-sac, and if the megaspore in O.muricata carries the rigens complex, it develops normally. If, however, the micropylar megaspore is curvans, the chalazal megaspore (which must then be rigens) outgrows the other spore and comes into position to form the embryo-sac. Only very occasionally does the curvans complex succeed in forming the embryo-sac.

Renner (1917) found that pollen tubes carrying one complex may tend to grow faster than those containing the competing complex. Thus in de Vries' O.biennis, the velans complex is carried faster to the ovules than curvans. This competition between pollen tube growth is more marked when the pollen is used for hybridisation on O.muricata stigmas. Here the velans complex fertilises four times as many ovules as curvans. The extreme case is reached when O.biennis is used on O.lamarckiana; no curvans pollen succeeds here, all the ovules being fertilised by the velans carrying pollen.

Nine of the subgenera of Oenothera exhibit self-incompatibility. The incompatibility is controlled by one gene, or gene complex, which usually has a large number of alleles, e.g. O.organensis has 45 known alleles of the s gene. Pollen behaviour is determined gametophytically, and the incompatibility may, or may not, be associated with small chromosome rings, depending on the species.

Thus the genus Oenothera shows a wide range of systems by which heterozygosity is maintained. In some instances, e.g. O.muricata, a species with a well developed structural heterozygosity, there are phenomena which strongly suggest a relationship with an incompatibility system. For this reason Crosby (1954) suggested that the evolution of the interchange system, with large chromosome rings held together by balanced lethals, may have depended initially on the presence of an incompatibility system, and eventually replaced it in many species. An incompatible species which is determined by an s oppositional system is permanently heterozygous for this gene, due to fertilisation taking place only when the s allele present in the pollen grain differs from the two alleles in the pistil. It follows that if this gene becomes incorporated into an interchange on a chromosome, it will enforce the heterozygosity of the chromosome. With the chromosome being linked in this manner, it is possible that lethal alleles could arise on it by mutation, but as the chromosome is always heterozygous due to the s allele, their presence would not be felt. However, if through the loss of the s incompatibility allele, the chromosome was present as a homozygote, the lethals would act and bring about post-fertilisation elimination of the homozygote. Thus the heterozygosity of the genes on the interchange would then be enforced not by gametic elimination, but by zygotic elimination of homozygotes. Once zygotic lethals have been established on the interchange chromosome, the importance of the s allele is lost, and the gene may be eliminated by mutation. Heterozygosity of the system becomes enforced by balanced lethals on a permanent interchange chromosome.

The experimental approach in *Oenothera* is difficult, for in no one species is there known to be a transitional stage in which self-incompatibility is known, associated with chromosome rings in some plants, but not in others.

It is of considerable relevance to the hypothesis of Crosby (1954), that interchange rings have been found (Darlington & Gairdner, 1938) in some natural populations in *Campanula persicifolia*, a species which has also been reported to be self-incompatible (Gairdner, 1926). It was decided to look for both an incompatibility system, and interchange heterozygotes in *C.persicifolia* to see if there is a correlation between the two, indicating a stage in the change from an oppositional factor system, to an interchange heterozygotic state. If a correlation was found, it was hoped to locate the mechanism by which it is maintained.

It was found necessary to modify the original project for two reasons. The first was the length of time that the majority of *Campanula* species take to reach maturity, flowering beginning only in the second year. The second was the difficulty encountered in elucidating the type of incompatibility system. Thus the project was modified to a study of the incompatibility system in the genus, and was approached by two means. The first was by a detailed examination of the breeding system of *C.persicifolia*, to determine the nature of the incompatibility system present, and if possible to deduce the controlling mechanism. The second was by examination of all other available species of *Campanula* to find the extent of self-incompatibility within the genus, and if possible to see if the incompatibility system was uniform throughout the genus.

II. THE BACKGROUND.

A. Incompatibility.

1) First record.

The first record of incompatibility was made by Kölreuter in 1764 on *Verbascum phoeniceum*. The term self-incompatibility was first used by Stout (1917). Although other terms have been applied to this phenomenon, such as 'self-impotence' (Darwin, 1876); 'adynamandry' (Loew, 1895), self-sterility is the only other alternative now in current use.

2) Definition.

Self-incompatibility may be defined as "the physiological inability of an organism possessing both male and female gametes to be self-fertilised. It refers only to instances in which a plant's own pollen and pistil come in contact with each other in the normal course of events, and excludes those cases in which self-fertility is prevented solely by some mechanical device" (Hughes & Babcock, 1950).

3) Occurrence.

Self-incompatibility is a widespread phenomenon, being found in 78 Angiosperm families in every major phylogenic line (Pandey, 1960 a). It has also been observed in *Pinus sylvestris* (Gustafsson unpub., cited by Bateman, 1952); *Pteridium aquilinum*, (Wilkie, 1956); *Larix laricina*,(Piatnitsky, 1934) and *Pseudotsuga taxifolia* (Allen, 1942).

Self-incompatibility is not confined to species with hermaphrodite flowers, as *Nicotiana* species, but is also found in monoecious species, e.g. *Euphorbia cyparissias* (Muenscher, 1936) and *Castanea crinita* (Clapper, 1954). Self-incompatibility has been reported with greater frequency from more specialised herbaceous plant groups, than from predominantly woody ones, even allowing for the fact that a larger proportion of herbaceous species have been investigated experimentally. Since the herbaceous form is considered to be derived from the more woody form, East (1940) has argued that self-incompatibility is a relatively recent innovation in sexual reproduction. However, Whitehouse (1950), while agreeing with the premise that the herbaceous form is more advanced than the woody form, postulates that the multi-alleleomorphic incompatibility system was originally held by all early angiosperms. He argues that those species which subsequently lost it evolved more slowly, retaining a greater range of ancestral characters, e.g. woody habit and multiple flower parts, than those which retained it. Thus the herbaceous families may be of recent origin due to the retention of incompatibility. However, neither of the above authors makes reference to the reported incompatibility in gymnosperms or Pteridium aquilinum, which, if it is of comparable form with that found in the angiosperms today, seems to fit with neither of these hypotheses. The success of self-incompatibility must be due at least partly, to the promotion and maintenance of heterozygosity, and must have played an important part in the evolutionary development of the flowering plants.

4) Types of incompatibility.

Incompatible species may be classified into two main groups, those with floral polymorphism, e.g. Primula vulgaris, Lythrum salicaria, and those without, e.g. species of Nicotiana, Trifolium and Prunus. The number of breeding groups present in the family or population of species exhibiting polymorphism in the stigma and style, may be determined by direct observation of their form without the need to examine pollen tube growth or seed set. Species of Campanula, however, are homomorphic and determination of the number of breeding groups has to be made by breeding experiments.

There are several distinct systems of incompatibility found in homomorphic species. The breeding patterns determined from crossing experiments in Campanula species will be compared below with those of other homomorphic species; and because of this, these are now reviewed.

The classical mechanism is that shown by Nicotiana spp. as found by East and Mangelsdorf (1925). The system is controlled by the action of one gene with a series of multiple allelomorphs, and inhibition of pollen occurs as a result of a reaction between diploid pistil tissue and haploid pollen grains. Each allele has individual action in the style, and the action of the pollen is determined gametophytically. Only pollen which contains an allele differing from the two within the style is able to grow down the style, and bring about fertilisation. Thus pollen with an allele s_1 is able to grow in a style containing s_2s_3 alleles and bring about fertilisation of the ovules. Pollen containing an s_2 allele, however, is unable to grow down this style. The incompatibility system may be upset in artificially produced tetraploids due to competition and dominance between alleles within the style. This gametic homomorphic incompatibility, also known as the oppositional factor system, is typical of species of Antirrhinum, Oenothera, Papaver, Solanum and Trifolium.

Another type of incompatibility system also controlled by one gene with a series of multiple alleles, is that in which pollen action is controlled by the incompatibility alleles present in the pollen mother cells, and not necessarily by those in the pollen itself; thus control of the behaviour of the pollen is sporophytic. Unlike the oppositional factor system, gene action in both pollen and style is not always individual, but it may be so, or it may exhibit dominance. Thus both pollen types s_1 and s_2 produced by a plant s_1s_2 may behave as s_1 if s_1 is dominant to s_2; as s_2 if s_2 is dominant to s_1; and as s_1s_2 if there is no dominance present. Plants showing this type of incompatibility system include Brassica spp., Crepis spp., Iberis amara and Parthenium argentatum.

A third type of system seems to be confined at present to the Gramineae. This system is controlled by two genes each with multiple alleles. There is individual action of the two genes and their alleles in both style and pollen. The nature of the pollen is determined gametophytically. For incompatibility, both alleles of the two genes in the pollen must be matched in the style. Examples of the system are Phalaris coerulescens and Secale cereale.

The fourth type of incompatibility system known to date, is at present confined to Physalis ixocarpa. This system, like the third, is determined by two genes each with multiple alleles. Gene action in the style is individual, though modified by epistasis. The nature of the pollen is determined gametophytically and there is individual action of the genes. Pollen is incompatible if one or both of the alleles present are common with those in the style.

Until recently it was generally held that there was a one-family-one-incompatibility-system relationship. The discovery of this last system by Pandey (1957) in Physalis ixocarpa shows that this idea is no longer tenable, for both Physalis and Nicotiana spp. are members of the Solanaceae. This could, however, be a reflection of the taxonomic grouping of the species.

5) Time of action of incompatibility.

The time of action of incompatibility varies greatly, though as Brewbaker (1957) indicated for homomorphic species, it is closely correlated with pollen cytology. Thus in species with binuclear pollen grains there is little or no inhibition of germination, inhibition occurs at the pollen tube stage. In species with trinuclear grains inhibition occurs at the time of germination. Exceptions to this are relatively few, but include Helianthus annuus, which is trinucleate with stylar inhibition (Habura, 1957), Beta vulgaris, which is also trinucleate but with ovular inhibition of the pollen tubes (Savitsky, 1959), and Theobroma cacao, which is an example of a plant with an incompatibility system unconnected with inhibition of germination, or growth of pollen tubes. Brewbaker (1957) also indicated a correlation between the two types of pollen (gametophytic and sporophytic) and incompatibility. Binucleate grains, he claimed, are associated with gametophytically determined incompatibility systems, and trinuclear ones with sporophytically determined incompatibility systems. The Gramineae, however, do not fit this hypothesis, being trinucleate and gametophytic.

6) Occurrence of self-compatibility in self-incompatible species.

Occasionally self-compatibility may be observed in an otherwise normally self-incompatible species. This may be due to compatibility allelomorphs of the incompatibility genes, or to non-allelic modification of the incompatibility genes. Compatibility allelomorphs have been described by East (1929), and Anderson & de Winton (1931) for Nicotiana Langsdorffii and other genera. Non-allelic modification leading to pseudo-compatibility, or pseudo-fertility as Mather (1943) prefers to call it, may be due to end-of-season effects, or to special pollination techniques. Occasionally pseudo-compatibility may be characteristic of any incompatibility system, but it may also be brought about by the action of non-allelomorphic genes, which have the effect of weakening or over-riding the incompatibility reaction (East, 1929). Strong pseudo-compatibility in an incompatible group is often associated with a hybrid origin of the plants or their ancestors (East, 1929).

7) Measurement of incompatibility.

Self-incompatibility may be measured by two methods. One method is by seed production in a controlled cross; and the other method is by the degree of germination or penetration of pollen tubes in a style by known pollen. The relative value and use of these methods will be discussed below.

B. The genus Campanula.

1) Taxonomic position.

The genus Campanula, along with those of Phyteuma, Wahlenbergia, Platycodon and Jasione, is a member of Campanulaceae. The genus is composed of about 300 species, which are mainly found in north temperate regions. The latest complete taxonomic work is that of de Candolle (1830) and because of this, and the great interest horticulturally, the taxonomic position within the genus is much confused.

2) The floral form.

The floral form is simple and relatively consistent, and because of this a detailed description of C.persicifolia can be used as a typical example.

The flowers open at the end of June and are markedly protandrous; the pollen is occasionally, though not usually, shed before the flower opens, (See Fig.1). (N.B. All Figures are placed in numerical order at the end of the text in this thesis). The pollen is shed from the five stamens on to the central pistil which they enclose (Fig.1,3). The stigmas are not mature at this stage and the three are held closely together as a continuation of the style, with their respective surfaces inward and adjacent to each other. The external surfaces of the stigmas and the top of the style are covered by large hairs which retain the pollen as it is shed by the anthers. After dehiscence both anthers and filaments wither, except the filament base, which covers and encloses the ovary and holds the secreted nectar (Fig.1,4). The stigmas mature one or two days after the flower opens, diverge and expose their receptive surfaces. Eventually the stigmas curl back on themselves to form a complete circle (Fig.1,7). Knuth (1909) claimed that self-pollination can be brought about by this means, but this has been observed only in one plant of C.rotundifolia in the present study.

Pollination and fertilisation do not seem to effect the length of life of the flower, and fertilisation may be brought about by cross-pollination, even four days after previous self-pollination. The mature ovary contains numerous ovules. The maximum number of seeds recorded in a mature capsule of C.persicifolia in the present study is 797. The capsule is ovoid, three-celled, and dehisces by apical pores. The seeds ripen between August and October in the north of England.

3) Source and treatment of material.

Most of the species of Campanula were obtained as seeds from botanic gardens in Britain and Europe, although a few plants were collected from the wild. (See Appendix I for full details of seed sources).

All seeds were germinated in the greenhouse. The seeds were sown between February and April, as it was found that those sown later in the year became insufficiently established to be able to overwinter successfully. Germination time is dependent on the weather, but in favourable conditions the seedlings were ready to prick out into boxes four weeks after sowing. They remained in boxes until large enough to be transplanted. Transplanting took place from June onwards. Twelve plants from each source of C.persicifolia were kept in boxes in an insect-proofed greenhouse. Delicate or half hardy species were also kept in boxes in a greenhouse. All other species were planted in flower-beds in the garden. Ten plants from each species were kept when available in the garden and planted in rows one foot apart, with nine inches between each plant in the row.

4) Pollination.

Pollination of C.persicifolia was carried out in an insect-proofed greenhouse. In 1961 and 1962 all flowers were emasculated as the buds opened, and the ripe pollen was collected and stored in cellophane seed packets. It was suspected that this method of storing pollen could give rise to contamination, and so in 1963, pollen was taken directly from flowers which were kept solely as a pollen source. In this way only a few plants were pollinated from each pollen source. It also ensured that only fresh pollen was used. All other flowers were emasculated just before the buds opened. Pollen was transferred on a mounted needle from one flower to another, and the needle was sterilised between each pollination in absolute alcohol.

Pollination of flowers not grown in an insect-proofed greenhouse was carried out in the laboratory. The flowers were picked in the bud stage, when the corolla was beginning to colour, and were placed in water in the laboratory. As flowers from each plant had to be kept distinct, the buds were placed in a marked area of a polythene grid which covered a tank of water. It was found necessary to pick buds, and not flowers in which the stigmas were immature, in order to ensure an adequate and uncontaminated supply of pollen. Flowers opening in the garden soon lost pollen from the outside of the immature stigmas, and were also liable to contamination with foreign pollen deposited by visiting insects. In most species some buds failed to open in water and in this way much material was lost.

The flowers were kept in the grid until the stigmas had diverged and were receptive. No flowers were emasculated as pollen was in short supply, and only those flowers which were self-sterile were of further interest. Pollen was transferred on a mounted sterilised needle from one flower to another. No pollen was stored. The cross was recorded and the pollinated flower was then placed in an individual container in water for 48 hours. After this time the

the pistil was removed from the rest of the flower and was stored until it could be examined for pollen tube growth.

5) Pollen viability.

The length of pollen viability was tested by collecting a large quantity of ripe pollen from a plant of *C.persicifolia* (T30), on July 3, and keeping it in a cellophane packet at room temperature. This pollen source was used throughout the summer to pollinate flowers of another plant of *C.persicifolia* (T36), with which it was known to be compatible. The flowers were removed two days after pollination and were scored as described below. The viability of the pollen is shown as the number of flowers with which it showed complete pollen compatibility. It can be seen from Table I that full viability of the pollen was retained until the 18th day, after which it became unpredictable.

6) Rate of growth of pollen tubes.

All plants in the garden were tested for incompatibility by pollen tube growth, and in 1963 cross and self-pollinations of *C.persicifolia* were tested in this way too. Cut flowers of *C.persicifolia* were kept in water at 23°C, in twelve hours of light and twelve hours of darkness. The length of penetration of the pollen tubes is expressed as a ~~decimal~~ percentage of the total pistil length of 100 units. Absolute measurement of the pistil length, and of penetration of the pollen tubes in it, was not possible due to distortion of the material during preparation. Thus the ratio of the length of the pollen tubes to the total pistil length was measured. Each reading is the mean of two repeated pollinations, in which all three stigmas were treated separately giving a total of six readings. The results are shown in Fig.2.

Table I.

Viability of stored pollen.

Number of days pollen stored. i.e. number of days between collection of pollen and pollination.	Number of flowers which set seed. Number pollinated on each day was 3.
0	3
7	3
9	3
10	3
18	3
19	2
21	2
22	3
23	2
24	1
25	1
26	2
27	2
28	1
29	3
30	1
32	0
33	1
35	0

The incompatible pollination was made by selfing plant T30, and the compatible pollination was made by crossing T30, as the female, with pollen of T36. Thus the female plant was the same in each pollination. The readings for the compatible pollinations at time 23½ hours and 25¼ hours are subject to doubt. The stain used is not sufficiently critical for accurate measurement of tubes at either the base of the stigma or the top of the style, and therefore these figures must be taken as approximations. No data are available for the compatible pollination at time 27¼ hours due to insufficiently critical staining. Similarly the reading at 48 hours for the compatible pollination may also be due to the staining technique used. The lower half of the style always stained lightly, except for the cut end at the very base, where a dark band formed. Hence any pollen tubes ending just at the style base were obscured by the band, while those just shorter were easily visible. This would account for the apparent failure of pollen tubes ever to reach the stigma base.

Germination of both compatible and incompatible tubes takes place within two hours of pollination. Only superficial penetration of the pollen tubes occurs with the incompatible pollen, though the exact degree of penetration is variable. From other material, penetration of incompatible pollen was occasionally observed as far as the stigma base or even the top of the style, though this was very rare. This variability of the extent of growth of incompatible pollen seems to be a fairly frequent event in incompatible plants. Emerson (1940) observed it in <u>Oenothera organensis</u> and found that the growth rate of incompatible tubes depended on the season, the actual flower on the plant, the actual plant when considering plants of the same incompatible allelic constitution, and according to the incompatible allelomorph present.

To check the rate of pollen tube growth and the fact that inhibition of the tubes did not take place at the style base or in the ovary, compatibility was measured by both pollen tube growth, and by seed set, on the same flowers. Flowers of C.persicifolia (12.4) were pollinated by compatible pollen (50.5) in the normal way in the greenhouse. The pistils of the flowers were removed at known regular intervals, but the ovaries were left on the plant to mature in the normal way. Thus only that pollen which had germinated, penetrated the whole length of the pistil, and had entered the ovary before pistil removal, would be able to bring about fertilisation and seed development. Examination of the pistil material showed pollen tubes near the style base 28 hours after pollination. However, there was no seed development in this flower. Seed development did not occur until the pollinated pistil had been left on the plant for 47 hours after pollination. As these plants were grown in a greenhouse, the results are not strictly comparable with those showing the rate of pollen tube growth under controlled temperature conditions. However, they do show that pollen has had time to penetrate the ovary sufficiently well 47 hours after pollination, to bring about seed development in the capsule. Thus it was decided that leaving a pistil on a flower for 48 hours after pollination would allow time for growth of compatible pollen to the style base in both cut flowers, and flowers pollinated on the plant.

7) Storage of pollinated material.

Staining of pistils was found to be unaffected by whether the pistils were killed or not before being stored in 70 per cent alcohol, in a sub-zero refrigerator. Hence pistils were stored in alcohol without being previously killed in boiling water. Over-crowding of storage space later compelled a new storage technique to be devised. The method was to store unkilled pistils, dry, in cellophane packets. Excessive drying-out of the material was prevented by placing these packets in a polythene bag, and storing it in a sub-zero refrigerator. No deterioration of the material was observed even after storage for six months.

8) Methods of measuring incompatibility in *Campanula* species.

There are two methods of measuring incompatibility, by seed production and pollen tube growth. Bateman (1943) has pointed out that measurement of incompatibility by seed production is the more sensitive over low ranges of fertility, when the differential rate of growth between compatible and incompatible pollen is too slight to be detected by direct observation. On the other hand, measurement by pollen tube growth does indicate the proportion of pollen which germinates, and the extent of growth, and thus provides more information than measurement by seed set. Measurement by seed set involves efficient insect proofing, either in an insect-proofed greenhouse, or by bagging. This method was used only on some *C.persicifolia* plants which were grown in an insect-proofed greenhouse. All other plants were tested for cross and self-incompatibility by pollen tube growth.

Hayman (1956) attempted to score pollen grain appearance as a measure of incompatibility. Thus he considered the pale, empty grains to be compatible, and the dark-staining, full ones, to be incompatible. He found that the observed dark:pale ratio differed from the expected ratio due to the large numbers of grains present; both due to masking of pale grains by dark ones, and also due to reduction in pollen germination when there is heavy pollination (Sears, 1937). He did, however, feel sufficiently confident in the method to score the ratio of the germinated to ungerminated grains into two classes, i.e. 1:1; 3:1. Bateman (1943) using Petunia concentrated not on the percentage germination of the pollen grains, but on the length of penetration of the pollen tubes into the pistil. He measured the degree of penetration, and found that he could score the pollen into completely compatible, or partially compatible types. He also observed that those pollen tubes which were incompatible showed a higher degree of abnormality at their tips.

In some preliminary experiments in Durham, measurement of incompatibility was tried by counting the proportion of full:empty grains in C.persicifolia. This was found to be unreliable as this system is based on the assumption that all empty grains are compatible. However, in C.persicifolia both incompatible and compatible grains germinate, and by careful study it was observed that in many cases the tubes of empty grains could be seen to have stopped growing in the stigmatic region. Bateman's (1943) method of scoring the exact length of penetration of the pollen tubes was also found to be unsatisfactory for Campanula species. Many of the pistils are thick with much mechanical tissue, and, in order to see the pollen tubes, it is necessary to flatten the pistils completely, thus bringing about a certain degree of distortion. It was possible to classify the length of the longest visible pollen tube into one of three categories: 'superficial', 'stigmatic' and 'stylar'. The 'superficial' group includes those tubes which had either not penetrated, or had just entered the external layers of the stigmatic tissue. The 'stigmatic' group includes those tubes which had penetrated well into the stigma, but not into the main stylar tissue, and the 'stylar' group covers tubes whose ends were visible at the style base. The three classes are shown in Plate 1. No attempt was made to classify the pollen into fully-compatible, half-incompatible, etc., as there was much variation in the lengths of the shorter tubes, even in repeated crosses. In this respect C.persicifolia may be compared to Abutilon 'Hybridum' (Pandey, 1960 b). Thus incompatibility was measured by the degree of penetration of the longest pollen tube. Although this method is obviously not as sensitive a measure as that of seed production it was found to be most useful for garden material; pollinations being made on cut flowers in the laboratory. It was also used on greenhouse material of C.persicifolia in 1963.

The ends of the pollen tubes of incompatible pollen were often found to be distorted and even branched. Polysiphonous pollen tubes have been recorded in the Campanulaceae (Maheshwari, 1949), but only incompatible tubes have been observed to branch in this study, and growth is limited after branching.

9) Staining techniques for measuring pollen tube growth.

The staining technique used for the measurement of incompatibility by pollen tube growth was developed on <u>C.persicifolia</u>. The chief difficulties involved were the thickness of the pistil, its toughness, and the presence of laticifers. After some initial trials it was decided to concentrate on the production of a squash preparation with stained pollen tubes, preventing the masking of tubes by mechanical tissue. The laticifers could be distinguished from the pollen tubes by their articulated and anastomosed character. The usual staining techniques were tried (Table 2); but all failed to give the softness required for squashing and satisfactory staining. Eventually a modified Buchholz' technique was devised (Table 3), which gave adequate preparations. Here the short 'superficial' and long 'stylar' tubes were moderately easy to see, but the intermediate lengths were more difficult. Confusion did also arise between the pollen tubes and the laticifers. This technique proved laborious when dealing with large numbers of pistils, and later an attempt was made to find a critical stain for the laticifers. This was not found, but in trying to do so a quicker and equally satisfactory method was found for staining pollen tubes, which also allowed more easy identification of the laticifers. The stain was chlorazol paper brown B, Gurr michrome number 94, an azo-dye, and was used as suggested by Verdcourt (1947) (Table 4). The full range of seven colours indicated by the manufacturers was not obtained in the pistils, but the cytoplasm of the pollen tubes and the laticifers stained orange, and the mechanical tissue pink. The rest of the pistil stained pale orange, except at the style base where it was dark orange. The pistils were easily squashed, and the pollen tubes and laticifers identifiable by observation.

Table 2.

List of staining techniques for pollen tube growth.

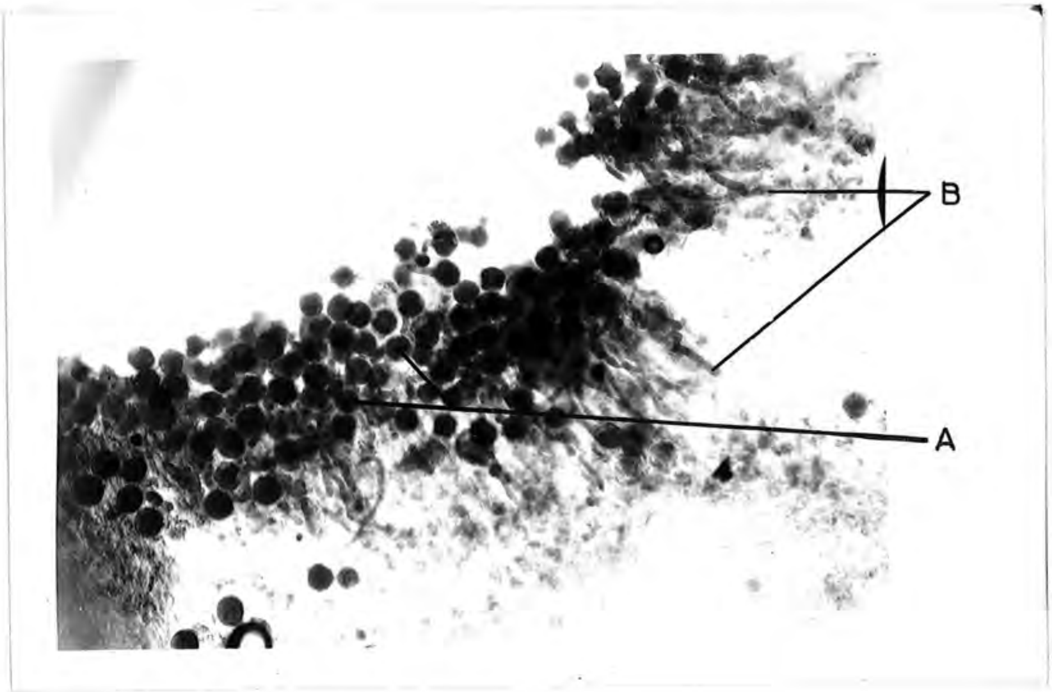
Staining agent.	Reference.
Acetocarmine	Anderson & Sax, 1934.
Acetocarmine & basic fuchsin	Chandler, 1931.
Acid fuchsin & light green	Buchholz, 1931.
Ehrlich's haematoxylin	Trankowsky, 1931.
Feulgen	Darlington & La Cour, 1947.
Lacmoid Martius yellow	Nebel, 1931.
Iodine	Emerson, 1940.

Table 3.

Modified Buchholz technique used to stain pollen tubes of *Campanula*.

1. Wash pistil, either fresh or pickled, in 30 per cent alcohol for ten minutes.
2. Split style into three or six parts, longitudinally.
3. Wash in 95 per cent alcohol for three minutes.
4. Boil in oxalic acid and Buchholz; 1:1; for one minute. (Buchholz, acid fuchsin : aqueous light green; 8:2).
5. Leave in hot stain for four minutes.
6. Wash in 95 per cent alcohol for ten minutes.
7. Wash in alcoholic light green for five minutes.
8. Boil in lacto-phenol for thirty minutes.
9. Mount on slide in glycerine, cover with a large cover slip, and squash.

PLATE I.



Pollination (50.7 / 50.13) -8 / -3. X 100.

'Superficial' penetration. The majority of the pollen grains have germinated, though they have retained some of their contents (A). The pollen tubes have penetrated the external layers of the stigma only (B) even 48 hours after pollination.

PLATE I.

Pollination (177.11 / 177.14) -16 selfed. **X 100.**

'Stigmatic' penetration ~~pollination~~. The pollen grains appear to be either full (A) or empty (B). Pollen tubes have entered the stigma to varying degrees (C and D), but have not entered the style.

PLATE I.

Pollination 181.11 / 181.15. **X 100.**

'Stylar' penetration. The ends of the pollen tubes are visible at the style base (A). The full pollen grains (B) are ungerminated grains which have been displaced from the stigmatic surface during preparation.

Table 4.

Staining technique for pollen tube growth using an Azo-dye.

A saturated aqueous solution of chlorazol paper brown was used; i.e. 30 g per litre at 14°C. The procedure indicated here produces temporary mounts, but the slides will keep for at least four months.

1. Thaw pistils in 30 per cent alcohol for one hour.
2. Boil in dye for one minute.
3. Leave in hot dye for four minutes.
4. Differentiate in 10 per cent nitric acid for five minutes.
5. Split pistils longitudinally into two or three parts.
6. Mount on a slide in glycerine, cover with a large cover slip, and squash.

10) Time of action of incompatibility.

A study of the nuclear content of the pollen grains of *C.persicifolia* was made to see if the Campanulaceae supported the hypothesis put forward by Brewbaker (1957), on the correlation between the time of action of incompatibility and pollen cytology. Brewbaker (1957) quotes the Campanulaceae as having both bi- and tri- nucleate grains. In this study the grains proved resistant to stain, but eventually two nuclei were counted in a mature grain of *C.persicifolia* using an alcoholic hydrochloric acid-carmine stain as shown by Snow (1963). The bi-nucleate condition of the mature pollen grain was confirmed in one slide stained to observe pollen tube growth. Here, division of the gametic nucleus was seen near the tip of the pollen tube, in the stylar region of the pistil.

Similarly, a bi-nucleate mature pollen grain was found in *C.rotundifolia*. Here, division of the gametic nucleus was observed in tubes grown on an artificial medium, of one per cent agar, thirty per cent sugar, with a trace of boron.

Thus both *C.persicifolia* and *C.rotundifolia* agree with Brewbaker's (1957) hypothesis, in that they have bi-nucleate mature pollen grains, and show stigmatic inhibition of incompatible pollen.

11) Occurrence of self-compatibility in self-incompatible species.

Self-incompatibility in many species may be overcome partially or completely by special pollination techniques, as follows:-

i) Bud pollination, e.g. *Nicotiana* spp. (East, 1923)
ii) End-of-season pollination, e.g. *Petunia violacea* (Yasuda, 1930)
iii) Mutilation of the stigma, e.g. *Brassica oleracea* (Kakizaki, 1930)
iv) Growing flower in the dark before pollination, e.g. *Oenothera organensis* (Emerson, 1930).
v) Use of growth regulating substances on the ovaries at the time of pollination, e.g. *Lilium longiflorum* (Emsweller & Stuart, 1948).

At no time in *Campanula* spp. could self-compatibility in an otherwise self-incompatible pollination be obtained by bud pollination, end-of-season pollination or mutilation of the stigma. In the latter case this result was to be expected because growth of pollen tubes in the pistil gave no indication of a distinct zone of inhibition in the pistil, as is found in *Brassica* spp. Pollination of pistils grown in the dark, and use of a growth regulating substance on the ovary were not attempted. However, cases of self-compatibility in an otherwise self-incompatible plant have been observed in some experimental results, and these are discussed below.

III. CAMPANULA PERSICIFOLIA.

A. Breeding patterns.

1) Introduction.

Material of C.persicifolia was obtained as seed from botanic gardens and from the John Innes Institute. The origin of the different seed sources is to be found in Appendix 1.

Each stock of seed was given a code number between 177 and 185 inclusive, as this followed the plant accession list of Crosby. The constituent plants of each stock were numbered by the decimal system. Progeny derived from known crosses were, at first, given a code number in a separate coding series and again each individual plant was numbered by the decimal system. Thus the eighth plant in stock 178 i.e. 178.8 ♀ crossed with the sixth plant in stock 177 i.e. 177.6 ♂ gave progeny to which was assigned the family code number 29. In all crosses quoted, the female is cited first. Later, the method of giving progeny a new code number was discontinued and the parents are cited, or simplified to a single letter. Each plant within a progeny still has an individual number. Thus the progeny of the cross 177.11/177.14 are either quoted as this, or simply called P.

Chromosome studies from 1961 onwards on pollen mother cells using an aceto-carmine freeze drying technique (Conger & Fairchild, 1953), showed that all the plants examined were diploid, with 2n = 16. This included a horticultural variety labelled Telham Beauty, showing that the stock obtained was not the true tetraploid Telham Beauty form. Some plants of C.persicifolia showed a single heterozygote interchange ring of four, but these were not present in all the pollen mother cells of the plants, and plants with such an interchange ring were in the minority.

In order to determine the number of breeding groups within a family an extensive crossing programme was carried out within the progeny derived from two known parents. Where possible the progeny were backcrossed with the parents to find out if there was a complete or partial incompatibility between parents and progeny.

All the material was grown in an insect-proofed greenhouse, and emasculation, pollination, removal of pistils, storage and staining were all enacted as previously described. Where incompatibility was measured by seed set, this is mentioned, otherwise it was measured by pollen tube growth. Due to the very large number of seeds formed in each capsule, the exact number of seeds developed was not counted. It was observed that either a low number of seeds (below 30) or a high number (above 300) were formed. Only rarely did the number fall between 30 and 300 and thus it was considered that if 50 or more seeds were set, there could be little or no incompatibility present. Numbers of seeds below 50 were counted and these are quoted where appropriate.

Some pollinations gave conflicting results when repeated. When a repeated pollination gave such a result and an equal number of successful and failed pollinations were obtained, it was assumed that the successful pollination was the correct one. Many factors affecting ante and post fertilisation stages may prevent seed development, e.g. bad pollen, ineffectual pollination and insect damage, but it may be assumed that if seeds have formed, or if pollen tubes are found at the style base, there is no incompatibility present. When a pollination was repeated more than twice and the results were conflicting, the successful pollination was always regarded as an indication of the absence of incompatibility, except when successful pollinations were outnumbered 4 : 1 or more. In this situation it was assumed that the successful pollination was due either to some freak physiological condition, or to contamination, and the overall result was interpreted as a failure.

2) Self-compatibility.

The majority of plants were consistently self-incompatible with no pollen tubes in the style, or seed formation, but a few plants had a low fertility when selfed. Thus out of a total of 628 self-pollinations, 42 set seed (6.7 per cent). It can be seen from Table 5 that in only nine pollinations out of a total of 42 (21.4 per cent) were 50 or more seeds set after any one pollination. In 19 pollinations (42.9 per cent) fewer than ten seeds were produced. The production of seed in the latter pollinations can be interpreted in one of three ways.

1) By the presence of a mechanism allowing a very low fertility; of the order of 1, 0.1 or 0.01 per cent.
2) By pseudo-compatibility.
3) Pollen contamination.

The first situation is unlikely as in all known incompatibility systems in homomorphic plants the reaction is generally all or nothing. In some cases a 50 per cent, or 25 per cent success of pollen placed on a stigma is known, but consistently lower percentages of success have not yet been recorded.

Table 5.

Plants showing degrees of self-compatibility, when measured by seed set.

Plant code number.	Number of times pollinations: failed	succeeded.	Number of seeds: good	bad
1.4	1	1	over 50	0
1.7	-	1	16	0
9.3	-	1	1	0
17.4	-	1	88	10
18.3	1	1	56	0
18.4	1	1	over 50	0
22.2	1	1	over 50	0
22.5	1	1	1	0
29.1	1	1	4	0
29.2	1	1	1	0
30.2	-	1	1	0
39.1	-	1	15	1
41.2	-	1	0	5
43.1	-	1	1	0
50.7	-	1	over 50	0
50.13	-	1	over 50	0
177.12	1	2	107	6
			5	0
178.11	3	1	3	0
179.16	-	2	0	2
			1	0

Table 5 continued.

Plant code number.	Number of times pollinations: failed	succeeded.	Number of seeds: good	bad.
180.17	-	1	15	1
181.14	-	2	35	3
			19	10
183.2	1	1	12	2
183.5	1	4	over 50	0
			8	1
			2	0
			2	0
183.8	-	2	30	3
			22	3
183.10	1	1	3	0
184.3	-	2	1	0
			1	0
184.5	1	3	74	0
			11	0
			2	0
185.2	1	1	7	6
185.3	1	1	6	0
185.5	2	2	38	0
			20	0
185.7	2	1	2	0.

Pandey (1960 b) showed in Abutilon 'Hybridum' that those plants which had low seed set on selfing in the normal flowering season (under 20 seeds out of a total maximum of 120 produced in cross-pollination) also showed varying degrees of pseudo self-compatibility in the autumn and winter. However, during the normal flowering season, in spring and summer, they were nearly all highly self-incompatible. End-of-season effects have not been found in C.persicifolia in the present study, but only the two ends of the natural flowering season were tested for this, and no out-of-season flowering was obtained. Pandey (1960 b) considered that unexpected self-compatibility in Abutilon is due to one of two possibilities. The first is mutation of the alleles affecting the behaviour of the pollen, style or both, but this he discards, as the rate would have to be impossibly high to account for the seasonal changes. He concludes that the difference is due to a combination of minor genes which affect the incompatibility reaction of the pollen tubes, allowing some of them to bring about fertilisation. The minor genes are inherited from both parents, and are unrelated to the s alleles. Pandey (1960 b) argues that an out-breeding mechanism based on self-incompatibility promotes heterozygosity of the whole genetype and results in the accumulation of recessive deleterious and lethal genes. Different combinations of these may disturb both incompatibility and compatibility reactions. Abutilon 'Hybridum' though, is a cultivar which has arisen through hybridisation of several species of Abutilon, including A.Darwinii and A.striatus. C.persicifolia is not a hybrid species, but the material used was obtained from botanic gardens, and probably had been bred as an attractive plant for herbaceous borders. It may therefore be compared to Abutilon in this respect, and the low seed set occasionally recorded on selfing may indeed be a reflection of segregating minor genes influencing the behaviour of the pollen, style or both. Lundquist (1961) found a low seed set in expected self-incompatible pollinations in Festuca pratensis to be associated with a lack of enforcement of incompatibility at the distal part of the stigma. In this region he found a concentration of emptied pollen grains. There was no evidence of such an area in C.persicifolia.

Low numbers of seeds produced on selfing C.persicifolia may however be due to experimental technique. The plants were all grown in boxes in the greenhouse, and of necessity were placed close together. A single flower remains receptive from seven to ten days depending on the temperature, and, as has been shown experimentally, is receptive to compatible pollen even a week after pollination by incompatible pollen. Thus these low numbers of seeds produced on selfing may simply be due to pollen contamination. This could have occurred by knocking plants during pollination, forcing one flower against another, or releasing dry, old pollen into the air. It was observed that the greenhouse was not insect-proof; green aphids, red spider mites and spittle-bugs were permanent inhabitants, while bees, flies and spiders were more rarely found. These, though many of them are not usually regarded as pollinators, may well have brought about a low level of contamination.

Self-pollinations with high seed set are more likely to be due to some physiological state of the pollen and, or, the style; either dependent on minor genes, or due to external factors.

When using pollen tube growth as a measure of compatibility, five compatible self-pollinations, out of a total of 174, were recorded. These are shown in Table 6. This low number is probably a reflection of the efficiency of using pollen tube growth as a method of measuring low fertilities of compatibility (Bateman, 1943). However, associated with this, is the fact that to measure compatibility by pollen tube growth, flowers were removed from the greenhouse two days after pollination. This had the dual effect of decreasing the time that the flower was available to stray pollen, and also decreased the total number of flowers present in the greenhouse at any one time. Thus there was a reduction in the source of stray pollen, and in overcrowding of plants.

Table 6.

Plants showing pollen tubes at the base of the style when selfed.

Example: $(181.14\ominus)$ - 2 means the second plant of the progeny produced by the self of 181.14

$(181.14\ominus)$ - 2
$(1.4\ominus)$ - 13
(50.7 / 50.13) - 6
(50.13 / 50.7) - 23
(177.14 / 177.20) C_1 - 3.

3) Intra-family crosses.

a) Families derived from seed source 177.

Progeny were raised from the reciprocal cross 177.14 / 177.11 and 177.11 / 177.14. The parent crosses were made in 1961 and were chosen as an example of good seed set in a reciprocal cross. Each parent was self-incompatible. Twenty-four seedlings of each cross were pricked out in 1962, but two plants of the cross 177.11 / 177.14 died during the winter of 1962-63. Not all these plants flowered in 1963, and so eight plants of each cross were used for intercrossing within the progeny. The breeding pattern obtained for the cross 177.11 / 177.14, (P), is shown in Fig.3. A diagonal line in the crossing-table indicates the presence of pollen tubes at the base of the style, which are interpreted as a successful pollination. A circle in the table indicates pollen tubes being observed in the 'superficial', or 'stigmatic' regions of the pistil only, this being interpreted as a failed pollination. Where a pollination was repeated, interpretation of the results was made as indicated above. Rearrangement of the order of the plants in the table has been made in an effort to group together those plants showing a similar breeding pattern, and the results of this, and of simplification of the repeated pollinations may be seen in Fig.3a.

It can be seen that each individual plant was self-incompatible. Plants 16 and 5 behaved in a similar manner when acting as females, but were distinguished by the fact that pollen of 16 was compatible on plant 5, while that of 5 was incompatible on plant 16. Plants 21 and 9 were reciprocally compatible and must therefore have had different incompatibility alleles. Similarly, plant 4 must have differed from all the above-mentioned plants as pollen of 4 was compatible on them all. Though pollen of plant 17 was incompatible on 4, the reciprocal cross was compatible, and the two plants also differed in behaviour with pollen of plant 21. Both plants 18 and 22 were incompatible as pollen on plant 9. However, they were cross-compatible with each other, and must therefore have differed genetically. The reciprocal cross-incompatibility of plant 22 with plant 9 suggests that these plants had an identical incompatibility constitution. They were distinguishable, though, by the fact that plant 18 was incompatible as pollen on plant 9, but was compatible on plant 22. It can be seen that both like and unlike reciprocal crosses were present, five pairs of crosses showing reciprocal differences. Each plant behaved in a manner differing from all the others. Thus there was a minimum of eight classes present in the progeny of the cross 177.11 / 177.14. The percentage compatibility was high, being 79.3 per cent.

Percentage compatibility is defined as the percentage of crosses in a crossing-table which are compatible out of the total number of cross and self-pollinations made. Even if plants have been tentatively grouped, because of similar experimental results, each individual result has been scored to produce the percentage compatibility, unless otherwise stated.

The presence of reciprocal differences in crosses within the progeny is a characteristic of a sporophytic pollen controlling mechanism. As the maximum number of breeding groups obtainable is four, when incompatibility is controlled by a single *s* gene, this cannot explain the data obtained. Thus the breeding pattern of the progeny of the cross 177.11 / 177.14 would not seem to be controlled by a single s gene.

Two types of incompatibility systems which are controlled by a two gene system have been described above. That described by Lundquist for Secale (1954), Festuca (1955, 1961) and Hordeum (1962), and that by Hayman (1956) for Phalaris coerulescens, are characterised by more than four breeding groups within progeny of a single cross, one group of which is identical to the behaviour of the male parent with the progeny. Reciprocal differences between crosses are present, and there is incomplete incompatibility between the progeny and the female parent.

Progeny derived from the cross 177.11 / 177.14 fit into this pattern by having more than four breeding groups. The behaviour of the male parent of the cross (177.14) is known only by its pollen reaction, but this was not identical to the behaviour of any of the progeny. However, as no one group was duplicated within the progeny it must be assumed that more than eight breeding groups could have been formed, and therefore the possibility that the pattern of the male parent might have been similar to a group not present must not be excluded. Due to an absence of flowering of the female parent of the cross (177.11) in 1963, the behaviour of the female parent with the progeny is not known. This however can be seen in the progeny of the reciprocal cross.

The pattern of the cross 177.14 / 177.11 differs from the one quoted above in that the percentage compatibility is much lower, i.e. 57.8 per cent, compared with 79.3 per cent above. The breeding pattern for the cross 177.14 / 177.11 is shown in Fig.4. Rearrangement of the plants within the table, in order to place similar ones together, and taking overall results to give a simplified picture, gives the pattern shown in Fig.4a. As in 177.11 / 177.14 examination of the breeding pattern shows that each plant had a distinct behaviour which was not duplicated by any other plant. Plants number 6, 4 and 22 were all cross-incompatible with each other, but number 22 was reciprocally compatible with plant 23, which distinguished it from plants 4 and 6. Similarly plant number 6 was compatible as pollen on plant 19, and it thus differed from plant 4, which was incompatible as pollen on plant 19. Plant 20 was cross-incompatible with plants 6 and 22 but was compatible with plant number 4, and so differed from all three. Similarly plant 23 was cross-incompatible with plants 6 and 4, but was compatible with plant 22, and thus differed again from them. Plants 19 and 5 were cross-incompatible, but must have been of different genotypes as plant 19 was incompatible as pollen on plant 23, while pollen of plant number 5 was compatible on plant 23. Plant 24 was compatible with all the other plants except number 6, with which it was incompatible when acting as a male.

Thus again, as with the reciprocal cross quoted above, each plant had its own characteristic breeding pattern, which suggests that the cross 177.14 / 177.11 might produce more than eight breeding groups within the F_1 generation, were more plants considered. The behaviour of the male parent of the cross is not known as flowering did not occur in 1963. The behaviour of the female parent with the F_1 progeny is of interest. It can be seen that it was reciprocally compatible with plants 6, 4, 5 and 24; was compatible as female with pollen of plants 22 and 19; and was cross-incompatible with plants 23 and 20. Only two reciprocally different crosses were present within the progeny, those of the crosses 24 x 6, 6 x 24, 19 x 23, and 23 x 19.

Studies on the progeny of the reciprocal cross 177.11 / 177.16 were made as the fertility of the cross seemed to be impaired in one direction. Thus only one seed of the cross 177.16 / 177.11 was produced, whereas about 500 seeds were set in the reciprocal cross (.11 /.16). Each of the parent plants were self-incompatible. The one plant derived from the cross.16 /.11 was self-incompatible, but as neither of the parents flowered in 1963, its behaviour with them is not known.

Ten plants of the cross .11 /.16 were extensively intercrossed amongst themselves and the results are shown in Fig.5. Simplification and rearrangement of the results are shown in Fig.5a. All the plants were self-incompatible. Though plants number 8 and 16 behaved identically with plants 9, 3, 1, 20 and 18, they were obviously not of identical genotypes as their reactions with plants 19, 12 and 17 differed. Plants 9, 3 and 1 were reciprocally cross-incompatible except for the crosses 3 / 9 and 3 / 1 which were compatible. However, unlike plants 3 and 1, pollen of plant 9 was compatible on plants 8 and 16. Plants 3 and 1 behaved similarly though were not reciprocally incompatible and were distinguished by pollen of plants 9, 20 and 19. Plant 20 was cross-incompatible with plant 1, but compatible with plant 3, thus differing from them both. Plant 19 was reciprocally cross-incompatible with plant 8 only, and because of this must have differed genetically from plant 8.
Plant 12 was cross-incompatible with 19, but was compatible with

plant 8. Like plant 17, it was reciprocally cross-incompatible with plant 16, but differed from 17 by being incompatible with 19. Plant 18 was predominantly compatible, being incompatible with pollen of plant 19 only. Thus the behaviour of any one plant was not identical with another. There were twelve pairs of crosses which showed reciprocal differences, and the percentage compatibility was low, 48.4 per cent. No data of the behaviour with the parents are available, but some cross-pollinations were made with the single progeny of the parental reciprocal cross. Thus the one plant from the cross 177.16 / 177.11 was reciprocally incompatible with plant number 12 of the cross 177.11 / 177.16, but was reciprocally compatible with number 16. It was compatible with pollen of plant 20 but the reciprocal cross was incompatible.

Studies were made on the progeny of the cross 177.13 / 177.11. Both parents were self-incompatible, and the reciprocal cross was incompatible. From 23 seeds sown, only 13 germinated and eight of these flowered in 1963. All the results are given in Fig.6, and a simplified picture is shown in Fig.6a. All the plants were self-incompatible. Plants 4 and 5 were cross-incompatible but differed in their reactions as pollen on plants 7, 10 and 6. Plant 8 was cross-incompatible as pollen on plant 4, and differed from it by being compatible as pollen on plant 2, thus indicating that it differed genetically from both plants 2 and 4. Plants 10 and 3 were cross-incompatible, but plant 3 was compatible as pollen on plant 7, whereas plant 10 was reciprocally incompatible with 7. Plant 7 must have differed genetically from 10 as shown by its action with plant 3. Plant 6 was cross-incompatible with plants 2 and 8, but pollen of plant 6 was compatible on plant 4, thus distinguishing it from plants 2 and 8. Thus all eight plants behaved differently from each other. There were eight pairs of reciprocal differences, and the cross-compatibility was 50 per cent. No information is available on the behaviour between the parents and the F_1 generation.

Progeny W are believed to be derived from a successful self-pollination of plant 177.12. Of 107 good seeds and 6 bad ones which were formed and sown, 36 germinated. Out of 24 pricked out, 11 were used for intercrossing in 1963. Fig.7 shows the total number of crosses made and Fig.7a gives a simplified picture of the breeding pattern produced. There is no evidence of any self-compatibility and cross-compatibility is moderately low, 49.1 per cent. Plants 3, 17, 16, 4 and 14 were all cross-incompatible except for the crosses 14 / 16 and 14 / 4. Though the plants behaved identically as females - except for the cross 4 / 22 - they differed when acting as pollen. Pollen of plants 3 and 14 were incompatible on plant 22, but plant 14 could be distinguished from 3 as its pollen was incompatible on plant 24 also. Plants 17 and 4 were distinguishable by the fact that pollen of plant 17 was incompatible on 14, while pollen of 4 was compatible. Pollen of 16, like that of 14, was incompatible on plant 10, but unlike 14 was compatible on plant 22. Thus though all 5 plants were cross-incompatible there were slight differences by which they could be distinguished. Pollen of plant 10 was incompatible on plant 6, but the two plants were otherwise dissimilar. Pollen of plant 6 was compatible on all the other plants, while pollen of plant 10 was cross-incompatible in 7 out of 10 cross-pollinations. Plants 5 and 18 were cross-incompatible and behaved extremely similarly, though they could be distinguished by the fact that pollen of 5 was compatible on plant 24, while that of 18 was not. Plants 24 and 22 were also cross-incompatible. There was a greater difference between these two, and they could be distinguished on any of five cross-pollinations. Each plant behaved in its own characteristic manner with no duplication of pattern, giving a total of eleven groups. It would seem, however, that the plants might be grouped into five classes, and even though the behaviour of plants within each class would not be identical, the group as a whole would show a general trend. Thus plants 3, 17, 16, 4 and 14 would form one class; plants 6 and 10 two additional classes, plants 5 and 18 a fourth class, and plants 24 and 22, a fifth class. However, in tho absence of further experimental evidence, the value of this grouping cannot be tested.

The progeny derived from the cross 177.14 / 177.20 seem to suggest a similar pattern. Each of the parent plants was self-incompatible and the reciprocal cross was also incompatible. The results of intercrossing the progeny (C_1) are shown in Fig.8. Rearrangement and simplification of the table is shown in Fig.8a. All plants except number 3 were consistently self-incompatible and it seems that the progeny may be split into three main groups. Plants 2, 17, 3, 21 and 15 were mainly intra-sterile, but were cross-compatible with plants number 8. 24, 12 and 1, which themselves were mainly intra-sterile. Plant number 9 differed from both groups. It was cross-incompatible as pollen on the first group of plants mentioned, subsequently to be called group I, but was mainly cross-compatible as a female with pollen of that group. Plant 9 was mainly cross-compatible with plants of group II. There are some anomalies in these main groupings. Thus in group I, plants 21 and 15 were receptive to pollen of plant number 3; and pollen of plant 21 was cross-incompatible with plant 12 of the second group. The cross-incompatibility of plant 12 (group II) with pollen of group III (i.e. plant number 9) might suggest that it should be removed from group II. However, the reciprocal cross was compatible, and so plant 12 has been retained in group II.

In this F_1 generation the reaction of both male and female parents may be examined with some of the progeny. It can be seen that the two parents behave in a dissimilar manner with the progeny, but that neither one nor the other was consistently compatible or incompatible with all the progeny. The female parent (177.14) was compatible to pollen from plants 2, 17, 8, 24 and 1, but in two of the reciprocal crosses, numbers 24 and 1, it was cross-incompatible. Thus pollen of the female parent split group II, placing plants 24 and 1 together, and dividing off plant 8. The reaction with plant 12 was unknown. The female parent also split the unity of group I, being pollen compatible with plants 2 and 17, but incompatible with number 21. Results with the other progeny were not known. The male parent (177.20) was cross-compatible with pollen of progeny 2 and 17, but was cross-incompatible with pollen of all the plants of group II. Like the female parent, the male parent split the unity of both groups I and II, though in a different manner. Thus pollen of 177.20 grouped plants 2 and 21 of group I together, but segregated off plant number 17.

Similarly it grouped plants 24 and 12 of group II, but segregated off plants 8 and 1. It can be seen from the above that the breeding pattern of the male parent is not similar to any of the three basic groups present. However, as both groups I and II have several anomalies, it looks as if the original grouping into three groups may have been made possible only because of a lack of other groups by which to separate them. Hence the fact that the male parent did not show a breeding pattern similar to that of any of the progeny must not be taken as definite evidence that the controlling incompatibility system present in this cross is not similar to that found in Phalaris coerulescens (Hayman, 1956), or Secale (1954), Festuca (1955, 1961), and Hordeum (1962) (Lundquist). The behaviour of the female plant showed well that there was compatibility between it and the progeny, as was found in Phalaris (Hayman, 1956). Thus the cross 177.14 / 177.20 (C_1) seemed to indicate a controlling system similar to that found in the Gramineae.

b) Families derived from plant collection number 50.

Plant collection number 50 was believed to be a true family derived from only two parents. The original box label was lost before the present study began, and so it was given a number following the sequence of box numbers already allocated. The identity of the parents was unknown. When plants 50.13 and 50.7 were crossed reciprocally over 200 seeds were set from each cross. Germination of the seeds was high, and in 1963 ten plants of each cross were intercrossed amongst themselves. The results of intercrossing the progeny of the cross 50.13 / 50.7 are shown in Fig.9.

Plant number 23 had pollen tubes at the style base when selfed on one occasion. Repetition of the cross produced normal 'stigmatic' pollen tube growth. The plant showed reciprocal incompatibility with plant number 1, and, as self-compatible but cross-incompatible plants have never been observed, it was assumed that the unexpected pollen tube growth was due either to contamination or to some freak physiological conditions. The self-pollination is therefore interpreted as incompatible. Rearrangements of the plants within the table, and simplification of the table is shown in Fig.9a. Plant numbers 1 and 2 were cross-incompatible and behaved in a similar fashion with pollen from the other members of the progeny, except for pollen from plant 23. They differed also when used as pollen on plants 21, 13 and 23, and must therefore be considered to be of different genotypes. Plants 9, 14, 21 and 13 were cross-incompatible except for the cross 9 / 13, which was compatible. However, these plants could not have identical genotypes as their reactions with other members of the progeny differed from each other. Hence plants 9 and 14 might be distinguished by differing behaviour with pollen of plant 13, as mentioned above, and by their reaction to pollen of plant 20. Plants 21 and 13 also differed, though only in a few crosses. When used as female they were distinguished by their reaction to pollen of plants 2 and 19. Thus, though all four plants 9, 14, 21 and 13 were cross-incompatible they must be considered to be of different genotypes. Plant 20 was incompatible with pollen of plants 9, 14, 21 and 13, but it was only cross-incompatible as pollen with plant 14. Plant 19 was alone in that it was reciprocally cross-incompatible with no other plant. Plants 23 and 24 were reciprocally cross-incompatible, but were distinguished in their reaction with number 1, plant 23 being reciprocally cross-incompatible with number 1, while plant 24 was not.

Thus the ten progeny derived from the cross 50.13 / 50.7 all behaved in a manner differing from each other when intercrossed amongst themselves. There were eight pairs of reciprocally differing crosses, and the percentage compatibility was 58 per cent.

When receiving pollen of plants 14, 21, 20 and 24, the action of the male parent (50.7) was similar to plants 9, 21 or 13. However the pollen reaction cannot be compared with that of any of the plants 9, 13 or 21, for the male parent was incompatible with plants number 2 and 23. This suggests a similarity to plant number 2, but even this explanation must be dismissed, as the pollen was compatible on plants 13 and 19. Thus the male parent cannot be grouped with any of the progeny considered.

The progeny of the reciprocal cross (50.7 / 50.13) show a much lower compatibility, i.e. 28.2 per cent, when intercrossing ten plants and considering all plants individually. The breeding pattern is shown in Fig.10. Here also one plant (number 6) showed 'stylar' pollen tubes when selfed on one occasion. This plant showed a very
7 high percentage of compatibility, but as it showed reciprocal cross-incompatibility with plant 24, it is argued that if it shows cross-incompatibility, it must also be self-incompatible. It is therefore shown as being self-incompatible in the figures derived from Fig.10. Fig.10a shows an initial grouping of the plants into three basic groups. Hence, in group I, all the plants 3, 15, 8, 13, 14 and 18 were cross-incompatible, and behaved identically with pollen from the other progeny.

However, when used as pollen on the other progeny they had differing patterns of behaviour. Plants 12, 20 and 24 were also all cross-incompatible, and they behaved identically as pollen, except in the cross 6 / 24. However, these plants differed, too, when acting as females. Rearrangement of the table may be seen in Fig.10b. Plants 3, 15 and 8 are now grouped together to form group I, and all show cross-incompatibility and identical behaviour with the other progeny. Group I may be distinguished from group II (plants 12 and 20) by being pollen compatible on group II. Plants 12 and 20 did not behave identically, but as they differed in only one cross (20 x 14) they have been grouped together. Groups II and III were reciprocally cross-incompatible, but were distinguished by their behaviour with pollen of group I. Group III differed from group I by being pollen compatible on group II. The plant forming group IV (number 24) was also cross-incompatible with group II, but was distinguished from it by being compatible to pollen not only of group I but also of group III. Plant 13 (group V) was cross-incompatible with both groups II and IV, but was unlike these two groups in that it was also cross-incompatible with groups I and III. Group VI showed a high degree of compatibility and was cross-incompatible only with group IV.

The progeny of the cross 50.7 / 50.13 may therefore be grouped into six classes, each of which shows a characteristic pattern when cross-pollinated by members of other groups. The reaction of the male parent with the progeny is not known, but the female parent showed both cross-compatibility and incompatibility with the progeny. When used as a pollen source it was incompatible with plants of group I, III and V, but when used as a female it split the uniformity of group I being incompatible with pollen of plant 3, but compatible with that of plant 8. This suggests that the total number of six groups is not the maximum which could have been produced from the original cross. There were only three pairs of reciprocally different crosses amongst these progeny.

Both parents of the reciprocal cross above set 100+ good seeds when selfed in 1961 (see Table 5), and eight plants of the self 50.13 were intercrossed in 1963. These plants were all diploid. The breeding pattern is shown in Fig.11, and overall results and rearrangement of the plants are shown in Fig.11a. Plants 3, 9 and 22 were reciprocally cross-incompatible and behaved identically both as male and female. These are grouped together to form group I. Plants 12 and 18 were also cross-incompatible and these are grouped to form group II, even though the two plants differed in the action of their pollen on plant 21. Plant 8 was cross-incompatible with group II but differed from it by being reciprocally incompatible with group I also. Plants 21 and 16 are grouped together to form group IV. They were cross-incompatible and though pollen of 18 was incompatible on number 21, but compatible on plant 16, the two plants behaved identically in all other crosses.

Thus the progeny of the self 50.13 may be grouped into four different incompatibility genotypes, as determined by their breeding behaviour. The small number of breeding groups confirms that the progeny are probably the result of a true self, but gives no information about the controlling mechanism. Four breeding groups derived from a self, could indicate either a one or a two gene controlling system. The percentage compatibility was low, i.e. 35.9 per cent, considering each plant individually. When calculated on a group basis, taking the cross ~~plant~~ 21 x 18 to be successful, the percentage compatibility was 31.2 per cent.

The breeding pattern of the progeny derived from the self of the plant 50.7 does little to clarify the position. The detailed results of interbreeding the progeny are shown in Fig.12, and the overall results with rearrangement of the order of the plants in an effort to group like patterns together, are shown in Fig.12a. Sixteen plants of the progeny were considered in an attempt to determine the maximum number of groups present, but due to the limited number of flowers produced by each plant few pollinations could be repeated, and several could not be made. Examination of the results showed that the behaviour of any one plant was not like that of any other. However there did seem to be a suggestion of a general pattern. Thus plants 1, 17, 13, 7 and 16 were mainly cross-incompatible, though plant 16 was compatible with pollen of plants 17, 13 and 7. Plants 19, 10, 5, 8 and 23 also showed a predominantly cross-incompatible behaviour, though six of the twenty-three pollinations made between them were cross-compatible. The behaviour of this group, i.e. group II, with pollen of group I was predominantly cross-compatible, and similarly the behaviour of group II with the rest of the progeny was predominantly cross-compatible. Plants 22, 4 and 14 of group III were intra-sterile except for the cross 22 x 14, which was compatible. However, pollen of plants 22 and 4 were incompatible on plant 20, which itself was pollen incompatible with plant 14, suggesting that they were genotypically similar. Plant 3 was incompatible as pollen on plant 20, suggesting that it had a common incompatibility allele which prevented fertilisation. Plant 11 on the other hand was reciprocally incompatible with both plant 10 (group II) and plant 22 (group III) and thus cut across any general group tendencies. No clear picture can be obtained from the experimental data, and elucidation of the incompatibility system present must be derived from other sources.

c) Other families derived from self-pollinations.

Only sixteen seeds were produced when plant number 1.7 was selfed in 1961, and of these nine germinated and seven flowered in 1963. They were all diploid. The results of repeated intercrossing among the progeny are shown in Fig.13. It can be seen that there was no indication of any self-fertility in the progeny. Regrouping the progeny to combine those plants with like behaviour and showing overall results of pollinations may be seen in Fig.13a.

Plants 2 and 4 were cross-incompatible and were also intra-sterile with the parent plant 1.7. These plants were cross-compatible with all the other members of the progeny except plant 6. Plants 2 and 4 were therefore grouped with the parent 1.7, to form group I. Plant 5 was the only plant which was cross-compatible with all the other members of the progeny and must therefore be classed alone in group II. Pollen of plant I was incompatible on plant 3, though the reciprocal cross was compatible. In the repeated pollinations of the cross 1 / 3, one pollination was successful and the other failed, indicating that the overall result may have been incorrectly interpreted. As no other difference was found between plants 1 and 3, the two plants have been placed together to form group III. Plants 7 and

6 were cross-incompatible, but plant 7 was compatible with the rest of the progeny, while plant 6 was reciprocally cross-incompatible with plants of group I. These two plants have been put into two separate groups, IV and V. Thus the progeny of the self of 1.7 may be divided into five groups, each of which has a characteristic behaviour. A point of interest in this family is that the parent plant had a breeding pattern with the progeny, which was comparable to one of the groups formed within the progeny, i.e. group I. Thus this fits one of the characteristics described by Hayman (1956), for Phalaris coerulescens, in that one of the classes formed within the progeny is identical to that of the male parent. Similarly, it fits another of the characteristics described by Hayman (1956) in that there is incomplete incompatibility between the progeny and the female parent, which in this pollination is the same plant, i.e. 1.7. It is of note that there are no instances of reciprocal differences between crosses in this family.

Thus selfing the plant 1.7 and examination of the breeding pattern within it, suggests that the controlling incompatibility system is a two gene system, perhaps similar to that found in the Gramineae.

The progeny derived from the self of plant 1.4 show a different breeding pattern, which may be seen in Fig.14. Only three plants flowered in 1963, though 22 seeds were pricked out in 1962. They were all diploid. Plants 10 and 16 were completely self and cross-incompatible, both between themselves and with the parent plant. Plant number 13, however, showed a tendency to both self and cross-fertility. In an overall interpretation of the results the pollination 13 selfed would be interpreted as incompatible, but the trend towards a very reduced fertility cannot be ignored. It may well be that plant 13 was exhibiting those characteristics by which the progeny were produced initially, i.e. those characters which permitted self-fertilisation of plant 1.4.

Plants numbered 181 were derived from seed obtained from Switzerland. The seed was the result of open pollination and therefore the parents were unknown. One of the plants, 181.14, set seed on selfing, 35 good seeds and 3 bad seeds were produced after self-pollination, and 19 good seeds and 10 bad seeds were produced in a repeated pollination. Of the second self-pollination 15 plants were pricked out in 1962, and nine of these flowered in 1963. They were all diploid. The results are shown in Fig.15.

Only plant 2 showed any tendency to self-fertilisation, and this occurred in only one of four repeated pollinations. As this plant also showed cross-incompatibility, it was assumed that it was usually self-sterile, and has been shown as such in Fig.15a. Rearrangement of the order of the plants within the table, and overall results are shown in Fig.15a. Plants 2, 11 and 10 were all intra-sterile, and behaved identically with pollen from the remainder of the progeny. However, when acting as pollen on the rest of the progeny, all behaved slightly differently. Thus pollen of plant 2 was compatible on all other plants, while pollen of 11 was incompatible on plant 7. Plant 10 was incompatible with both plants 1 and 7, while the parent plant which was cross-incompatible with all three plants 2, 11 and 10, differed from them by being incompatible as pollen on plants 1, 7 and 4. Plants 6 and 12 were cross-incompatible but differed in their pollen reactions on plants 7 and 3, and must therefore be considered to be of different genotypes. Likewise, plants 1 and 7 were cross-incompatible, but were distinguishable by their pollen reaction on plant 6. Plant 4 was reciprocally cross-incompatible with plants 6 and 12 but differed from plant 6 by being receptive to pollen of plant 7, and from plant 12 by being pollen compatible on plant 7. Plant 3 was predominantly cross-compatible, being unreceptive to pollen of plant 6 only. Thus all plants behaved independently, and though the action of the parent with the progeny is similar to that of plant 10, the difference in behaviour as pollen on plant 4 makes it impossible to state that they are of the same genotype.

The similarity between plants 2 and 11, 10 and 181.14, 6 and 12, and 1 and 7, suggests that the progeny of the self 181.14 may fall into six groups when intercrossed amongst themselves. If this were so it would indicate that a two or more gene incompatibility system were present, but no information on whether the system was like that of *Physalis* or the Gramineae is obtainable.

B) Tetraploidy.

1) Introduction.

Some information on the type of incompatibility system present in a species may be obtained by studying the behaviour of autotetraploids. Information may be obtained by self-pollinating and interbreeding tetraploids, by examination of their breeding behaviour with diploid progeny of the same cross, and with the parents from which they were derived.

Gairdner (1926) discovered that though the diploid *C.persicifolia* was self-sterile, the horticultural variety Telham Beauty was self-fertile. The origin of Telham Beauty is obscure, but it was first recognised as a new variety in 1916, and is believed to be an autotetraploid of *C.persicifolia*, though it forms no quadrivalents at meiosis (Gairdner, 1926). This, however, cannot be taken as conclusive evidence against Telham Beauty being an autotetraploid, for artificial tetraploids of *C.persicifolia* produced by Gairdner (1926) did not form quadrivalents at meiosis. In the present study, one or two quadrivalents were formed at meiosis in artificially produced tetraploids, but complete quadrivalent production was never observed.

Gairdner (1926) observed that the cross between Telham Beauty and *C.persicifolia* could be made in one direction only, i.e. with Telham Beauty (the tetraploid) as the female. This is in contrast with the results obtained by Crane and Thomas (1939), and Crane and Lewis (1942) who found that in *Pyrus communis* var. 'Fertility' the cross between the diploid and the autotetraploid was successful only when the tetraploid was used as the male parent. Lewis and Modlibowska (1942) explained these results on the basis that when diploid pollen produced by a tetraploid plant carried two different *s* alleles, fertility of the pollen on the diploid style (produced from a diploid plant) was due to competition between the alleles in the pollen grain. Competition allows complete fertilisation in some species, e.g. *Petunia* sp., *Pyrus communis*, but only partial fertilisation in others, e.g. *Oenothera organensis*. By this means self-fertilisation of a tetraploid species may be brought about. However, in some diploid grains, dominance may occur between the alleles present. Self-fertilisation may be brought about by the action of dominance if both pollen incompatibility alleles have to be matched in the style for incompatibility. If, however, only one common allele is sufficient to enforce incompatibility, dominance cannot allow self-fertilisation. It can permit cross-fertilisation between two plants in which one of the incompatibility alleles in the pollen is held in common with that in the style, provided that the common allele is recessive in either the pollen or the style.

Atwood (1944) found a slightly different situation in tetraploid Trifolium repens in which an F_1 family contained both self-compatible and self-incompatible plants. He explained this as being due to some new effect caused by the presence of many different pollen genotypes in the style. It may be concluded that the presence, strength and effect of competition and dominance between incompatibility alleles of a diploid pollen grain, and between the grain and the tissues of the pistil, depends largely on the species concerned.

2) Production of artificial tetraploids.

Seeds derived from four different crosses were used in the production of artificial tetraploids. Colchicine was used as described by Levan (1940), and ten seeds of each cross were used for each treatment. Six methods of treatment were devised to try to ensure the production of tetraploids.

Treatments 1, 2 and 3 were carried out on young germinating seedlings growing on pads in a petri dish. The seedlings were soaked in a known concentration of colchicine solution, for a controlled length of time, as shown in Table 7. The dishes were then washed thoroughly by flooding them with water and draining them four times.

Table 7.

Colchicine treatments for the production of polyploids in C.*persicifolia*.

Treatment	Percentage concentration of colchicine solution	Number of hours bathed in colchicine solution
1	0.05	5
2	0.1	4
3	0.2	2

The seedlings were left in the dishes for twenty-four hours after treatment, and were then transferred to pots with seed compost. The pots were kept well watered at laboratory temperature, to ensure optimum growing conditions.

Treatment 4 was carried out on ungerminated seeds. These were soaked in 0.2 per cent colchicine solution for twenty-four hours, before being soaked in distilled water for two days. They were then sown.

Treatments 5 and 6 were made on individual seedlings in seed boxes when three months old, and just beyond the first leaf stage. For treatment 5, 0.2 per cent colchicine solution was used, and for treatment 6, 0.1 per cent. One drop of the solution was placed on to the growing point of each plant, three times a day, for six days, and was allowed to dry on the plant. In the controlled conditions the seeds were sown in pots as normal, before being pricked out into boxes. The success of the various treatments is shown in Tables 8 and 9.

Table 8.

Success of various treatments of seeds and seedlings of C.persicifolia with colchicine solution.

Parentage of seed.	Number treated.	Type of treatment.	Number surviving treatment.	Number of tetraploids.	Number of diploids.	Number of plants with unknown ploidy.
177.14 / 177.20	10	1	0	0	0	0
	10	2	0	0	0	0
	10	3	2	0	1	1
	10	4	4	0	1	3
	10	5	1	1	0	0
	10	6	7	0	6	1
	24	control	23	0	23	0
179.14 / 179.15	10	1	4	0	4	0
	10	2	1	0	1	0
	10	3	1	0	1	0
	10	4	0	0	0	0
	10	5	8	1	4	3
	10	6	10	1	6	3
	18	control	18	0	18	0
180.15 / 180.16	10	1	0	0	0	0
	10	2	0	0	0	0
	10	3	0	0	0	0
	10	4	0	0	0	0
	10	5	7	1	2	4
	10	6	6	1	1	4
	18	control	15	0	15	0

..... continued

Table 8 continued.

Parentage of seed.	Number treated.	Type of treatment.	Number surviving treatment.	Number of tetraploids.	Number of diploids.	Number of plants with unknown ploidy.
180.17 / 180.13	10	1	0	0	0	0
	10	2	0	0	0	0
	10	3	1	0	1	0
	10	4	3	0	3	0
	10	5	7	1	4	2
	10	6	10	0	9	1
	24	control	23	0	23	0

3) Success of production of tetraploids.

Measurement of the ploidy of the treated plants was by examination of stained pollen mother cells. It was regarded as essential to make chromosome counts on the sexual material of each plant in case the colchicine had affected only limited areas of the plant, producing polyploid chimaeras. Due to this limiting condition those plants which did not flower could not be scored, and these have been included in the final column in Table 8.

Examination of the table shows that one tetraploid was produced in each of the progeny of the crosses 177.14 / 177.20 and 180.17 / 180.13, and two tetraploids were produced in the progeny of each of the other crosses, i.e. 179.14 / 179.15 and 180.15 / 180.16. A higher ploidy was not found in any of the other plants. Table 9 indicates that treatments 5 and 6 were the most successful for tetraploid production, and

Table 9.

Percentage success of tetraploid production in C.persicifolia for each treatment used.

Type of treatment.	Total survival in all four crosses.	Percentage survival.	Percentage tetraploid production, of total treated.
	Total = 40		
1	4	10.0	0
2	1	2.5	0
3	4	10.0	0
4	7	17.5	0
5	23	57.5	10
6	33	82.5	5

Total success = 2.5 per cent.

that these treatments also gave the highest survival rate. Though treatment 5 gave a lower plant survival percentage than 6, it gave a higher tetraploid production, i.e. ten per cent. The total percentage tetraploid production of treated seeds and seedlings was relatively high, i.e. 2.5 per cent.

4) Behaviour of artificially produced tetraploids.

a) Tetraploids produced in family 177.14 / 177.20 (C_1).

The one tetraploid produced in this family was by treatment 5. The plant was self-incompatible, and showed both compatibility and incompatibility when crossed with untreated plants of the same family, and with its parents. The crossing behaviour of the tetraploid may be seen in Fig.16. Examination of Fig.16 shows that the tetraploid was reciprocally incompatible with its female parent, but was compatible with pollen of the male parent. When crossed with the untreated progeny of the same cross, the tetraploid plant was compatible with pollen of plants C_1 -2 and -17, though it was reciprocally incompatible with these plants. Pollen of plant -21 was incompatible on the tetraploid. The action of pollen of plants -8 and -12 was not known, but pollen of the tetraploid was compatible on plant -8, though incompatible on -12. A comparison of the breeding pattern of the tetraploid with the pattern produced by intercrossing the diploid progeny (Fig.8a.) shows that that of the tetraploid is identical with that of group III (plant 9). Unfortunately the behaviour of plant 9 with its parents is not known and so a comparison on this point cannot be made.

b) Tetraploids produced in family 179.14 / 179.15 (C_2).

Treatment of young plants with colchicine solution produced two tetraploid plants T 15 and T 27. Their overall behaviour is shown in Fig.17. Examination of the crossing-table shows that the two plants behaved very differently. Plant T 15 was self-incompatible, but T 27 showed a tendency towards self-compatibility, for in two out of a total of nine repeated self-pollinations, pollen tubes were found at the style base. Plant T 27 was predominantly cross-compatible, being incompatible only with pollen of plant -3 of the diploid progeny. (This cross was repeated three times with identical results). Pollen of plant T 27 was compatible on plant T 15, though the reciprocal cross was incompatible. The tetraploid T 27 was also fully compatible with both parents. Pollen of plant T 15 was incompatible on both parents, though the reciprocal crosses were compatible. This result with T 15 agrees with the results found by Gairdner (1926) in crosses between Telham Beauty 4n and C.persicifolia 2n, in that the cross was successful with the tetraploid plant as the female. Pollen of plant T 15 was incompatible on all the diploid progeny of the same cross, except with C_2 -15, with which it was reciprocally compatible. T 15 was receptive to pollen from -3, but was incompatible to pollen of plants -16 and -18. Thus it can be seen that the two tetraploids retained their incompatibility reaction, though the evidence suggests that it was greatly weakened in T 27.

c) Tetraploids produced in family 180.15 / 180.16 (C_3).

Colchicine solution treatment of young plants produced two tetraploids. Diploid progeny of this cross showed a reduced pollen production and this character seemed to be accentuated in the tetraploid. Hence the breeding pattern shown in Fig.18 probably shows an over-estimate of incompatibility, infertility being due to bad pollen rather than an incompatibility reaction. Both tetraploids produced (T 1 and T 4) were self-incompatible, but were receptive to pollen from the female parent of the cross. Unfortunately pollen from the other parent was not available. Pollen of plant T 1 was compatible on the female parent, though that of T 4 was not. Pollen of plant T 1 was compatible on only two of the diploid progeny, i.e. on numbers C_3 - 10 and -14, being incompatible on the rest. Unfortunately many of the reciprocal crosses could not be made due to the shortage of tetraploid material, but pollen of plants -1 and -14 was found to be incompatible on the tetraploid T 1, while that of -3 was compatible. The pattern shown by

T 4 was of greater incompatibility. T 4 was incompatible to pollen from the diploid progeny and was incompatible as pollen on the progeny, except for plants -1 and -14. Thus here, also, both self and cross-incompatibility is a characteristic of the tetraploid plants.

d) Tetraploid produced in family 180.17 / 180.13 (C_4).

Only one plant was formed as the result of colchicine treatment of young plants. This plant was unlike the other tetraploid plants as it was the only one in which a tendency to a 'gigas' character was present (Stebbins, 1950). The plant had a thickened inflorescence spike and the individual flower stalks were reduced in length and thickened. The basal leaves were slightly thicker than those of the diploid plants and also darker green. The breeding behaviour of the tetraploid plant T 40 may be seen in Fig.19. Both self and cross-incompatibility were present in this plant. It was completely self-incompatible, but was reciprocally compatible with the female parent. The male parent was not available for breeding. When crossed with diploid progeny of the same family T 40 was receptive to pollen from each plant. In the reciprocal crosses, though, pollen of T 40 was incompatible on plants C_4 -1 and -14, but compatible on plant -18. Here again the artificially produced tetraploid showed both self and cross-incompatibility.

5) Conclusion.

Five of the six tetraploids produced retained self and cross-incompatibility comparable to that found in diploid plants of the same cross. In only one plant (T 27) was the self-incompatibility markedly reduced. Previous evidence suggested that more than one incompatibility gene must be controlling the breeding system. The lack of breakdown of incompatibility in the tetraploids produced supports this conclusion, for in neither Physalis (Pandey, 1957) nor in the Gramineae (Hayman, 1956), (Lundquist, 1954, 1955, 1961, 1962) was incompatibility broken down in tetraploids. The behaviour of plant T 27 may be explained by a genetic upset due to duplication of the chromosomes, but this upset is insufficient to cause a complete breakdown of the incompatibility system.

C. Discussion of the breeding system in *C.persicifolia*.

Plants of *C.persicifolia* are predominantly self-incompatible and only 6.7 per cent of the self-pollinations set seed. Of these plants 42.9 per cent set fewer than ten seeds and 21.4 per cent set over 50 seeds when self-pollinated. This self-fertility does not seem to be due to end-of-season effects, which have not been observed in *C. persicifolia*, nor has self-incompatibility been overcome by bud pollination or pistil damage. *C.persicifolia* is a horticultural species and all the plants obtained were derived from botanic garden sources. It is concluded that the presence of some self-fertility in the stock examined may be due to segregation of minor genes, which are unrelated to *s* alleles, but which nevertheless influence the effect of *s* alleles and allow self-fertilisation. Low seed set on selfing may also be due to contaminated pollination, the pollen being carried by stray insects in the greenhouse, or being knocked from one flower to the next during pollination. Full seed development on selfing is more difficult to explain, and in the absence of any definite evidence, it is suggested that self-compatibility may be due to some physiological state of the pollen, or style, or both, dependant either upon external factors, or upon minor genes unrelated to the s alleles, or to a combination of both these factors.

All the progeny examined (except that of the self 1.7) showed reciprocal differences between crosses. Considering a one gene incompatibility system, reciprocal differences are indicative of sporophytic determination of pollen behaviour. Reciprocal differences can only occur between crosses when the influence of more than one gene or allele is present in the pollen. In plants which have sporophytic determination of pollen behaviour, the nature of the pollen is determined in the pollen mother cell before the individual grains are produced, with their different incompatibility alleles. Thus in a plant with a one gene incompatibility system, though the incompatibility alleles of the pollen are haploid, the pollen behaviour is determined by the diploid nature of the pollen parent. Crepis Foetida shows this phenomenon (Hughes and Babcock, 1950). Here, there is dominance between alleles in the pollen mother cells, though not in the style. S_1 is recessive to all the other incompatibility alleles in the pollen mother cells; S_4 is dominant to S_3 and S_2; and S_3 is dominant to S_2. The behaviour of the pollen is determined solely by the dominant gene in the pollen mother cell. Thus in the cross ♀ S_1S_2 and ♂ S_2S_3 the pollen behaviour is determined by S_3 and therefore all the pollen is fertile on the style S_1S_2. In the reciprocal cross, however, the pollen behaviour is determined by S_2, an allele which is held in common with the diploid style, and thus the pollen is incompatible.

Reciprocal differences between crosses can also occur when incompatibility is determined by a two gene system with gametophytic determination of pollen behaviour, provided that the allelic or genic relationships differ in the pollen and style. This is discussed in more detail below.

A one gene incompatibility system with sporophytic determination of pollen behaviour gives rise to four breeding groups only when the progeny are intercrossed. The experimental evidence obtained from all the progeny examined showed that there were more than four breeding groups present, and hence it must be concluded that a one gene incompatibility system is not present in C.persicifolia.

In the two types of two gene incompatibility systems known, the notation used for the genes and their alleles is $S_1 \ldots\ldots_n$ $Z_1 \ldots\ldots_{n'}$, where n and n' may or may not be equal (Lawrence, 1930; Lundquist, 1954; Hayman, 1956). Self-pollination of a plant in which incompatibility is temporarily overcome, gives rise to nine different progeny genotypes. Intercrossing these progeny with incompatibility functioning when either or both alleles in the pollen are held in common with those in the style, gives rise to nine breeding groups, each with a characteristic behaviour, as shown in Fig.20.

Crossing a plant with four different incompatibility alleles with another with which it has no incompatibility alleles in common, gives rise to a maximum number of sixteen different progeny genotypes. Each parent, i.e. $S_{1.2}Z_{5.6}$ x $S_{3.4}Z_{7.8}$ produces four different, viable gametes, i.e. (S_1Z_5); (S_1Z_6); (S_2Z_5); (S_2Z_6); x (S_3Z_7); (S_3Z_8); (S_4Z_7); (S_4Z_8). When all gametes are compatible with each other, sixteen genotically different progeny are produced. Intercrossing these progeny, when one or both alleles of the pollen held in common with those in tho style causes incompatibility, gives rise to sixteen different breeding groups (Table 10, section a). The number of breeding groups obtained when intercrossing progeny depends on the type of incompatibility system, the number of alleles held in common by the parents, and the relationship of one allele to another (dominance), and one gene to another (epistasis). The variation of the number of breeding groups formed by intercrossing the progeny, and the percentage compatibility which may be obtained when the conditions above are varied, are shown in Table 10. Here incompatibility is present when one or both alleles are held in common between the pollen and the style. The nature of the pollen is determined gametophytically.

The only known incompatibility system which is determined by two genes with gametophytic pollen determination, and incompatibility present when one allele is held in common between pollen and style is that in Physalis (Pandey, 1957). In this species interpretation of the results involved different genic relationships in the pollen and style. Hence, there was epistasis and individual action between alleles of the pollen, but reduced epistasis and individual action of alleles in the style. Thus S_2 and S_3 were epistatic to Z_1 and Z_2 in the pollen; S_2 was usually epistatic over Z_1 and Z_2 in the style; and S_1 was independent in both pollen and style. As no clue was obtained from the experimental results quoted above about the relationships between alleles and genes in

C.persicifolia, the relationships found in Physalis have been taken as a basis for theoretical considerations. However, Pandey (1957) found that in one of his parental stocks (plant 2) the relationship between the genes differed. Here, S_2, Z_1 and Z_2 all acted independently in the style. Different gene relationships within parents or progeny have not been considered in Table 10. The situation in which alleles of both genes are equal has been shown in Table 10, sections a, b, c, d and e. Two other situations have been considered. The first is where some S alleles in the parent plants are epistatic to one of the Z alleles in the male parent (Table 10, sections b_2, b_3, c_2, c_3, d_2, d_3, e_2 and e_3) as opposed to the situation where the S alleles are epistatic to both of the Z alleles in the male (Table 10, sections b_4, b_5, c_4, c_5, d_4, d_5, e_4 and e_5).

Examination of Table 10 shows that the only conditions which give rise to different patterns between progeny derived from a reciprocal cross are those shown by d_4 and d_5 (Fig.21 and 22). In this situation only one allele is common to both parents (Z_6), and the behaviour of this allele is modified by the effect of both S_2 and S_3 in the pollen, and S_2 in the style, both alleles being epistatic to Z_6. This epistasis influences the results of the cross. In the situation shown by d_4, four types of male gametes are compatible in the parent cross, giving rise to sixteen different progeny genotypes. When intercrossed these fall into ten breeding groups. In the reciprocal parental cross, d_5, only three types of male gametes are compatible. Twelve different genotypes are formed within the progeny, and when these are intercrossed they form six breeding groups. The percentage compatibility of both the progeny groups is high, i.e. 72.6 and 70.8 per cent, respectively.

Table 10.

Variations in the number of progeny breeding groups formed by different parental types, and different gene relationships. Incompatibility is present when one or both alleles are common to pollen and style, and pollen behaviour is determined gametophytically.

* Indicates reciprocal differences formed when intercrossing progeny.

Parents.	Compatible male gametes.	Relationship between genes.	Number of groups within progeny.	Percentage compatibility.
a. $S_{1.2}Z_{5.6}$ $S_{3.4}Z_{7.8}$	S_3Z_7: S_3Z_8: S_4Z_7: S_4Z_8.	equal	16	56.2
b. $S_{1.2}Z_{5.6}$ $S_{2.3}Z_{7.8}$	S_3Z_7: S_3S_8:	equal	8	37.5
b2. $S_{1.2}Z_{5.6}$ $S_{2.3}Z_{7.8}$	S_3Z_7: S_3Z_8:	S_1 independent S_2S_3 epistatic to Z_5Z_8 pollen S_2 epistatic to Z_5Z_8 style	4	46.8
b3 (reciprocal of b2). $S_{2.3}Z_{7.8}$ $S_{1.2}Z_{5.6}$	S_1Z_5: S_1Z_6.	as b2	4	46.8
b4. $S_{1.2}Z_{5.6}$ $S_{2.3}Z_{7.8}$	S_3Z_8	S_1 independent S_2S_3 epistatic to Z_7Z_8 pollen S_2 epistatic to Z_7Z_8 style	2	50.0

..... continued

Table 10 continued.

Parents.	Compatible male gametes.	Relationship between genes.	Number of groups within progeny.	Percentage compatibility.
b5. (reciprocal of b4).				
$S_{2.3}Z_{7.8}$				
$S_{1.2}Z_{5.6}$	S_1Z_5: S_1Z_6.	as b4	2	50.0
c.				
$S_{1.2}Z_{5.6}$				
$S_{2.3}Z_{6.7}$	S_3Z_7	equal	4	25.0
c2.				
$S_{1.2}Z_{5.6}$		S_1 independent		
$S_{2.3}Z_{6.7}$	S_3Z_7	S_2S_3 epistatic to Z_5Z_7 pollen		
		S_2 epistatic to Z_5Z_7 style	2	50.0
c3. (reciprocal of c2)				
$S_{2.3}Z_{6.7}$				
$S_{1.2}Z_{5.6}$	S_1Z_5	as c2	2	50.0
c4.				
$S_{1.2}Z_{5.6}$		S_1 independent		
$S_{2.3}Z_{6.7}$	S_3Z_6: S_3Z_7	S_2S_3 epistatic to Z_6Z_7 pollen		
		S_2 epistatic to Z_6Z_7 style	2	50.0
c5. (reciprocal of c4)				
$S_{2.3}Z_{6.7}$				
$S_{1.2}Z_{5.6}$	S_1Z_5: S_1Z_6.	as c4	2	50.0
d.				
$S_{1.2}Z_{5.6}$				
$S_{3.4}Z_{6.7}$	S_3Z_7: S_4Z_7.	equal	8	37.5

..... continued

Table 10 continued.

Parents.	Compatible male gametes.	Relationship between genes.	Number of groups within progeny.	Percentage compatibility.
d2.				
$S_{1.2}Z_{5.6}$		S_1 independent		
$S_{3.4}Z_{6.7}$	S_3Z_7: S_4Z_7.	S_2S_3 epistatic to Z_5Z_7 pollen		
		S_2 epistatic to Z_5Z_7 style	6*	71.8
d3. (reciprocal of d2)				
$S_{3.4}Z_{6.7}$	S_1Z_5: S_2Z_5			
$S_{1.2}Z_{5.6}$	S_2Z_7	as d2	6*	70.8
d4.				
$S_{1.2}Z_{5.6}$	S_3Z_6: S_3Z_7:	S_1 independent		
$S_{3.4}Z_{6.7}$	S_4Z_6: S_4Z_7.	S_2S_3 epistatic to Z_6Z_7 pollen		
		S_2 epistatic to Z_6Z_7 style	10*	72.6
d5.				
$S_{3.4}Z_{6.7}$	S_1Z_5: S_2Z_5			
$S_{1.2}Z_{5.6}$	S_2Z_6.	as d4.	6*	70.8
e.				
$S_{1.2}Z_{5.6}$				
$S_{1.2}Z_{6.7}$	--	equal	-	--
e2.				
$S_{1.2}Z_{5.6}$		S_1 independent		
$S_{1.2}Z_{6.7}$	--	S_2S_3 epistatic to Z_5Z_7 pollen		
		S_2 epistatic to Z_5Z_7 style	-	--

..... continued

Table 10 continued.

Parents.	Compatible male gametes.	Relationship between genes.	Number of groups within progeny.	Percentage compatibility.
e3. (reciprocal of e2)				
$S_{1.2}Z_{6.7}$				
$S_{1.2}Z_{5.6}$	--	as e2	-	--
e4.				
$S_{1.2}Z_{5.6}$		S_1 independent		
$S_{1.2}Z_{6.7}$	--	S_2S_3 epistatic to Z_6Z_7 pollen		
		S_2 epistatic to Z_6Z_7 style	-	--
e5. (reciprocal of e4)				
$S_{1.2}Z_{6.7}$				
$S_{1.2}Z_{5.6}$	--	as e4	-	--
f.				
$S_{1.2}Z_{5.6}$				
$S_{1.3}Z_{5.6}$	--	equal	-	--
f2.				
$S_{1.2}Z_{5.6}$		S_1 independent		
$S_{1.3}Z_{5.6}$	S_3Z_5: S_3Z_6	S_2S_3 epistatic to Z_5Z_6 pollen		
		S_2 epistatic to Z_5Z_6 style	4	50.0
f3. (reciprocal of f2)				
$S_{1.3}Z_{5.6}$				
$S_{1.2}Z_{5.6}$	S_2Z_5: S_2Z_6	as f2	4	50.0
g.				
$S_{1.2}Z_{3.4}$	S_1Z_3: S_1Z_4:	Incompatibility overcome in		
self.	S_2Z_3: S_2Z_4.	parents only. Genes independent, and equal.	9*	19.7

Plants with an incompatibility system comparable to that found in the Gramineae, i.e. where the two alleles present in the pollen have to be matched in the style for incompatibility, show a reduced variation in the number of progeny groups formed when the number of alleles held in common between the parents is varied; see Table 11. This is due to the fact that in the system described for the Gramineae the two loci are apparently independent with no epistasis, and there is no dominance between alleles of one gene. A theoretical self (with incompatibility overcome in the parents only)is shown in Fig.23.

A comparison of Tables 10 and 11 shows that the number of breeding groups formed by intercrossing the progeny with an incompatibility system requiring the presence of two common alleles for incompatibility (Table 11) is greater than the number formed when one common allele between pollen and style is sufficient to cause incompatibility (Table 10).

Table 12 shows the number of breeding groups obtained experimentally.

It can be seen that there is a tendency for a relatively large number of groups to be formed within the progeny derived from two genetically different parents. The smallest number was found within progeny of the cross 177.14 / 177.20. Ten plants were intercrossed and these fell into three groups, though the behaviour of the progeny with the parent plants indicated that three was not the maximum number which was formed. Ten progeny of the cross 50.7 / 50.13 when intercrossed could be grouped into six classes, the plants within each class showing great uniformity. Of the two self-pollinations which produced progeny which could be classified into distinct breeding groups, the selfs 50.13 and 1.7 gave rise to four and five breeding groups respectively.

Table 11.

Variations in the number of progeny breeding groups and percentage compatibility formed by different parental types. Incompatibility occurs when both alleles present in the pollen are held in common with those in the style. Pollen behaviour is determined gametophytically.

* Indicates reciprocal differences formed when intercrossing progeny.

Parents.	Compatible male gametes.	Number of groups formed by inter-breeding progeny.	Percentage compatibility.
a. $S_{1.2}Z_{3.4}$	S_1Z_3; S_1Z_4: S_2Z_3: S_2Z_4.	9* Incompatibility overcome in the parental cross only.	69.1
b1. $S_{1.2}Z_{5.6}$ $S_{3.4}Z_{7.8}$	S_3Z_7: S_3Z_8: S_4Z_7: S_4Z_8;	16	93.6
b2. $S_{3.4}Z_{7.8}$ $S_{1.2}Z_{5.6}$	S_1Z_5: S_1Z_6: S_2Z_5: S_2Z_6;	16	93.6
c1. $S_{1.2}Z_{5.6}$ $S_{2.3}Z_{7.8}$	S_2Z_7: S_2Z_8: S_3Z_7: S_3Z_8;	16*	90.4
c2. $S_{2.3}Z_{7.8}$ $S_{1.2}Z_{5.6}$	S_1Z_5: S_1Z_6: S_2Z_5: S_2Z_6;	16*	90.4
d1. $S_{1.2}Z_{5.6}$ $S_{2.3}Z_{6.7}$	S_2Z_7; S_3Z_6; S_3Z_7;	12*	86.6
d2. $S_{2.3}Z_{6.7}$ $S_{1.2}Z_{5.6}$	S_1Z_5; S_1Z_6; S_2Z_5;	12*	86.6
e1. $S_{1.2}Z_{5.6}$ $S_{1.2}Z_{6.7}$	S_1Z_7; S_2Z_7;	6*	72.2
e2. $S_{1.2}Z_{6.7}$ $S_{1.2}Z_{5.6}$	S_1Z_5; S_2Z_5;	6*	72.2

Table 12.

Number of breeding groups formed by interbreeding true families.

Parents.	Number of plants considered.	Number of breeding groups formed.
177.11 / 177.14	8	8
177.14 / 177.11	8	8
177.11 / 177.16	10	10
177.13 / 177.11	8	8
177.14 / 177.20	10	3
50.13 / 50.7	10	10
50.7 / 50.13	10	6
50.13 ⊖	8	4
1.7 ⊖	7	5

It was felt that many of the progeny studied showed trends towards distinct breeding classes, and it was regretted that a further flowering season was not available in which to confirm these trends. Repeated pollinations would have confirmed the results obtained, and determined which of the pollinations resulted in random failure, or contamination.

The number of breeding groups formed within the progeny of known pollinations does not give a definite indication of the type of controlling incompatibility system, but suggests that it is nearer to that of the Gramineae than that of Physalis.

Consideration of the number of breeding groups with the presence of reciprocal differences formed when intercrossing progeny eliminates some of the theoretical considerations studied. Thus in Table 10 only three of the pollinations quoted give rise to reciprocal differences when intercrossing the progeny, that of the self g (Fig.20), and the situations described by d_2, d_3 (Fig.24), and d_4, d_5 (Figs.21 and 22). When both alleles present in the pollen have to be matched in the style for incompatibility, an increased number of parental pollinations gives rise to reciprocal differences within the progeny. Thus all the crosses quoted in Table 11, except that of b_1 and b_2, in which no alleles are held in common by the two parents, give rise to reciprocal differences when the progeny derived from the quoted crosses are intercrossed. With self-incompatibility overcome in the parents only, intercrossing the progeny of a self also gives rise to reciprocal differences (Fig.23). In the experimental results all the progeny of the cross-pollinations and self-pollinations, (except that of the self 1.7) showed reciprocal differences when they were intercrossed amongst themselves. Hence, this gives no indication of the type of incompatibility system present. The fact that the self-pollinations of 1.7 showed no reciprocal differences suggests that the incompatibility system present differed from both the known two gene systems.

Consideration of the percentage compatibility obtained when the progeny are intercrossed does little to clarify the situation. Table 10 shows that the percentage compatibility which may be obtained in the pollinations indicated range from 19.7 (g) to 72.6 (d_4) per cent. The percentage compatibilities shown in Table 11 are much higher and range from 69.1 (a) to 93.7 (b) per cent. Table 13 shows the percentage compatibility obtained experimentally when intercrossing progeny.

Table 13.

Percentage compatibility formed by interbreeding true families.

Parents.	Percentage compatibility.	Parents.	Percentage compatibility.
177.11 / 177.14	79.3	50.13 / 50.7	58.0
177.14 / 177.11	57.8	50.7 / 50.13	28.2
177.11 / 177.16	48.4	50.13 ⊖	35.9
177.13 / 177.11	50.0	1.7 ⊖	64.0
177.12 ⊖	49.1	181.14 ⊖	64.1

The experimental results show that the percentage compatibility formed when progeny are intercrossed ranges from 28.2 to 79.3 per cent. The surprising fact about these figures are those indicated for the self-pollinations. The figures for the four self-pollinations quoted all differ to a remarkable degree and range from 35.9 to 64.1 per cent. The figure 64.1 per cent approaches that shown for the self in Table 11 section a, i.e. 69.1 per cent, but the other figures cannot be explained in this way.

Hayman (1956) showed that one of the classes obtained by interbreeding progeny of a known cross was identical in behaviour to that of the male parent with the progeny. Examination of the data shows that in one set of progeny this phenomenon was obtained. The progeny were the result of a self-pollination, i.e. 1.7 ⊖, and the male parent behaved identically with group I of the progeny (Fig.13a). It is of interest to note that this situation was not found within any of the progeny derived from a cross-pollination.

It was also observed by Hayman (1956) that the female parent of the cross was both compatible and incompatible with the progeny. This occurrence is more widespread in the data obtained and may be seen in the breeding patterns of the following crosses.

177.14 / 177.11	(Fig.4)
177.14 / 177.20	(Fig.8)
50.7 / 50.13	(Fig.10b)
1.7 ⊖	(Fig.13a)
181.14 ⊖	(Fig.15)

A general trend found throughout the experimentally produced crossing-tables was the similarity of the behaviour of plants when acting as female, but the dissimilarity when donating pollen to the rest of the progeny, (Fig.7a, plants 3, 17 and 16; Fig.10a, group I; Fig.15a, plants 2, 11 and 10). A similar situation was found by Pandey (1957) in one of the experimental families in *Physalis*, suggesting that a two gene system, with incompatibility present when one allele is held in common between pollen and style, may be similar to the system found in *C.persicifolia*.

Both Lundquist (1954) and Pandey (1957) found that doubling the chromosome number of *Festuca* and *Physalis ixocarpa* failed to remove the incompatibility system. Experimental results show that the majority of artificial tetraploids produced in *C.persicifolia* also retained their incompatibility systems. Most of the tetraploids were self-incompatible, but T 27 showed a low self-fertility. In reciprocal crosses made between the related diploid progeny and the tetraploids, each tetraploid was both compatible and incompatible with its diploid relatives. This is in contrast to the situation found by Gairdner (1926) who observed that a cross between the believed tetraploid C.persicifolia var Telham Beauty, and the diploid form, was successful only when the tetraploid was the female. Cross-pollination of the two tetraploids T 1 and T 4 was unsuccessful, but when T 15 and T 27 were cross-pollinated T 15 was receptive to pollen of T.27, though the reciprocal cross was not compatible. The behaviour of a tetraploid plant with its parent varied, depending on the tetraploid plant considered.

Thus, though the form of the incompatibility system within the tetraploid plant is not known, it is possible to conclude from the behaviour of the tetraploids, that an incompatibility system was present, though it is not known to what extent it differed from a diploid plant containing the same alleles.

The type of incompatibility system present in C.persicifolia has not been elucidated, though it may be concluded that it is a system based on two or more incompatibility genes. Whether or not these genes are related has not been determined. Examination of the systems found in Physalis and the Gramineae suggest that that of C.persicifolia has perhaps a slightly closer affinity with that of the Gramineae, though obviously the systems are not identical.

IV. SOME OTHER SPECIES OF CAMPANULA.

A. 1) Introduction.

The second approach to the problem of incompatibility in the Campanulaceae is a survey of the breeding systems found in other species of Campanula.

Seeds of as many species as possible of known origin were obtained; these however were limited and seeds of Campanulas long grown in botanic gardens were also collected. Some species obtained in this way had obviously been incorrectly named, and the majority of names given had no authority after them. The taxonomic position of the genus Campanula is confused, the latest complete work being that of de Candolle (1830). Where possible the names given were checked in floras, in Chittenden (1951), or in descriptions given by Crook (1937-1940), but where no clarification of the situation could be obtained, the name given by the botanic garden was taken. Those names which may be subject to correction have been marked with an asterisk in Appendix 1.

The seeds from botanic gardens were the result of open pollination, and therefore liable to contamination. Also it was appreciated that the seeds sent were unlikely to be the result of a single cross. However, due to the usual delay of two years between germination and flowering it was considered impracticable to make controlled crosses from which to raise a true family and to investigate the breeding system present. Hence it was decided to raise the plants from the seeds sent, and to look at the breeding system within these. It was hoped that there would be sufficiently few alleles present in the ten plants considered from each seed source to indicate the nature of the controlling breeding system, if one was present.

Each species obtained was given a code number over 100. Where two or more samples of the same species were obtained from different sources these were numbered on the decimal system. Individual plants within a species were also numbered. These numbers were placed after the decimal numbering and were separated from them by a dash. Hence, number 101.2 -3 indicates, working from left to right, C.*alliariaefolia*, the second sample of this species, and the third plant within this sample.

The seedlings were raised in a greenhouse in 1961, and then planted in beds in the botanic garden. Flowering began in late June 1962, continuing through to September. Extensive self- and cross-pollinations were carried out within plants of a species. Pollinations were made in the laboratory and incompatibility was measured by pollen tube growth except when otherwise stated.

2) Self-compatible species.

The following species were found to be self-compatible:

Campanula erinus
Campanula Hybrid Z 2
Campanula mollis

Campanula erinus (120.1)

This species was believed to be half-hardy and was therefore grown in the greenhouse, with incompatibility being measured by seed production. All self and cross-pollinations gave rise to good seed production, except for two crosses which failed. These were believed to be due to damage of the flowers during emasculation, as the plant was very brittle, and the flowers small, i.e. 6 mm long.

Flowers not emasculated set seed naturally and abundantly. Unlike the majority of Campanulas, *C.erinus* is an annual. The lack of incompatibility and the annual habit of the plant may well be correlated, though there is no experimental evidence for this. Certainly in a plant relying on good seed production the presence of incompatibility with its resultant heterozygosity may not be sufficiently advantageous to overcome the disadvantage of a perhaps reduced seed production.

Campanula Hybrid Z 2.

This is a horticultural hybrid *C.* x E.K. Toogood, obtained from the Northern Horticultural Society, Harrogate, which closely resembles *C.cochlearifolia*. The lack of an incompatibility system here confirms East's (1929) hypothesis, that compatibility in an incompatible group is often associated with a hybrid origin of the plant.

Campanula mollis (*199.1*).

The incompatibility reaction in *C.mollis* is interesting. In the ten plants considered, three were consistently self-incompatible, four consistently self-compatible and three showed conflicting results. However, all showed some cross-incompatibility. The name of the plant is in doubt. Crook (1938) maintains that *C.malacitana* is the accepted name for the plant called *C.mollis* by de Candolle (1830), and Beddome (1907). However, his description given for *C.malacitana* does not fit the plant under consideration. A synonym of *mollis* is given as *velutina* in Chittenden (1951), but neither does this description fit the plant. The dictionary states that *velutina* is a name sometimes used for *C.lanata*, though Crook (1937) regards the two (*velutina* and *lanata*) as indistinguishable. His description of *C.lanata* describes the plant grown. _C.lanata_ is recorded as being monocarpic and certainly all the plants which flowered in 1962 died before the spring of 1963. If the plant is considered to be monocarpic, the advantage of a lack of an incompatibility system would be similar to that of an annual species. Further work on this species would be required to elucidate whether the results obtained indicated the interesting situation of incomplete self-incompatibility.

3) Self-incompatible species.

All the rest of the _Campanula_ species studied were predominantly self-incompatible, see Table 14. Examination of the crossing pattern produced by intercrossing plants of a single species and source failed to reveal any obvious and consistent pattern. As the plants were derived from open pollination and the parent plants might well have been grown together with other _Campanula_ plants with a large gene pool of _s_ incompatibility alleles, it is possible that each plant of a species grown in this study might have a different _s_ incompatibility complement. Thus intercrossing plants of a species might well shown no cross-incompatibility, though self-incompatibility would be present. This could explain the position shown in the crossing-table of _C.thrysoidea_ (Fig.25). _C.thrysoidea_ is completely self-incompatible. It is of interest to note that though this species, like that of '_C.mollis_' above, is monocarpic, it differs from it in its breeding behaviour. The majority of the cross-pollinations made between plants of _C.thrysoidea_ were compatible, only four (1 x 8; 5 x 2; 6 x 3; and 8 x 6) being incompatible. Each of the reciprocal crosses was successful. It is deduced that the _s_ alleles present in the nine plants

Table 14.

Self-incompatible species of *Campanula*.

Code number.	Name.
101.2	*C.alliariaefolia*
101.3	*C.alliariaefolia*
103.1	*C.alpina*
193.1	*C.aucheri*
105.1	*C.autraniana*
106.1	*C.barbata*
106.2	*C.barbata*
204.1	*C.bellidifolia*
205.1	*C.betulaefolia*
108.2	*C.bononiensis*
114.4	*C.cochlearifolia*
115.1	*C.collina*
194.1	*C.elatinoides*
212.1	*C.fenestrellata*
195.1	*C.filicaulis*
123.1	*C.garganica*
124.3	*C.glomerata*
124.5	*C.glomerata*
125.1	*C.grandis*
190.2	*C.grossekii*
191.1	*C.hondoensis*
--	*C.isophylla*
213.1	*C.istraica*
128.1	*C.kemulariae*
208.1	*C.kolenatiana*
130.2	*C.lactiflora*
143.1	*C.lanata*
132.6	*C.latifolia*
197.1	*C.linifolia*
137.1	*C.medium*
200.1	*C.nobili-macrantha*
192.1	*C.ochroleuca*
141.1	*C.patula*
201.1	*C.phytidocalyx*
209.1	*C.pilosa*
198.1	*C.piperi*
145.1	*C.portenschlagiana*
202.1	*C.pseudo-raineri*
148.1	*C.punctata*
151.1	*C.raddeana*
154.1	*C.rapunculoides*
155.1	*C.rapunculus*
157.1	*C.rotundifolia*
157.3	*C.rotundifolia*
157.4	*C.rotundifolia*
157.6	*C.rotundifolia*
159.1	*C.saxatilis*
160.1	*C.scheuchzeri*
160.2	*C.scheuchzeri*
162.1	*C.sibirica*
163.1	*C.speciosa*
164.1	*C.spicata*
210.1	*C.spruneriana*
166.1	*C.thrysoidea*
168.1	*C.trachelium*
203.1	*C.tridentata*
214.1	*C.vanhoutteri*
170.1	*C.versicolor*.
172.1	*C.waldsteiniana*
172.2	*C.waldsteiniana*

considered were either different in each of the plants, or that their combinations were such that there was little inhibition to fertilisation. No information can be obtained from these results about the nature of the incompatibility mechanism present, other than that it is probably multiallelic.

The other extreme was shown by the breeding pattern of *C.barbata* (106.1). These plants showed a large proportion of failed cross-pollinations, suggesting that there were some incompatibility alleles in common (see Figs.26 and 26a). Out of a total of 55 cross-pollinations, only 15 were compatible. (This has not been expressed as percentage compatibility so as to avoid confusion between plants of a species, i.e. *C.barbata*, and intercrossing progeny of a single family, i.e. *C.persicifolia*). Plants 10, 5 and 11 were very similar in behaviour and were provisionally assumed to have the same genotype, but these were the only plants which could be grouped together. It is of interest to note that though the majority of *Campanula* species showed reciprocal differences as a common occurrence, there were only two examples of this in *C.barbata*, cross 3 x 1, 1 x 3; and cross 5 x 9, 9 x 5.

C.medium (137.1) also showed a large number of incompatible crosses, see Figs.27 and 27a. Examination of the simplified crossing-table (Fig.27a) shows that out of a total of 59 cross-pollinations, only 23 were compatible. Plants 4, 7, 1 and 3 were all cross-incompatible, except for the cross 4 x 7, which was compatible. Pollen of plant 9 was incompatible with plants 4 and 7, and split them from the other two with which it was compatible. The behaviour of pollen of plant 8 differentiated between plants 1 and 3, being incompatible with plant 1, but compatible with plant 3. Plants 4 and 7 were distinguished when used as pollen on plants 5 and 6, pollen of plant 4 was compatible on these two, but that of plant 7 was incompatible. Thus all four plants, 4, 7, 1 and 3 behaved in a different manner and must have had different genotypes. Plant 9 was reciprocally incompatible with plants 4 and 7 but differed from them in that it was reciprocally compatible with plant 1. Plant 5 was reciprocally incompatible with plant 9 but was compatible with pollen of plant 4, with which plant 9 was incompatible. Plant 6 was reciprocally incompatible with 7, but unlike 7 it was reciprocally compatible with plants 1, 3, 9 and 5. The behaviour of plants 2 and 8 was not known in detail but plant 2, though similar in its activity as pollen to that of plant 6, could be distinguished from it by being compatible with plant 6. Plant 8 was pollen incompatible on plants 4, 7 and 1 but differed from them in that it was compatible on plant 3. Thus all nine plants behaved in a different manner and must have had different genotypes. The large number of cross-incompatible crosses, however, suggests that there must be some factors in common between them preventing cross-compatibility.

The breeding pattern of C.lanata (143.1) (Figs.28 and 28a) also showed a high degree of cross-incompatibility. Two plants (numbers 3 and 10) were self-compatible, but as these plants were cross-incompatible it is suggested that the self-fertility was due to some freak conditions, and was not a normal phenomenon. Plants 4, 5 and 7 were cross-incompatible with each other, but must have differed genetically as pollen of plants 5 and 7 was compatible on plant 6, but pollen of 4 was incompatible. Plants 5 and 7 also differed, pollen of plant 5 being compatible on plant 1, but that of plant 7 being incompatible. Plant 10 was unlike the other plants in that it was self-fertile, but incompatible with pollen of all other plants. Plants 6, 1, 3 and 9 were all reciprocally incompatible with plant 10, but all differed from it by being compatible with pollen from other plants. The plants also differed from each other. Both plants 1 and 3 were compatible with pollen of plant 4, but plant 1 was compatible with pollen of plant 5, while that of plant 3 was not. Plant 6 was compatible to pollen of plant 5, but unlike plant 1 it was incompatible to pollen of plant 4. Plant 9 was incompatible to pollen of plant 5 (as was 3) but differed from 3 by being compatible with pollen of plant 7. Plant 2 was reciprocally incompatible with plant 9, but differed from it by being reciprocally compatible with plant 3. Pollen of plant 2 was incompatible on plant 8, though the reciprocal cross was compatible.

Thus all the ten plants considered behaved in a different manner. Like the pattern shown by C.medium there are a large number of cross-incompatible pollinations. Out of a total of 76 cross-pollinations only 24 were compatible. Unlike C.barbata both these species showed many instances of reciprocally different crosses; C.medium had seven pairs of reciprocally different crosses, and C.lanata eleven pairs.

No other self-incompatible species seemed to have sufficient plants with identical or even related incompatibility alleles in common for there to be a definite pattern in the crossing-table. Although the species were almost always consistently self-incompatible, cross-compatibility and incompatibility seemed almost to be distributed at random, with a large number of reciprocal crosses giving different results. This may be seen by examination of the crossing pattern produced when plants of C.grossekii were intercrossed amongst themselves (Fig.29). All the plants were consistently self-incompatible but out of a total of 45 reciprocal crosses, 19 showed reciprocally different results, i.e. 42 per cent. Plants 3 and 4 were reciprocally incompatible, but must have differed in their incompatibility genotype as pollen of plant 3 was compatible on plant 5, whereas pollen of 4 was incompatible on 5. The pollen reactions of the two plants also differed on plants 1, 8, and 10. Plants 4 and 5 were reciprocally incompatible, but these plants must have differed in their incompatibility genotype, as they could be distinguished by the action of pollen of plants 1, 2, 3, 6 and 10 on them. Plants 5 and 6 were cross-incompatible, but were distinguished by their action as pollen on plants 1, 4, 8 and 10. Plants 7 and 8 were cross-incompatible and though these two plants behaved identically as pollen on the remainder of the plants, their reaction to pollen from those plants differed considerably. Thus plant 7 was receptive to pollen from all the plants, except 8, but plant 8 was incompatible to pollen from plants 2, 4, 5, 7, 9 and 10. Plants 9 and 10 were reciprocally cross-incompatible, but these two could not have had identical genotypes either, for they could be distinguished by differing reactions with pollen of plants 1, 3, 5 and 6.

It is of interest to note that the results of cross-pollinations of C.grossekii failed completely to fall into discrete groups, and that there was even a chain of plants which were incompatible with their neighbours. This is shown in Table 15.

Table 15.

To show that the results of incompatible and compatible pollinations of <u>C.grossekii</u> do not fall into discrete groups.

Pollen parent.	Plants with which the pollen is incompatible.	Plants with which the pollen is compatible.
1	3,4,9,10.	2,5,6,7,8.
2	5,6,8,9,10.	1,3,4,7.
3	2,4; 1,9.	5,6,7,8,10.
4	3,5; 2,8,9,10.	1,6,7.
5	4,6; 1,8.	2,3,7,9,10.
6	5; 10.	1,2,3,4,7,8,9.
7	6,8; 2,3,4,5,10.	1,9.
8	7; 2,3,4,5,6,10.	1,9.
9	8,10; 1,2,3,4,5,6.	7.
10	9; 2,4,8,10.	1,3,5,6,7.

It seems as if the crossing successes and failures shown in Fig.29 were distributed at random. A statistical investigation of the results of each pollination seemed to support this. It was assumed that if failed pollinations were distributed at random over the table, and the chance of any cross failing was the proportion of failures in the table, then scoring the results of reciprocal crosses would give the ratio of $p^2 : 2pq : q^2$; where $p + q$ is 1, p^2 is the number of consistently successful reciprocal crosses, q^2 is the number of consistently failed reciprocal crosses, and 2pq is the number of reciprocal crosses showing different results. A Chi- square test on the difference between the observed and calculated ratios allows the calculation of the probability of such a difference being found. The probability of the observed ratio being found in C.grossekii is calculated below (Table 16), and it can be seen that there was a 50 per cent chance or more that this result would be obtained considering the conditions above. This would suggest that if an incompatibility system was present, it was not sufficiently obvious to affect the crossing pattern produced, due to dissimilarity between the plants considered. If this was so, and if the distribution of failures was at random with no obvious incompatibility, it would be expected that repetition of the pollinations in the following year would give a similar ratio, but a different pattern in the breeding table.

Table 16.

To show the method used to calculate the significance between the observed and the calculated ratios obtained in C.grossekii.

	Number of successful reciprocal pollinations.	Number of dissimilar reciprocal pollinations.	Number of failed reciprocal pollinations.
observed ratio	10.0	19.0	16.0
calculated ratio (e)	8.4	22.1	14.4
deviation (d)	1.6	3.1	1.6
d^2	2.4	9.6	2.6
d^2/e	0.29	0.47	0.17

$\chi^2 = 0.93$. $n = 2$, therefore $p > 0.5$.

Repeated pollinations were carried out the following year, but scoring the pollen tubes was not possible due to the poor quality of staining of the pollen tubes with a new batch of stain from a different source.

4) Statistical investigation of results.

It has been shown for C.grossekii that a comparison between the calculated ratio of the results of reciprocal crosses, and the observed ratio, indicated that there was a fifty per cent chance or more that the observed ratio would be found. If an incompatibility system was present, and was functioning due to genetical similarity between the plants considered, it would be expected that the observed result would deviate considerably from the calculated result, giving a low probability value. A Chi- square test was carriod out on the differonce between the observed and calculated values of reciprocal crosses in many of the self-incompatible species, and the probability of that result being obtained was found. Table 17 shows the individual results for the species considered. Examination of the probability values obtained shows that only for three species, i.e. C.barbata, C.lanata and C.modium do these indicate a significant difference between the observed and calculated values of reciprocal crosses. As has been shown above, these three species cannot be interpreted in the light of any known incompatibility system. Table 17 also shows the probability value obtained when the total result of all the species is considered. The value of p greater than 0.2 was obtained. If, however, the three species showing significant deviation are removed from the total, or even if each one is removed in turn, the new probability value is greater than 0.3. Thus it may be concluded that for the majority of species considered

Table 17.

To show the probability of obtaining the experimental results found.

Code no.	Name.	Experimental ratio.			Calculated ratio.			χ^2	p.
		++	+-	--	++	+-	--		
101.1	*alliariaefolia*	11	22	9	11.5	20.9	9.5	0.1	>0.5
101.3	*alliariaefolia*	11	20	4	12.6	16.8	5.6	1.27	>0.5
106.1	*barbata*	6	2	17	2.0	13.0	10.1	65.6	<0.001
205.1	*betulaefolia*	3	6	5	2.6	6.9	4.6	0.12	>0.5
108.2	*bononiensis*	28	14	0	29.1	11.6	1.2	1.71	≏0.4
114.4	*cochlearifolia*	3	23	19	4.7	19.6	20.6	1.3	≏0.5
194.1	*elatinoides*	6	3	1	5.6	3.7	6.3	0.4	>0.5
212.1	*fenestrellata*	5	3	1	4.7	3.6	0.7	0.48	>0.5
195.1	*filicaulis*	9	11	7	7.8	13.6	6.0	0.87	>0.5
123.1	*garganica*	8	1	0	8.0	0.9	0.3	1.69	≏0.45
124.5	*glomerata*	15	12	6	13.4	15.3	4.4	1.52	≏0.45
125.1	*grandis*	9	16	12	7.8	18.3	10.8	0.5	>0.5
190.2	*grossekii*	10	19	16	8.4	22.1	14.4	0.93	>0.5
191.1	*hondoensis*	5	17	7	6.3	14.4	8.3	0.91	>0.5
213.1	*istraica*	5	10	16	3.2	13.5	14.2	2.13	>0.3
128.1	*kemulariae*	2	16	24	2.4	15.2	24.4	0.99	>0.5
130.2	*lactiflora*	1	5	21	0.5	6.1	20.5	0.87	>0.5
143.1	*lanata*	6	12	20	3.0	16.4	17.7	4.48	>0.1
132.6	*latifolia*	0	4	13	0.2	3.5	13.2	0.84	>0.5
197.1	*linifolia*	6	5	5	4.5	7.9	3.5	2.24	>0.3
137.1	*medium*	7	6	11	4.2	11.7	8.2	5.7	≏0.05
192.1	*ochroleuca*	16	14	6	14.7	16.6	4.7	0.89	>0.5

.... continued

Table 17 continued.

Code no.	Name.	Experimental ratio.			Calculated ratio.			χ^2	p.
		++	+−	−−	++	+−	−−		
201.1	<u>phytidocalyx</u>	15	17	11	12.8	21.3	8.8	1.76	≃0.4
209.1	<u>pilosa</u>	8	15	5	8.6	14.0	5.6	0.19	> 0.5
145.1	<u>portenschlagiana</u>	12	7	0	12.6	5.6	0.6	0.99	> 0.5
202.1	<u>pseudo-raineri</u>	5	8	6	4.2	9.5	5.3	0.46	> 0.5
151.1	<u>raddeana</u>	3	22	15	4.9	18.2	16.9	1.74	≃0.4
154.1	<u>rapunculoides</u>	21	19	4	21.0	18.7	4.4	0.16	> 0.5
157.3	<u>rotundifolia</u>	3	12	2	4.7	8.5	3.7	2.94	≃0.25
159.1	<u>saxatilis</u>	13	17	4	13.6	15.8	4.6	0.19	> 0.5
160.1	<u>scheuchzeri</u>	13	21	6	13.8	19.4	6.8	0.27	> 0.5
160.2	<u>scheuchzeri</u>	15	12	2	15.2	11.6	2.2	0.22	> 0.5
162.1	<u>sibirica</u>	6	12	11	4.9	14.0	9.9	0.43	> 0.5
168.1	<u>trachelium</u>	13	16	9	11.6	18.8	7.6	0.84	> 0.5
214.1	<u>vanhoutteri</u>	15	16	5	14.7	16.6	4.7	0.11	> 0.5
170.1	<u>versicolor</u>	1	4	1	1.5	3.0	1.5	0.66	> 0.5
172.1	<u>waldsteiniana</u>	4	6	10	2.5	9.1	8.5	2.31	> 0.3
	Total	319	445	311	303.5	475.6	300.3	3.14	> 0.2

there were insufficient incompatibility alleles in common in the plants considered to show a definite breeding pattern which could be distinguished from a random one, in which the chance of any particular pollination failing was determined by the frequency of failures in the table.

A comparison between the probability values determined from observed and calculated results, between reciprocal crosses and within a repeated cross-pollination was made. Each individual result of a repeated pollination was paired with the other results for that pollination. Thus, when a cross was repeated three times, with two successful pollinations and one failure, it was scored as one repeated success and two mixed results. Individual results for seven species are shown in Table 18. Three species, C.aucheri, C.barbata and C.rotundifolia show a probability value which is greater than 0.5 , while the rest show lower values. Consideration of the combined results gives a total value of p approximately equal to 0.04. The figures for C.barbata are much larger than the others as this species was studied both in 1962 and 1963. In the total result, therefore, the probability value is bound to be determined largely by the result of C.barbata.

The probability values for the same species as above were considered for the results of reciprocal crosses, see Table 19. Here, four species, C.aucheri, C.thrysoidea, C.glomerata and C.rotundifolia showed probability values greater than 0.5, but unlike the situation in the repeated pollinations quoted above, the combined results show a total value of p greater than 0.5. The results obtained for the repeated pollinations indicate that the outcome of the cross is not at random. The observed value differs significantly from the expected value, which would be obtained considering the proportion of failures in repeated pollinations, derived from the crossing tables. It is argued that if the result of a repeated pollination is not at random, then the result of a reciprocal cross-pollination should not be either. It is concluded that though the results of reciprocal cross-pollinations appear to be at random, this is spurious. It is the outcome of

Table 18.

To show the probability of repeated pollinations giving the same results.

Code no.	Name.	Experimental ratio.			Calculated ratio.			χ^2	p.
		++	+-	--	++	+-	--		
193.2	aucheri	1	3	6	0.6	3.75	5.6	0.65	>0.5
106.2	barbata	99	212	134	94.4	221.1	129.4	0.75	>0.5
124.3	glomerata	16	9	6	13.5	13.8	3.5	3.85	≏ 0.15
157.2	rotundifolia	5	8	6	4.3	9.5	5.3	0.45	>0.5
210.1	spruneriana	5	3	11	2.2	8.5	8.2	8.06	≏ 0.02
166.1	thrysoidea	51	8	4	48.0	13.9	1.0	11.54	≏ 0.005
172.2	waldsteiniana	7	5	6	5	9	4	3.54	≏ 0.18
	Total	184	248	173	168.0	279.6	157.0	6.39	≏ 0.04

Table 19.

To show the probability of reciprocal pollinations giving the same result.

Code no.	Name.	Experimental ratio.			Calculated ratio.			χ^2	p.
		++	+-	--	++	+-	--		
193.2	aucheri	1	3	3	0.9	3.2	2.9	0.03	>0.5
106.2	barbata	14	17	15	11.0	22.9	12.0	3.08	>0.2
124.3	glomerata	14	14	5	13.3	15.2	4.4	0.22	>0.5
157.2	rotundifolia	17	21	3	18.4	18.0	4.4	1.04	>0.5
210.1	spruneriana	2	3	6	1.1	4.7	5.1	1.56	≏ 0.45
166.1	thrysoidea	31	12	1	31.0	11.7	1.1	0.09	>0.5
172.2	waldsteiniana	3	6	3	3	6	3	no deviation	
	Total	82	76	36	78.7	81.7	32.9	0.85	>0.5

considering plants which, though of the same species, have too few incompatibility alleles in common, when the plants are interbred, for the crossing pattern produced to reflect the presence of the incompatibility system in the majority of the crosses.

The most satisfactory method of checking this conclusion would be to consider a true family of one of the species considered above. The incompatibility system could be examined in the family obtained, knowing that the number of alleles present would be limited by the number present in the parent. This was not possible, but the method was tried on a family of _C.rotundifolia_, the breeding pattern of which is considered below in Section IV B 2. Here, the probability that repeated cross-pollinations gave the same result was highly significant, p being less than 0.001; but the pattern produced by reciprocal crosses falls well within the bounds of probability, i.e. p is between 0.99 and 0.98. Though the plants considered were the progeny of a single cross, the majority of cross-pollinations were compatible, and the total compatibility was 70.8 per cent. Hence, again, as with the other species of _Campanula_ which are considered above, the presence of cross-incompatibility within the table was insufficient to be differentiated from random failures.

B. 1) Detailed investigation of _C.patula_ (141.1).

Some plants of _C.patula_ when grown under sub-optimal conditions behave as annuals, although the species is normally biennial. A preliminary crossing programme in 1961 with incompatibility measured by pollen tube growth, indicated that there were at least four different breeding groups amongst the selected plants. Seeds of a known cross

(141.1 -14 / 141.1 -12) were collected and sown in order to find the breeding groups within a true family. Out of a total of 180 seedlings, 27 plants were forced to flower sufficiently to enable a restricted crossing programme to be carried out. Each plant was selfed, and then crossed as the female with pollen of four selected plants. Where possible all crosses were repeated. The plants were grown in a greenhouse; incompatibility was measured by seed set, and the results are given in Fig.30. Examination of the crossing-table shows that the progeny may be divided into eight breeding groups which are self-sterile, and one group which is self-fertile. Plant 21 in this latter group is infertile with pollen of plant 8. As there is no record of any plant being cross-incompatible, but self-compatible, it is assumed that either the failure or the self-fertility is due to reasons other than incompatibility. Pollen from two progeny of the cross 141.1 -12/ -13 were also placed on the plants of the family studied above, and the results obtained split the original grouping to form ten different self-incompatibility groups. The pollen parents for these pollinations are those with ringed numbers in Fig.31. Comparison of Figs.30 and 31 shows that apart from the removal of plant 16, the first three groups are identical. Group IV of Fig.30 was, however, split by the additional pollinations to form two groups, which differed in their reactions with pollen ㉙. From the available data it is impossible to know to which of groups 4 and 5 of Fig.31 to assign plants 103, 40, 105 and 113, for their reactions with pollen ㉙ were not known. They have been placed arbitrarily in group 5. Group 6 of Fig.31 is identical with group V of Fig.30, containing plant 51 only. Group 7 is a new group composed of plant 69 (previously in group IV) and plant 16 (from group 1, in Fig.30). Plants 7, 114 and 8 are still unique in their behaviour and are all classed separately. Thus pollen from two progeny of the cross 141.1-12 / -13, splits the eight self-incompatible groups from the cross 141.1-14 / -12 into ten different groups. It is unlikely that the limited range of pollen used was sufficient to segregate all the breeding groups and it can only be concluded that there is a minimum of ten different breeding groups formed within the progeny of a true family of C.patula. It is of interest to note that the few reciprocal crosses made, i.e. 43 x 8; 43 x 80; 43 x 114; 8 x 80; 8 x 114; 80 x 114, were all reciprocally identical and compatible. It can thus be concluded that the pollen genotypes used in plants 8, 43, 80 and 114 were all different. Seeds of the reciprocal cross 43 x 8, and 8 x 43 were all sown in order to try to determine the maximum number of breeding groups within each of the families, and if possible to see if the groups were identical between the families; unfortunately the plants could not be forced into flower in 1963.

2) Detailed investigation on C.rotundifolia (157)

Three plants were obtained from Glen Coe, Scotland, and one from St. David's Island, Pembrokeshire, and these were intercrossed amongst themselves. The plant from St. David's Island was the only one which was self-incompatible, though the other plants had a reduced fertility. The average number of seeds set when the three plants were self-pollinated was 28.7, compared with a seed set of 91.9 when the plants were cross-pollinated. Individually the three plants from Glen Coe showed very different degrees of self-fertility. Plant 157.5 -1 showed the highest fertility with an average of 66 seeds on selfing (result of three repeated pollinations); plant 157.5 -3 had an average of 18 seeds per self-pollination (two repeated pollinations); and plant 157.5 -2 an average of two seeds per pollination (two repeated pollinations).

Progeny were raised from parents from two widely different sources, the female parent (157.4 -1) was from Cronkley Fell, in County Durham, and the male parent (157.2 -2) was obtained from seed from the botanic garden in Stuttgart. Both parents were self-incompatible. Preliminary experiments on the progeny showed that a few plants, (numbers 11, 19 and 24) had a reduced self-fertility, though most of the plants were self-incompatible. Thus the average number of seeds set in those three self-pollinations was 7.1, while the average produced out of fifteen cross-pollinations was 148.5 seeds per pollination.

Detailed intercrossing of eleven progeny was made in 1963 and the results are shown in Fig.32. Six additional plants of the same family were tested for self-fertility and all were found to be self-incompatible. It can be seen from Fig.32 that three plants showed a tendency towards self-fertility, plants 3, 11 and 20. Plant 3 showed vigorous pollen germination when selfed, and though the majority of the pollen tubes were superficial, a few were present at the style base. Out of thirteen repeated self-pollinations of plant 11 only one showed any self-fertility. The capsule was left on the plant after pollination and six seeds developed.

It is of interest that this was the one plant observed in which self-pollination seemed to be brought about by the plant. Non-emasculated flowers were left for a week after the stigmas had diverged and were examined for pollination. All five plants so treated showed germinated pollen on the backward curved stigmas, suggesting that this had been transferred to the stigma by contact between the diverging and receiving stigma tip and the pollen covered stigma base. Plant 20 showed a low degree of self-fertility, four pollinations out of eleven being successful. Pollen tubes were observed at the base of the style in two of the flowers, and in flowers which were left for seed set, two seeds were produced in one capsule and one in another. It is of interest that the four successful self-pollinations were all made in May, i.e. in 16, 20 and 27 (two), which was the beginning of the flowering season, the first flowers being mature for pollen on 16 May. Flowers continued to be produced until July 24, and repeated self-pollinations on the plant on June 4 (three), June 15 (two) and June 28 (two), all failed. This was the only indication of end-of-season effects in all the Campanula species considered.

Fig.32a shows the overall results obtained by intercrossing the progeny of C.rotundifolia. Those plants which showed an incomplete self-incompatibility have been ringed, and it can be seen from Fig.32a that cross-incompatibility was still present in these plants. Thus plant 11 was cross-incompatible as pollen on plant 18, and was unreceptive to pollen of plant 17; plant 3 was incompatible as pollen on plant 1, and was unreceptive to pollen of plant 2; and plant 20 was incompatible to pollen of plant 15, and was itself incompatible as pollen on plants 2, 16, 17 and 23. Examination of the table shows that though plants 23 and 18 were reciprocally cross-incompatible, their behaviour as pollen on plants 6 and 17 differed. Pollen of plant 17 was incompatible on plant 23, but the reciprocal cross was successful. Plant 17, however, was reciprocally cross-incompatible with plant 18, and behaved in a similar fashion to it in many crosses. Plants 17 and 18 though, could be distinguished by their behaviour with pollen of plants 11, 2, 20 and 23. Plant 16 was incompatible with pollen of plant 20, but the reciprocal cross was compatible. The two plants differed in their behaviour when used as pollen on plants 2 and 18. Similarly pollen of plant 2 was incompatible on plant 3, though the reciprocal cross was compatible. These two plants were distinguished by their behaviour as pollen on plants 1 and 18. Pollen of plant 1 was incompatible on plant 6, but was reciprocally compatible. Both plants showed a high degree of cross-compatibility but were distinguished by their reaction with pollen of plants 3 and 23. Plant 15 was incompatible as pollen on plants 2, 20 and 16 but was otherwise cross-compatible. There was a high degree of reciprocal differences between crosses of the plants considered above, there being eighteen pairs of crosses showing different results.

It may be concluded that as each plant had a peculiar and characteristic behaviour, each must have had a different incompatibility genotype. A minimum of eleven groups may therefore be formed when intercrossing progeny of a true family of _C.rotundifolia_. The number of compatible pollinations was high; 87 out of a total of 109 cross-pollinations (79.9 per cent) were successful, suggesting that there were few factors in common between the parents. Further work on progeny derived from seeds produced in a cross with reciprocally different results, would ensure that there were factors in common between the parents, and should thus provide progeny with more incompatibility alleles in common. Interbreeding these progeny would be expected to produce fewer breeding groups, and might thus help to elucidate the controlling mechanism behind the breeding system.

3) Notes on C.isophylla.

One plant each of C.isophylla and C.isophylla var alba were available for breeding. C.isophylla var alba was self-sterile; pollen germination was good, but penetration of the stigmatic tissue by the pollen tubes was negligible. C.isophylla was pollen sterile and it was not known if there was an incompatibility mechanism present. C. isophylla var alba produced abundant pollen, and out of eight repeated cross-pollinations with C.isophylla two were successful, producing one and three seeds each. These were sown and the single seed set germinated in March 1963, but the other three had failed to do so by September 1963.

4) Notes on C.carpatica.

Many plants of C.carpatica were collected, but the majority of these showed partial or complete male sterility, confirming Pellew (1917). It was decided that this feature would obscure any incompatibility system present, and would be a complete study in itself, so it was not continued in the present study.

5) Conclusion.

From the experimental results obtained it may be concluded that the majority of Campanula species studied have an incompatibility system. In some species: C.erinus, C.mollis and the hybrid C. X E.K. Toogood this seems to have been lost. In a few species, as was observed in the detailed studies of C.patula and C.rotundifolia, incompatibility seems to be reduced in a few plants allowing a very low percentage of self-fertility, but these plants were in the minority.

True families were only available for inbreeding in two of the species considered, which was a limiting factor to the study. The breeding patterns produced by intercrossing plants of a single species cannot be explained in the light of any known incompatibility system, and it is considered that the incompatibility system can only be elucidated by interbreeding a true family of known parentage.

The consideration of the true families of *C.patula* and *C.rotundifolia*, however, did little to indicate the type of controlling breeding system, except to show that at least 10 or 11 breeding groups respectively may be formed within a true family. Further work on these two families considering progeny derived from two parents with some incompatibility alleles in common, and experimentation with much larger families would help to elucidate the problem. Vegetative propagation of each individual plant would be essential to ensure an adequate supply of material. The scoring of material by pollen tube growth would increase the supply of material as removal of flowers from a plant stimulates further flower production. Consideration of plant material from the wild only, might overcome the chance of low seed set on selfing, which results from an upset incompatibility genotype due to hybridisation or plant breeding.

V. DISCUSSION.

The present study has indicated that self-incompatibility is of widespread occurrence in the genus Campanula. A few species are self-compatible, as has been shown in section III, A, 2; and a few isolated plants within self-incompatible species show self-fertility under some conditions, e.g. C.persicifolia, 185.5. There is no evidence that self-incompatibility may be overcome by bud pollination, pistil damage, or end-of-season effects in C.persicifolia.

Work on C.persicifolia has revealed several of the characteristics of incompatibility, but the system has not been elucidated. The study of true families has indicated that the number of groups formed within the progeny of a known cross is large: too large to be the result of an incompatibility system controlled by one locus, even with many alleles.

It was found that both male and female parents of a family were compatible to some extent with the progeny. The female parent was compatible with some of the progeny but incompatible with others, while the behaviour of the male parent could be identified with one of the breeding groups formed by interbreeding the progeny themselves. All the progeny except that of 1.7 selfed, showed some reciprocally different results when intercrossed amongst themselves. Whether two families derived from a reciprocal cross behaved identically or not, was not shown. This would form a good starting point for future work. Determination of whether reciprocal differences produced when interbreeding progeny were spurious or not, is also most important.

Work with artificially produced tetraploids of C.persicifolia showed that self-incompatibility is generally retained in these plants. This is in agreement with the results found when examining material of some other species of Campanula. Table 20 shows chromosome numbers of some of the Campanula species considered in the present study. They are all taken from Darlington and Wylie (1955), as no counts were made on species other than C.persicifolia in the present study. It does not follow that all the species considered had the number quoted in the table, but in the absence of personal counts these are taken as a guide.

Table 20 indicates that many of the species considered are of polyploid origin. There are two main basic numbers found in the genus Campanula, (Sugiura, 1942), x = 8 or 17; but x = 10, 12, 13, 14 and 15 are also found. It is of interest to note that the only self-compatible species listed, C.erinus, is a diploid and the absence of self-incompatibility therefore cannot be considered to be due to competition or dominance arising from chromosome doubling. The retention of self-incompatibility in natural polyploids is in agreement with the behaviour of artificially produced polyploids in C.persicifolia.

A comparison between the results found in C.persicifolia and those of known incompatibility systems has been made in detail in section III, C. Investigations on other species of Campanula have shown the extent of self-incompatibility within the genus, but have added little knowledge about the type of system present.

It is of value to consider here the complexity of incompatibility systems, the type of variation that may occur due to changes within the controlling gene itself, and to linked or related genes.

Table 20.

Chromosome numbers in <u>Campanula</u> species (Darlington and Wylie, 1955).

Basic number.	Name.	Somatic number.
x = 8	*C.isophylla*	32
	C.thrysoidea	48
	C.alliariaefolia	96
x = 10	*C.patula*	20
x = 14	*C.erinus*	28
x = 17	*C.barbata*	34
	C.trachelium	34
	C.thrysoidea	34
	C.glomerata	34, 68
	C.portenschlagiana	34, 102
	C.garganica	68, 102
	C.scheuchzeri	68
	C.speciosa	68
	C.rapunculoides	102
	C.sibirica	102

Incompatibility is of widespread occurrence throughout the plant kingdom, and until recently has been believed to be controlled by one or two loci, with either two or many alleles. Modern evidence, though, seems to suggest that this simple interpretation is no longer tenable, and that the controlling system is more complex.

Recent work on the fungi has brought some of this complex system to light. The terms used here to describe the mating types of the fungi are those defined by Burnett (1956). Incompatibility only occurs in fungi in which genetically different nuclei derived from different thalli fuse to form a sexual reproductive body, i.e. heteromictic fungi. In most heteromictic fungi differentiation of cross-compatible but self-incompatible groups is independent of the differentiation of the sex organs (Fincham and Day, 1963). The simplest form of incompatibility system is found in dimictic fungi in which mating is controlled by two allelomorphs at a single incompatibility locus. The two alleles are termed A and a, and form complementary nuclei with obligatory cross-fertilisation. This type of system is widespread throughout the Phycomycetes and Ascomycetes, and is found in _Saccharomycetes cerevisiae_, _Puccinia graminis_ and _Neurospora sitophila_ (Burnett, 1956).

The incompatibility locus in the Ascomycete, _Chromocrea spinulosa_ seems to be closely associated with spore size. Each ascus normally gives rise to eight large and eight small spores (Mathieson, 1952). The small spores are consistently self-sterile, but occasionally cultures of large spores show a reduced fertility producing perithecia which bear asci with eight large and eight small spores, which are perfectly cross-fertile. This has been explained by the assumption that mutation from large to small spores occurs in the material believed to be derived from large spores only, and hence fertile fruit bodies are able to be formed. It is significant to note the close correlation between the spore size and the mating type.

In diaphoromictic Basidiomycetes mating is controlled by a series of multiple alleles at either one locus, A in bipolar series; or two loci, A and B in tetrapolar series. Mycelia which carry different

A alleles in bipolar series or which differ with respect to both A and B alleles in tetrapolar series are compatible. The number of alleles at any one locus has been estimated by Whitehouse (1949), as being about 100 in the Hymenomycetes, but fewer in the Nidulariales. Burnett and Boulter (1963), after work on Mycocalia denudata, estimated the number of alleles in one mating group as 7 or 8, and in the other as 12 or 13. Recent work on Schizophyllum commune (Raper et al, 1958, 1960) and Coprinus lagopus (Day, 1960) has shown that the A locus of the tetrapolar series is composed of two sub-units, i.e. Aa and Ab, and only A factors which have identical a and b are incompatible. Preliminary reports suggest that the B factor is equally complex. It is not known whether the sub-units are sites in the same functional region of the chromosome or whether they are distinct genes.

The studies by Crowe (1963) have shown that though the incompatibility system in Schizophyllum commune is based on two incompatibility loci, there is competition between compatible nuclei. Thus in some compatible dimon matings the two new dikaryotic genotypes were recorded with equal frequency, while from other matings there was a marked preponderance of one class of dikaryon in relation to another (Kimura, 1958). No dikaryon was established if either the A or B alleles, or both, were homologous, but combinations of nuclei with different A and B factors occurred and showed different degrees of success in establishing dikaryotic mycelium. Dikaryons with maximum success were those with maximum heterozygosity of the incompatibility alleles. In many tetrapolar Basidiomycetes the loci A and B control different parts of the process of dikaryon formation, e.g. Coprinus lagopus (Swiezynski and Day, 1960), Schizophyllum commune (Parag, 1962), *Cyathus stercoreus* (Fulton, 1950). The formation of clamp connections seems to be controlled by the A locus and nuclear migration by the B locus. Crowe (1963) suggests that heterozygosity of the B factor is more important than that of the A factor. Thus here the incompatibility system is no longer concerned solely with the ability of two nuclei to fuse, but also determines the dikaryotising ability of a pair of nuclei. Crowe (1963) concluded that there were at least two mechanisms involved which depend on the internal organisation of the incompatibility factors themselves. This complex of genes is not confined to *Schizophyllum*, but has been demonstrated in *Pleurotus oesteatus* (Terakawa, 1960), *Coprinus lagopus* (Day, 1960) and *Collybia velutipes* (Takemaru, 1961).

The diversity of incompatibility alleles in the fungi appears to be more complex than that found in the angiosperms. In species of *Nicotiana* it is possible to identify incompatibility alleles which are held in common by several related species (East, 1929). The Basidiomycetes, in contrast to the Phycomycetes and Ascomycetes, are notoriously sterile in interspecific and even intervarietal crosses. Each species seems to have a completely different set of incompatibility factors, or have the same incompatibility factors with an overriding sterility factor (Papazian, 1958). This is probably not an artifact of taxonomy for in the genus *Coprinus*, which has been extensively investigated by Lange (1952), he claims that 'intersterility barriers divide the studied strains into groups corresponding to, or in some cases narrower than, the species defined according to morphological characters'.

Until recently it was believed that incompatibility in the heterothallic Myxomycetes was determined by a one locus, two allele mating system, e.g. Physarum polycephalum (Dee, 1960), Physarum pusillum (Collins, 1962). However, when investigating the breeding behaviour of Didymium iridis from Honduras and Panama, Collins (1963) found that the breeding behaviour was determined by two genes, each with more than two alleles. Thus here again, recent work has shown that the determining incompatibility mechanism is more complex than originally supposed.

The heterostylic Angiosperms are a good illustration of the complexity of the controlling mechanism of incompatibility. The simplest is the distylic form found in Primula species. Bateson and Gregory (1905) showed that the control of the incompatibility system is determined by a single gene with two alleles, and pollen behaviour is determined sporophytically. Ernst (1936) pointed out that associated with the pistil length and anther height were other characters, such as the pollen size and the size of the stigmatic papillae. He showed that these factors are controlled by three sub-units of the s gene which are closely linked, and only rarely does crossing-over break this linkage to produce abnormal flowers. The mechanism of control of incompatibility is similar in Primula sinensis, but is modified by two independant genes with pleiotropic effects. One of these, a, Primrose eye, shortens the style length, and alters the size of the stigmatic cells and the incompatibility reaction of the style; and the other, m, Fertile Double, raises the anther level without altering the size or incompatibility reaction of the pollen (Beale, 1939; Mather, 1950). Thus these genes affect the incompatibility relationships of the pollen and style. In Linum grandiflorum the anther and stigma height is associated not with pollen size, but with turgor pressure of the pollen (Lewis, 1943). Thus here another factor is associated with the incompatibility gene.

In tristylic species, e.g. Lythrum salicaria, Oxalis valdiviensis, each flower has anthers at two different levels, the stigma being borne at a third level. Compatible pollination only occurs between pollen and stigma borne at the same height on different plants. Genetical control in Lythrum is based on two independent genes S and M. The short style character is determined by dominant S, irrespective of the Mm constitution. The long style is controlled by the recessive form of both genes ssmm. Determination of the mid-style length has been the subject of much controversy (Barlow, 1923; East, 1927, 1932; Fisher and Mather, 1940, 1942; Haldane, 1934; von Ubish, 1925) but is controlled by recessive s and dominant M. Inheritance of both Mm and Ss is tetrasomic (Fisher and Mather, 1943; Fisher and Martin, 1947). Thus an understanding of the inheritance of incompatibility in Lythrum salicaria was not solved until 80 years after Darwin (1864) first wrote about it. The situation in Oxalis valdiviensis, though similar, differs in that the two controlling loci S and M are linked (Fyfe, 1950).

Control of incompatibility in Narcissus triandrus is not yet elucidated, though commented upon by Henriques in 1887. Recently Bateman (1952 b) observed that though the stigmas were of three heights in N.triandrus var. concolor, the anthers were borne at two heights only. He found that no good seed was set when flowers were selfed with pollen borne on the same plant. This contrasts with the results of pollinations on *N.triandrus* var. *cernuus* made in Durham by the writer, where anthers at three heights were observed and where low seed sets were consistently obtained on selfing. Bateman (1952 b) concluded that the incompatibility system is independent of the heterostyly, and is probably due to a multipolar system. Obviously the controlling system is complex and requires further work on much larger samples of material.

The controlling systems of incompatibility in homostylic plants have been outlined in section II, A, 4. Since East and Mangelsdorf (1925) first elucidated the basic controlling system in *Nicotiana*, many genera with the same type of incompatibility system have been found, and in the process some anomalies in the controlling systems have brought information on the basic mechanism to light. There seems to be two levels of control. There is control of incompatibility from within the gene, or series of very closely linked genes, and modification of control due to the relationship of the incompatibility complex to the linkage group within which it is held, and to the rest of the chromosomes.

Anderson and de Winton (1931) found anomalous behaviour in the crosses between strains of self-incompatible *N.alata* and self-compatible *N.langsdorffii*. More recent work on *Oenothera organensis* (Lewis, 1951), *Prunus avium* (Lewis and Crowe, 1954) and *Trifolium pratense*, *T.repens* (Pandey, 1956), on spontaneous and irradiation induced mutants involving *S* alleles, show that the *S* allele complex has at least two independently mutable units.

Crowe (1955) and Lewis and Crowe (1958) have shown that there are at least two kinds of self-compatible species. There are those plants which are recently self-compatible (Sc), in which fertility is maintained by an S_f allele, allelomorphic to the *S* incompatibility alleles, but comparable to an experimentally produced pollen-part mutant. There are also long established self-compatible species (S_C), in which compatibility is maintained by a self-fertile allele which has not yet been recovered in mutation studies in self-incompatible species .

Pandey (1962 a) has tentatively proposed a hypothesis for the *S* gene structure which accounts for some of the anomalies found when interbreeding incompatible plants. He suggests that the basic incompatibility allele consists of four substances in both pollen and style. These substances include a growth substance, a protective growth substance, primary specificity and secondary specificity. The growth substance he concludes may or may not be a part of the *S* gene complex, as it is basic to all functional pollen; the protective growth substance is attached to the growth substance and prevents its inactivation after both specificities are lost. These four components are attached to the pollen and style in the order given, the secondary specificity being added last. By mutation each component may be lost sequentially from the pollen and style. Theoretically, therefore, it is possible to have sixteen different types of *S* incompatibility alleles, and Pandey (1962 a) believes that he has identified six of these.

The normal self-incompatible allele contains all four parts in both pollen and style. Loss of secondary specificity in the pollen is believed to account for the behaviour of mutants of *Oenothera organensis*, *Trifolium pratense*, *T. repens* and *Prunus avium* and is termed S_f. Loss of secondary specificity in the style alone accounts for the behaviour of the self-fertile allele found in recently self-compatible species, i.e. mutants of *T.pratense*, and *Prunus avium*. Loss of secondary specificity in both pollen and style indicates the position in *N.alata*. Lewis and Crowe (1958) and Pandey (1959) explained the fact that some strains of *N.alata* reject pollen of the self-compatible *N. langsdorffii* as being due to two factors. The first is that *N.langsdorffii* is believed to be a recently self-compatible species (Sc) and to contain the self-fertility allele S_f. Pollen of this plant, though, is rejected by *N.alata* which contains the self-incompatibility allele S_F. This is interpreted by assuming that some specificity is retained in the S_f allele of the pollen, suggesting that though secondary specificity is lost, primary specificity is retained.

Pandey (quoted 1962) found that some strains of *Solanum pinnatisectum* and *S.ehrenbergii* which had homologous alleles (S_{1C}) in the style, rejected all self-incompatible pollen. He explained this by suggesting that secondary, primary and protective growth substances are lost in the style, and that the growth substance has been inactivated by the specific substance in the self-incompatible pollen.

The sixth type of form identified in the *S* gene complex is the type found in many wild self-compatible species. This *S* gene complex retains the minimum requirements of a dynamic species, with the loss of both specificities in pollen and style, but loss of the protective substance in the pollen only. If the protective substance had been lost in both pollen and style, the species having such an allele would be completely incapable of exchanging genes with other species with any pollen specificity, and would prevent all hybrid development with species retaining pollen specificity.

Thus Pandey (1962) has indicated that the S incompatibility locus is probably of great complexity which, by loss or inactivation of one or more of its component parts may change its breeding behaviour.

Grun and Radlow (1961) found that though usually pollen of the self-compatible Solanum verrucosum will not grow down styles of self-incompatible species of Solanum, the same pollen would grow down styles of other equally self-incompatible species, but from a different geographical location. This condition was not due to Solanum verrucosum being a recently self-compatible species, but is interpreted (Grun and Radlow, 1961) as resulting from a loss, due to pleiotropy or linkage, of their unused breeding barrier against introduction of genes from self-compatible species. Thus it can be seen that incompatibility is controlled both by the state of the incompatibility allele itself, and by the linkage group in which it is held.

Studies on Vicia faba L. by Holden and Bond (1960) resulted in a proposal of two alternative mechanisms for the spontaneous fertility which was a property of cross-bred plants, and certain inbred individuals and lines. They suggested that this could be due to the presence of specific genes in inbred lines, which must be related to the incompatibility locus though not closely linked to it. The other method suggested was that this fertility was due to heterozygosity, being a specific expression of hybrid vigour, which overcomes the necessity of tripping. This observation of spontaneous self-fertility seems to be similar to that recorded by Denward (1963). He found that pollination of Trifolium pratense L. between ordinary plants and stigmas of plants derived from shoots of the same clone, but grafted on to other clover material, gave rise to 20 to 40 times higher fertility than normally derived by spontaneous fertility. He assumed that spontaneous fertility is normally genotypically controlled in Trifolium, but that its effect is greatly accentuated due to grafting. Another unexplained peculiarity of incompatibility alleles is the situation that was found in Physalis ixocarpa by Pandey (1957). Here, intercrossing progeny derived from a reciprocal cross gave rise to a different number of breeding groups within each of the progeny. This was explained by a differing relationship between the two genes involved in the parent plants, as shown above. The alleles involved were identical so the difference must lie either in the relationship of the incompatibility allele to the rest of the genic complement, or to a cytoplasmic influence. Perhaps the phenomenon of end-of-season effects may eventually be explained in these terms.

As investigation on incompatibility proceeds not only does the complexity of the incompatibility locus become more apparent, but also the system which the locus controls. Bateman in 1952 a considered it doubtful whether two or more loci controlling an incompatibility system could give rise to an efficient system. However, Hayman (1956), Lundquist (1954, 1955, 1961, 1962) and Pandey (1957) have elucidated two different systems controlled by two genes, one of which is present in the very successful Gramineae family. The complex nature of the incompatibility system in Theobroma cacao (Cope, 1962) has at last been elucidated, the barrier to fertilisation lying in the ovary, with the pollen behaviour having characteristics both of gametophytic and sporophytic determination. The system of incompatibility in Coffea canephora canephora is still unsolved. Self-incompatibility is present in varying degrees, but no evidence of cross-incompatibility has been obtained (Archibald and Pritchard, 1960).

From a knowledge gained in this study, it is suggested that the incompatibility system present in Campanula persicifolia may be elucidated by considering the progeny derived from a reciprocal cross, between two self-incompatible plants, preferably from the wild. At least 40 plants within each of the progeny groups should be examined. If a two gene system is present a maximum number of 16 different breeding groups within the progeny could theoretically be obtained. From a consideration of 40 plants, duplication of plants within breeding groups would be ensured, and most breeding groups would be represented. Intercrossing the plants within a family would reveal the number of groups present, and intercrossing the plants from the two families would indicate whether the groups present in each individual family were identical. Crosses between parents and progeny would indicate the exact relationship between them. A large supply of material would be required for these studies but this should be readily available by vegetative propagation of each plant.

It is suggested that all means of overcoming self-sterility should be attempted, i.e. out-of-season flowering, raising flowers in the dark, and the use of growth regulating substances on the ovary at the time of pollination. The behaviour of progeny raised from a known true self would provide valuable information about the nature of the controlling incompatibility system in C.persicifolia.

Fig.1. MATURATION OF A FLOWER OF CAMPANULA PERSICIFOLIA.

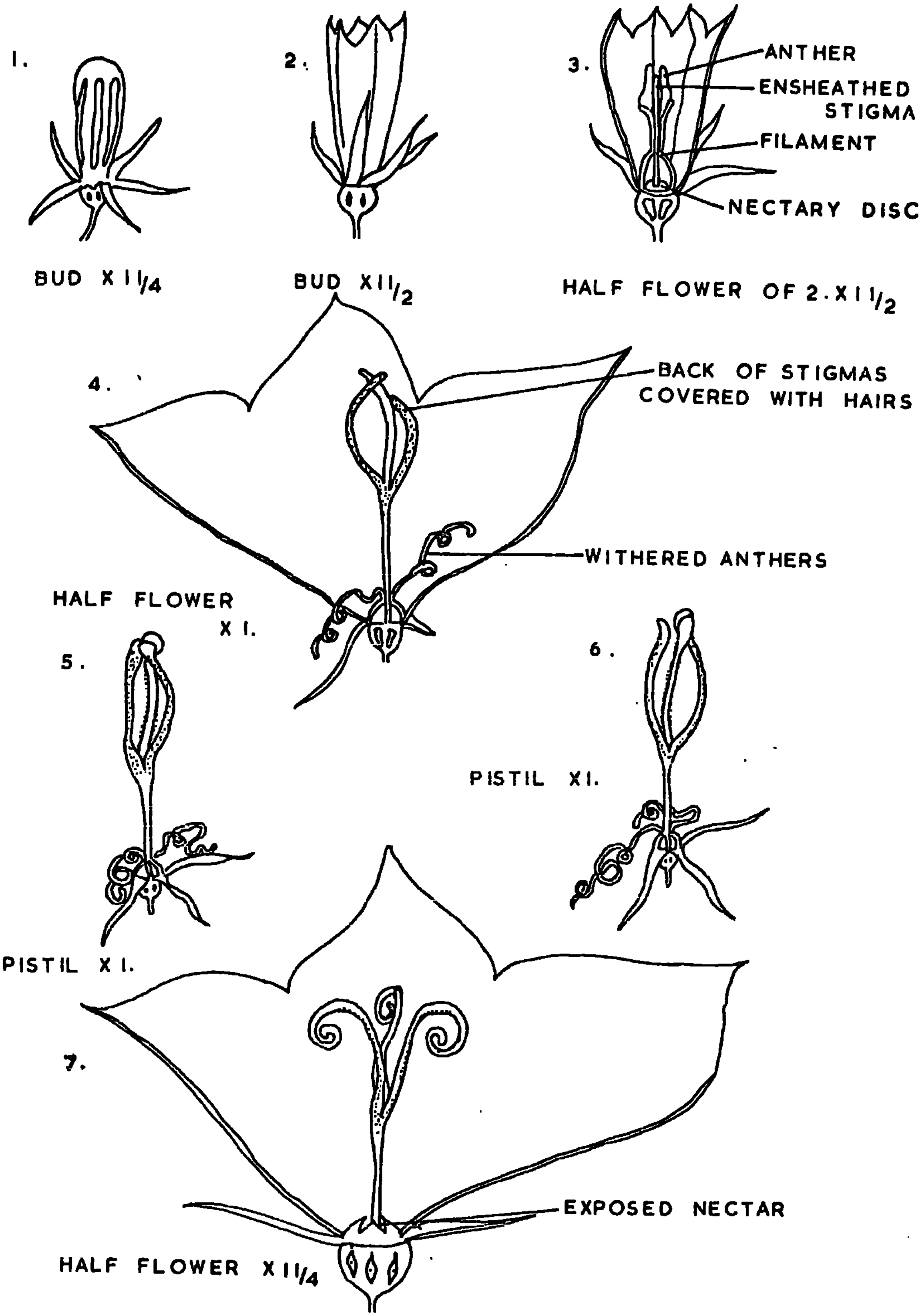
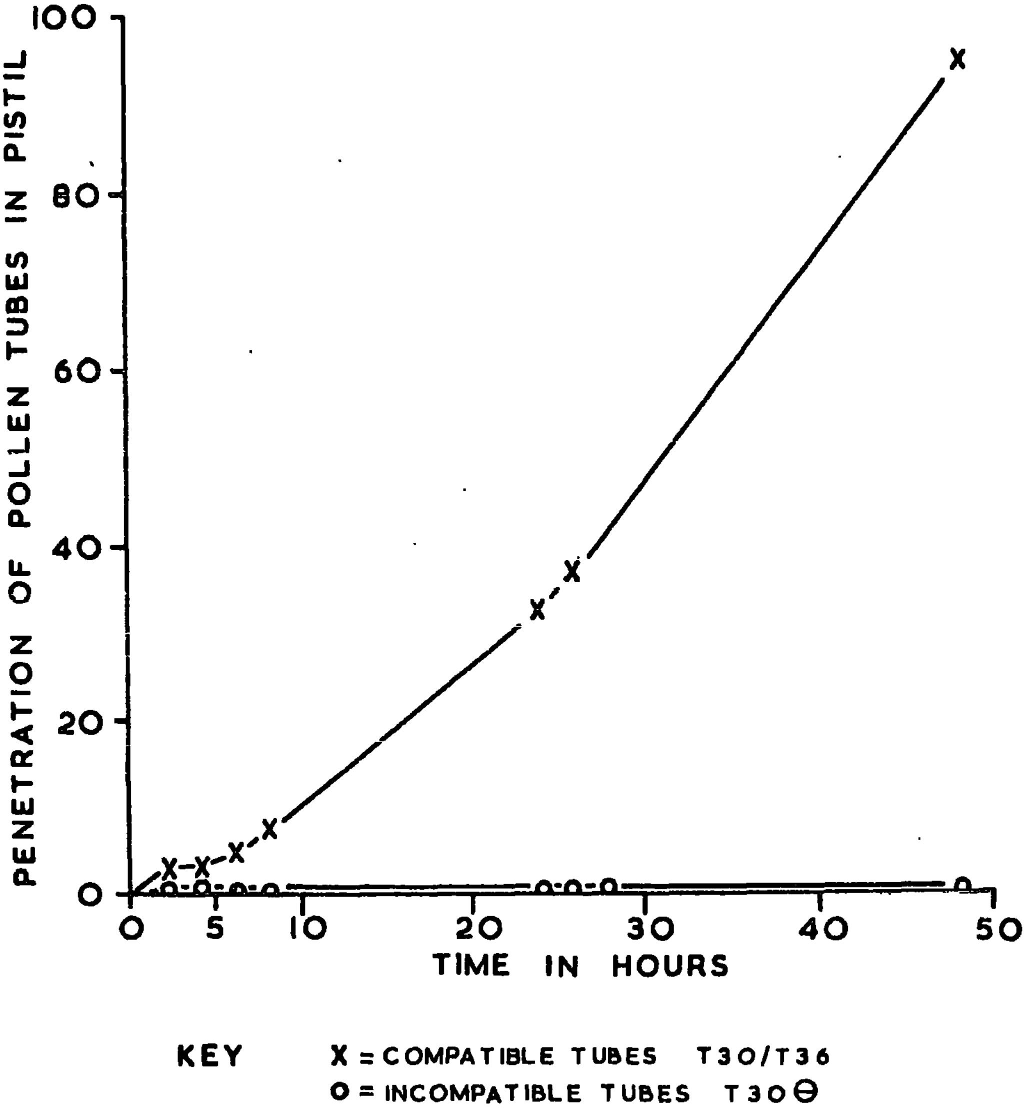


Fig.2.

Fig.2.

RATE OF GROWTH OF INCOMPATIBLE AND COMPATIBLE POLLEN TUBES IN PISTIL TISSUE.



Figs.3 & 3a.

Fig.3.

BREEDING PATTERN OF PROGENY OF CROSS 177.11 / 177.14 (P).

	4	5	9	16	17	18	21	22	177 .14
4	0|	/	//	0//	0	/	00	//	00
5	/	0|	/	/0/	/	/	00	/	0
9	//	/	0|	/000	/	00	0/	0	-
16	00/	00	/	0|	//	//	0	/	0
17	/	/	/	/	00|	/	/	//	0
18	/	/	//	0/	/	0|	/	/	0/
21	//	//	0/	0	/	/	0|	//	0/
22	0//	/	0	/	//	/	/	00|	0/
177.14	-	-	-	-	-	-	-	-	0|

Fig.3a.

SIMPLIFIED BREEDING PATTERN OF CROSS 177.11 / 177.14 DERIVED FROM FIGURE 3.

	16	5	21	9	4	17	18	22	177 .14
16	0|	0	0	/	/	/	/	/	0
5	/	0|	0	/	/	/	/	/	0
21	0	/	0|	/	/	/	/	/	/
9	/	/	/	0|	/	/	0	0	-
4	/	/	0	/	0|	0	/	/	0
17	/	/	/	/	/	0|	/	/	0
18	/	/	/	/	/	/	0|	/	/
22	/	/	/	0	/	/	/	0|	/
177.14	-	-	-	-	-	-	-	-	0|

KEY.
0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Figs.4 & 4a.

Fig.4.

BREEDING PATTERN OF PROGENY OF CROSS 177.14 / 177.11 (0)

	4	5	6	19	20	22	23	24	177 .14
4	0|	/	00	/	0/	0	0	/	//
5	/	00|	/	0	/	////	0/	0//	/
6	00	/	0|	/	0	0	0	0	/
19	0	0	/	0|	/	0/	//	0/	0
20	/	0/	0	/	0|	0	/	//	0
22	0	0/	00	//	0	0|	0/	0/	0
23	0	/	0	00	/	//	0|	/	0
24	/	0/	/	///	0/	/	/	0|	0/
177.14	/	/	/	0/	00	/	00	/	00|

Fig.4a.

SIMPLIFIED BREEDING PATTERN OF CROSS 177.14 / 177.11 DERIVED FROM FIGURE 4.

	6	4	22	23	19	5	20	24	177 .14
6	0|	0	0	0	/	/	0	0	/
4	0	0|	0	0	/	/	/	/	/
22	0	0	0|	/	/	/	0	/	0
23	0	0	/	0|	0	/	/	/	0
19	/	0	/	/	0|	0	/	/	0
5	/	/	/	/	0	0|	/	/	/
20	0	/	0	/	/	/	0|	/	0
24	/	/	/	/	/	/	/	0|	/
177.14	/	/	/	0	/	/	0	/	0|

KEY 0 = incompatible pollination.
/ = compatible pollination
- = pollination not made.

More than one symbol per square, indicates the result of each repeated pollination.

Figs.5 & 5a.

Fig.5.

BREEDING PATTERN OF PROGENY OF CROSS 177.11 / 177.16.

	1	3	8	9	12	16	17	18	19	20	[177.16/ .11]
1	0|	00	0	0	//	0	0/	/	//	0	-
3	/	0|	00	0/	/	0	0/	/	0	/	-
8	0	00	0|	0/	//	0	/	//	0	0	-
9	0	0	00	0|	//	0	/	00	0/	00	-
12	/	/	/0	00	0|	0	00	0/	0	0	0
16	0	0	0	0/	0	00|	0	/	00	0	/
17	//	/	0/	/	0/	0	0|	/	0/	00	-
18	/	/	0/	//	/	/	//	0|	00	/	-
19	/	0//	00	//	0	//	0/	0/	0|	//	-
20	00	/	0	0	-	000	//	/	00	0|	0
(177.16/.11)	-	-	-	-	0	/	-	-	-	/	0|

Fig.5a.

SIMPLIFIED BREEDING PATTERN OF CROSS 177.11/177.16 DERIVED FROM FIGURE 5.

	8	16	9	3	1	20	19	12	17	13	[177.16/ .11]
8	0|	0	/	0	0	0	0	/	/	/	-
16	0	0|	/	0	0	0	0	0	0	/	/
9	0	0	0|	0	0	0	/	/	/	0	-
3	0	0	/	0|	/	/	0	/	/	/	-
1	0	0	0	0	0|	0	/	/	/	/	-
20	0	0	0	/	0	0|	0	-	/	/	0
19	0	/	/	/	/	/	0|	0	/	/	-
12	/	0	0	/	/	0	0	0|	0	/	0
17	/	0	/	/	/	0	/	/	0|	/	-
18	/	/	/	/	/	/	0	/	/	0|	-
(177.16/.11)	-	/	-	-	-	/	-	0	-	-	0|

KEY
0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig.6.

BREEDING PATTERN OF PROGENY OF CROSS 177.13 / 177.11.

	2	3	4	5	6	7	8	10
2	0|	00	0	00	0	//	//	/
3	//	0|	0/	//	0/	00	0/	0
4	0	0/	0|	0	//	//	00/	0
5	0	00/	0	00|	//	0/	//	/
6	0	/	00	0/	00|	/	0	//
7	0/	0/	00	//	///	0|	/	0
8	00	00	00	000	00	/	0|	/
10	/	0	0	/	00/	000	0//	00|

Fig.6a.

SIMPLIFIED BREEDING PATTERN OF CROSS 177.13 / 177.11 DERIVED FROM FIGURE 6.

	2	8	4	5	7	10	3	6
2	0|	/	0	0	/	/	0	0
8	0	0|	0	0	/	/	0	0
4	0	/	0|	0	/	0	/	/
5	0	/	0	0|	/	/	/	/
7	/	/	0	/	0|	0	/	/
10	/	/	0	/	0	0|	0	/
3	/	/	/	/	0	0	0|	/
6	0	0	0	/	/	/	/	0|

KEY 0 = incompatible pollination.
/ = compatible pollination.

More than one symbol per square indicates the result of each repeated pollination.

Fig.7.

BREEDING PATTERN OF PROGENY OF SELF 177.12 (W).

	3	4	5	6	10	14	16	17	18	22	24
3	00|	0	/	/	000	0	0	0	/	0	00
4	0	00|	/	/	00	000	0	00	/	/	00
5	/	/	0|	0/	/	/	/	/	0	//	00
6	/00	/	000	0|	00	/	0/	/	00	/	/
10	///	//	/	//	0|	0	0	//	/	0	00
14	0	//	/	0/	0	0|	/	0	0/	0	0
16	-	0	/	//	00	0	0|	0	/	000	0
17	0	00	/	/	00	0	0	0|	/	00	00
18	/	/	0	0/	/	0/	/	/	0|	0/	//
22	00	/	00	//	0	0	//	//0	00	0|	0
24	//	0/	//0	0/	0/	0	///	0/	00	0	0|

Fig.7a.

SIMPLIFIED BREEDING PATTERN OF THE SELF 177.12 DERIVED FROM FIGURE 7.

	3	17	16	4	14	6	10	5	18	24	22
3	0|	0	0	0	0	/	0	/	/	0	0
17	0	0|	0	0	0	/	0	/	/	0	0
16	-	0	0|	0	0	/	0	/	/	0	0
4	0	0	0	0|	0	/	0	/	/	0	/
14	0	0	/	/	0|	/	0	/	/	0	0
6	/	/	/	/	/	0|	0	0	0	/	/
10	/	/	0	/	0	/	0|	/	/	0	0
5	/	/	/	/	/	/	/	0|	0	0	/
18	/	/	/	/	/	/	/	0	0|	/	/
24	/	/	/	/	0	/	/	/	0	0|	0
22	0	/	/	/	0	/	0	0	0	0	0|

KEY
0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Figs.8 % 8a.

Fig.8.

BREEDING PATTERN OF PROGENY OF CROSS 177.14 / 177.20 (C_1)

	1	2	3	8	9	12	15	17	21	24	177 .14	177 .20
1	0	//	/	0	/0/	0	//	/	0/	//	0	/
2	//	000	0	0/	00	/	0	0	0	/	/	/
3	//	0	00/	0/	0	/	00	0	00	0/	-	-
8	0	/	///	0	/	0	/	/	/	0	//	00/
9	0/	/	//	/	0	0/	-	///	0	/	-	-
12	0	//	/	0	00	0	0/	/	000	0	-	00
15	0/	0	/	/	0	//	0	0	0	/	-	-
17	//	00	0	/	00	/	0	0	0	00/	/	00
21	//	0	0/	/	0	//	0	0	0	0/	0	/
24	00	//	//	0	/	0	/	//	0/0	0	000	00
177.14	/	/	-	/	-	-	-	/	-	/	000	///
177.20	00	/	-	0	-	0	-	/	-	0	/000	00

Fig.8a. SIMPLIFIED BREEDING PATTERN OF CROSS 177.14 / 177.20 DERIVED FROM FIGURE 8.

	2	17	3	21	15	8	24	12	1	9	177 .14	177 .20	
2	0	0	0	0	0	/	/	/	/	0	/	/	
17	0	0	0	0	0	/	/	/	/	0	/	0	
3	0	0	/	0	0	/	/	/	/	0	-	-	Group 1.
21	0	0	/	0	0	/	/	/	/	0	0	/	
15	0	0	/	0	0	/	/	/	/	0	-	-	
8	/	/	/	/	/	0	0	0	0	/	/	/	
24	/	/	/	/	/	0	0	0	0	/	0	0	
12	/	/	/	0	/	0	0	0	0	0	-	0	Group 11.
1	/	/	/	/	/	0	/	0	0	/	0	/	
9	/	/	/	0	-	/	/	/	/	0	-	-	Group 111
177.14	/	/	-	-	-	/	/	-	/	-	0	/	Parents.
177.20	/	/	-	-	-	0	0	0	0	-	/	0	

KEY 0 = incompatible pollination.

/ = compatible pollination.

- = pollination not made.

More than one symbol per square indicates the result of repeated pollinations.

Figs.9 & 9a.

Fig.9.

BREEDING PATTERN OF PROGENY OF CROSS 50.13 / 50.7

	1	2	9	13	14	19	20	21	23	24	50.7
1	0|	0	/	0	/	0/	/	0	0	/	-
2	0	0|	0/	0	//	/0//	/	0	0/	0/	0
9	/	//	0|	0/	0	//	//	0	/	0/	/
13	0	0	00	00|	0	00	//	0	/	0/	/
14	0/	0/	00	0	0|	/	00	0000	/	/	/
19	000	//	00	0/	/	0|	0/	0/	/	/	/
20	0/	/	00	00	0000	0/	0000|	0	0/	0/	-
21	0	0/	0	00	0	0/	0/	00|	/	/	0/
23	0	//	/	/	0/	/	//	/	0/|	0	0
24	/	//	0/	//	/	/	0/	/	0	0|	///0
50.7	-	-	-	-	0	-	/	0	-	/	-|

Fig.9a. **SIMPLIFIED BREEDING PATTERN OF CROSS 50.13 / 50.7 DERIVED FROM FIGURE 9.**

	1	2	9	14	21	13	20	19	23	24	50.7
1	0|	0	/	/	0	0	/	/	0	/	-
2	0	0|	/	/	0	0	/	/	/	/	0
9	/	/	0|	0	0	/	/	/	/	/	/
14	/	/	0	0|	0	0	0	/	/	/	/
21	0	/	0	0	0|	0	/	/	/	/	/
13	0	0	0	0	0	0|	/	0	/	/	/
20	/	/	0	0	0	0	0|	/	/	/	-
19	0	/	0	/	/	/	/	0|	/	/	/
23	0	/	/	/	/	/	/	/	0|	0	0
24	/	/	/	/	/	/	/	/	0	0|	/
50.7	-	-	-	0	0	-	/	-	-	/	-|

KEY

0 = incompatible pollination.

/ = compatible pollination.

- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig.10.

BREEDING PATTERN OF PROGENY OF CROSS 50.7 / 50.13.

	3	6	8	12	13	14	15	18	20	24	50.7
3	00|	0//	-	0	0	0	0	0	0	00	0
6	0/	0/|	/	/	0/	/	//	//	//	0	/
8	0	/	0|	00	0	00	0	0	0	00	00
12	//	/	//	00|	0	0	//	00	0	0	/
13	0	0/	00	00	0|	0	0	0	00	0	0
14	0	/	0	0	0	0|	0	00	0	0	0
15	0	0/	00	00	0	0	0|	0	00	00	0
18	0	/	0	0	0	0	0	0|	0	00	0
20	/	/	/	0	00	0/	//	0	0|	0	/
24	//	0	///	00	0	/	//	//	0	0|	00/
50.7	0	0	/	-	-	-	-	-	-	-	-|

Fig.10a.

SIMPLIFIED BREEDING PATTERN OF CROSS 50.7 / 50.13 DERIVED FROM FIGURE 10.

	3	15	8	13	14	18	12	20	24	6	50.7	
3	0|	0	-	0	0	0	0	0	0	/	0	Group 1.
15	0	0|	0	0	0	0	0	0	0	/	0	
8	0	0	0|	0	0	0	0	0	0	/	0	
13	0	0	0	0|	0	0	0	0	0	/	0	
14	0	0	0	0	0|	0	0	0	0	/	0	
18	0	0	0	0	0	0|	0	0	0	/	0	
12	/	/	/	0	0	0	0|	0	0	/	/	Group 11
20	/	/	/	0	/	0	0	0|	0	/	/	
24	/	/	/	0	/	/	0	0	0|	0	/	
6	/	/	/	/	/	/	/	/	0	0|	/	Group 111
50.7	0	-	/	-	-	-	-	-	-	0	-|	Parent.

KEY
0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the results of each repeated pollination.

Fig.10b.

BREEDING PATTERN OF PROGENY OF CROSS 50.7 / 50.13

DERIVED FROM FIGURE 10a.

	3	15	8	12	20	18	14	24	13	6	50.7	
3	0	0	-	0	0	0	0	0	0	/	0	Group 1.
15	0	0	0	0	0	0	0	0	0	/	0	
8	0	0	0	0	0	0	0	0	0	/	0	
12	/	/	/	0	0	0	0	0	0	/	/	Group 11
20	/	/	/	0	0	0	/	0	0	/	/	
18	0	0	0	0	0	0	0	0	0	/	0	Group 111
14	0	0	0	0	0	0	0	0	0	/	0	
24	/	/	/	0	0	/	/	0	0	0	/	Group 1V
13	0	0	0	0	0	0	0	0	0	/	0	Group V
6	/	/	/	/	/	/	/	0	/	0	/	Group V1
50.7	0	-	/	-	-	-	-	-	-	0	-	Parent

KEY

0 = incompatible pollination.

/ = compatible pollination.

- = pollination not made.

Fig.11

BREEDING PATTERN OF PROGENY OF SELF 50.13 (L).

	3	8	9	12	16	18	21	22
3	0|	0	0	/	00	/	0	0
8	0	0|	0	0	//	0	0	0
9	0	0	0|	/	0	/00	0	0
12	/	0	/	0|	/	0	/	00/
16	0	0/	0	/	0|	/	0	0
18	/	0	//0	0	/	0|	///	00/
21	0	//	00	/	0	0000	0|	0
22	0	0	0	//0	0	/	0	0|

Fig.11a. SIMPLIFIED BREEDING PATTERN OF PROGENY OF SELF 50.13

DERIVED FROM FIGURE 11.

	3	9	22	12	18	8	21	16	
3	0|	0	0	/	/	0	0	0	
9	0	0|	0	/	/	0	0	0	Group 1
22	0	0	0|	/	/	0	0	0	
12	/	/	/	0|	0	0	/	/	
18	/	/	/	0	0|	0	/	/	Group 11
8	0	0	0	0	0	0|	/	/	Group 111
21	0	0	0	/	0	/	0|	0	
16	0	0	0	/	/	/	0	0|	Group 1V

KEY 0 = incompatible pollination, / = compatible pollination.
More than one symbol per square indicates the result of
each repeated pollination.

Fig.12.

BREEDING PATTERN OF PROGENY OF SELF 50.7.

	1	3	4	5	7	8	10	11	13	14	16	17	19	20	22	23	50.7
1	0	0/	/	0//	0	/	00	/	0	/	0	0	/	/	0	000	/
3	/	0	///	//	/	-	-	-	-	-	-	-	-	-	-	-	-
4	/	/	0	/	/	0	/	/	/	0	/	/	0/	0/	0	0/	/
5	/	/	/	0	//0	00	00	/	/	/	0/	/	0	/	/	00	/
7	00	/	/	/	0	0	/	/	0	0/	0	0	/	/	/	/	/
8	/	0	/	0/	/	0	/	/	//	//	/	/	-	/	/	-	/
10	/	000	-	/	0	0	0	0	/	/	/	/	0	/	0	0	0
11	/	/	00	/	/	/	0	0	/	/	/	//	/	/	0	-	-
13	0	//	/	/	0	0	/	/	0	-	00	00	0	/	/	0/	0
14	0/	//	0	/	0/	0	-	0/	0	0	00	-	-	0	0	0	/
16	0	/	//	/	/	/	/	/	0/	/	0	/	0	0/	/	0	/
17	0	0/	/	/	0	/	0	/	0	/	0	00	/	/	-	-	/
19	/	/	/	0	/	0	0	/	0	0	0	0	0	00	/	/	/
20	/	00	0	/	/	/	0	0/	0/	/	/	0	/	0	00	/	0
22	0	0/	0	/	/	/	00	0	/	/	/	0	//	/	0	/	0
23	/	0	/	/	/	00	/	/	/	-	-	-	00	-	/	00	-
50.7	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

KEY

0 = incompatible pollination.

/ = compatible pollination.

- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig 12a.

SIMPLIFIED BREEDING PATTERN OF SELF 50.7 DERIVED FROM FIGURE 12.

	1	17	13	7	16	19	10	5	8	23	22	4	14	20	3	11
1	0|	0	0	0	0	/	0	/	/	0	0	/	/	/	/	/
17	0	0|	0	0	0	/	0	/	/	-	-	/	/	/	/	/
13	0	0	0|	0	0	0	/	/	0	/	/	/	-	/	/	/
7	0	0	0	0|	0	/	/	/	0	/	/	/	/	/	/	/
16	0	/	/	/	0|	0	/	/	/	0	/	/	/	/	/	/
19	/	0	0	/	0	0|	0	0	0	/	/	/	0	0	/	/
10	/	/	/	0	/	0	0|	/	0	0	0	-	/	/	0	0
5	/	/	/	/	/	0	0	0|	0	0	/	/	/	/	/	/
8	/	/	/	/	/	-	/	/	0|	-	/	/	/	/	0	/
23	/	-	/	/	-	0	/	/	0	0|	/	/	-	-	0	/
22	0	0	/	/	/	/	0	/	/	/	0|	0	/	/	/	0
4	/	/	/	/	/	/	/	/	0	/	0	0|	0	/	/	/
14	/	-	0	/	0	-	-	/	0	0	0	0	0|	0	/	/
20	/	0	/	/	/	/	0	/	/	/	0	0	/	0|	0	/
3	/	-	-	/	-	-	-	/	-	-	-	/	-	-	0|	-
11	/	/	/	/	/	/	0	/	/	-	0	0	/	/	/	0|

KEY

0 = incompatible pollination.

/ = compatible pollination.

- = pollination not made.

Fig.13. BREEDING PATTERN OF PROGENY OF SELF 1.7.

	1	2	3	4	5	6	7	1.7
1	0|	/	0/	/	0/	/	/	-
2	/	0|	/	0	/	00	/	-
3	0	/	0|	0/	/	/	//	0/
4	/	0	0/	0|	/	0	/	0000
5	/	0//	/	/	0|	/	//	/
6	/	0	/	0	/	0|	0	-
7	//	/	//	/	/	0	0|	//
1.7	-	0	/	00	//	-	//	-|

Fig.13a. SIMPLIFIED BREEDING PATTERN OF SELF 1.7 DERIVED FROM FIGURE 13.

	2	4	1.7	5	1	3	7	6	
2	0|	0	-	/	/	/	/	0	
4	0	0|	0	/	/	/	/	0	Group 1
1.7	0	0	-|	/	-	/	/	-	
5	/	/	/	0|	/	/	/	/	Group 11
1	/	/	-	/	0|	/	/	/	
3	/	/	/	/	0	0|	/	/	Group 111
7	/	/	/	/	/	/	0|	0	Group 1V
6	0	0	-	/	/	/	0	0|	Group V

Fig.14. BREEDING PATTERN OF PROGENY OF SELF 1.4.

	10	13	16	1.4
10	00|	00	00	00
13	//00	/0000|	/00	0
16	00	00	00|	00
1.4	00	0	00	-|

KEY
0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig.15 **BREEDING PATTERN OF PROGENY OF SELF 181.14.**

	1	2	3	4	6	7	10	11	12	181 .14
1	00|	0//	//	00/	//	00	000	//0	0/	00
2	//00	/000|	/	0//	//	//	00	00	//	0
3	//	//	00|	//	00	//	0//	//	0/	0//
4	0//	///	00/	00|	00	/0/	00/	//0	00	0
6	//	//	0///	00	00|	00	//	//	00	//
7	00	//	//	//0	//0	00|	00	000	000	0
10	//	00	///	//	0///	0/	00|	00	//0	0
11	//	00	//	//	//0	//0	00	00|	//	0
12	//	//	///	00	00	///0	//	//	00|	//
181.14	-	-	-	-	-	-	-	-	/	0|

Fig.15a. **SIMPLIFIED BREEDING PATTERN OF SELF 181.14 DERIVED FROM FIGURE 15.**

	2	11	10	181 .14	6	12	1	7	4	3
2	0|	0	0	0	/	/	/	/	/	/
11	0	0|	0	0	/	/	/	/	/	/
10	0	0	0|	0	/	/	/	/	/	/
181.14	-	-	-	0|	-	/	-	-	-	-
6	/	/	/	/	0|	0	/	0	0	/
12	/	/	/	/	0	0|	/	/	0	/
1	/	/	0	0	/	/	0|	0	/	/
7	/	0	0	0	/	0	0	0|	/	/
4	/	/	/	0	0	0	/	/	0|	/
3	/	/	/	/	0	/	/	/	/	0|

KEY
0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig.16.

BREEDING BEHAVIOUR OF TETRAPLOID PROGENY OF CROSS

177.14 / 177.20 (C_1).

					C_1				
		T	177 .14	177 .20	-2	-8	-12	-17	-21
tetraploid		0|	0	/	/	-	-	/	0
parents	177.14	0	0|	/					
	177.20	0	/	0|					
diploid progeny	C_1- 2	0							
	C_1- 8	/							
	C_1-12	0							
	C_1-17	0							
	C_1-21	-							

Fig.17

BREEDING BEHAVIOUR OF TETRAPLOID PROGENY OF CROSS

179.14 / 179.15 (C_2).

						C_2					
		T15	T27	179 .14	179 .15	-3	-10	-12	-15	-16	-18
tetraploid	T15	0|	/	/	/	/	-	-	/	0	0
	T27	0	/|	/	/	0	-	/	/	/	/
parents	179.14	0	/	0|	/						
	179.15	0	/	/	0|						
diploid progeny	C_2- 3	0	/								
	C_2-10	0	/								
	C_2-12	0	/								
	C_2-15	/	/								
	C_2-16	0	/								
	C_2-18	0	/								

KEY 0 = incompatible pollination, / = compatible pollination,

- = pollination not made.

Fig.18. **BREEDING BEHAVIOUR OF TETRAPLOID PROGENY OF CROSS**

180.15 / 180.16(C_3).

				180	C_3						
		T1	T4	.15	-1	-3	-9	-10	-12	-13	-14
tetraploid	T1	0|	0	/	0	/	-	-	-	-	0
	T4	0	0|	/	0	0	-	-	-	-	0
parent.180.15		/	0	0|							
diploid progeny	C_3- 1	0	/								
	C_3- 3	0	0								
	C_3- 9	0	0								
	C_3-10	/	0								
	C_3-12	0	0								
	C_3-13	0	0								
	C_3-14	/	/								

Fig.19. **BREEDING BEHAVIOUR OF TETRAPLOID PROGENY OF CROSS**

180.17 / 180.13 (C_4).

			180	C_4				
		T40	.17	-1	-3	-5	-14	-18
tetraploid T40		0|	/	/	/	/	-	/
parent 180.17		/	0|					
diploid progeny	C_4- 1	0						
	C_4- 3	-						
	C_4- 5	-						
	C_4-14	0						
	C_4-18	/						

KEY 0 = incompatible pollination, / = compatible pollination,

- = pollination not made.

Fig. 20. THEORETICAL CROSSING TABLE DERIVED FROM SELF $S_{1.2}Z_{3.4}$

All genes and alleles are equal, pollen behaviour is gametophytic, and incompatibility is present when one or more alleles of the pollen are matched in the style. Self-incompatibility is overcome in the parents only. Table 10, section g.

Parents $S_{1.2}Z_{3.4} \times S_{1.2}Z_{3.4}$

Gametes: $(S_1Z_3),(S_1Z_4),(S_2Z_3),(S_2Z_4) \times (S_1Z_3),(S_1Z_4),(S_2Z_3),(S_2Z_4)$

Progeny:

$S_{1.1}Z_{3.3}$ = A $S_{1.1}Z_{3.4}$ = B $S_{1.2}Z_{3.3}$ = C $S_{1.2}Z_{3.4}$ = D
$S_{1.1}Z_{3.4}$ = B $S_{1.1}Z_{4.4}$ = E $S_{1.2}Z_{3.4}$ = D $S_{1.2}Z_{4.4}$ = F
$S_{1.2}Z_{3.3}$ = C $S_{1.2}Z_{3.4}$ = D $S_{2.2}Z_{3.3}$ = G $S_{2.2}Z_{3.4}$ = H
$S_{1.2}Z_{3.4}$ = D $S_{1.2}Z_{4.4}$ = F $S_{2.2}Z_{3.4}$ = H $S_{2.2}Z_{4.4}$ = J

Intercrossing the nine different progeny A - J inclusive.

	A	B	C	D	E	F	G	H	J
A	0|	0	0	/	0	/	0	/	/
B	0	0|	0	0	0	0	0	0	0
C	0	0	0|	0	0	0	0	0	0
D	0	0	0	0|	0	0	0	0	0
E	0	0	/	/	0|	0	/	/	0
F	0	0	0	0	0	0|	0	0	0
G	0	/	0	/	/	/	0|	0	0
H	0	0	0	0	0	0	0	0|	0
J	/	/	/	/	0	0	0	0	0|

KEY 0 = incompatible pollination,
/ = compatible pollination.

Fig.21. TO ILLUSTRATE THE WAY IN WHICH PROGENY DERIVED FROM A RECIPROCAL CROSS MAY HAVE A DIFFERENT NUMBER OF BREEDING GROUPS.

Example taken from Table 10, sections d_4 and d_5.

d_4 Gene relationships. S_1 independent,
S_2S_3 epistatic to Z_6Z_7 in pollen
S_2 epistatic to Z_6Z_7 in style

Parents $S_{1.2}Z_{5.6} \times S_{3.4}Z_{6.7}$

Compatible male gametes: (S_3Z_6), (S_3Z_7), (S_4Z_6), (S_4Z_7)

Progeny

$S_{1.3}Z_{5.6}$ = A	$S_{1.3}Z_{5.7}$ = E	$S_{1.4}Z_{5.6}$ = J	$S_{1.4}Z_{5.7}$ = N
$S_{1.3}Z_{6.6}$ = B	$S_{1.3}Z_{6.7}$ = F	$S_{1.4}Z_{6.6}$ = K	$S_{1.4}Z_{6.7}$ = O
$S_{2.3}Z_{5.6}$ = C	$S_{2.3}Z_{5.7}$ = G	$S_{2.4}Z_{5.6}$ = L	$S_{2.4}Z_{5.7}$ = P
$S_{2.3}Z_{6.6}$ = D	$S_{2.3}Z_{6.7}$ = H	$S_{2.4}Z_{6.6}$ = M	$S_{2.4}Z_{6.7}$ = Q

Intercrossing the progeny A - Q inclusive.

	A	B	C	D	G	H	E	F	J	K	L	M	P	Q	N	O	
A	0	0	/	/	/	/	0	0	0	0	/	/	/	/	/	/	Gp.1
B	0	0	/	/	/	/	0	0	/	0	/	/	/	/	/	/	Gp.2
C	/	/	0	0	0	0	/	/	/	/	/	/	/	/	/	/	Gp.3
D	/	/	0	0	0	0	/	/	/	/	/	/	/	/	/	/	
G	/	/	0	0	0	0	/	/	/	/	/	/	/	/	/	/	
H	/	/	0	0	0	0	/	/	/	/	/	/	/	/	/	/	
E	0	0	/	/	/	/	0	0	/	/	/	/	/	/	0	/	Gp.4
F	0	0	/	/	/	/	0	0	/	0	/	/	/	/	/	0	Gp.5
J	/	/	/	/	/	/	/	/	0	0	/	/	/	/	0	0	Gp.6
K	/	/	/	/	/	/	/	/	0	0	/	/	/	/	0	0	Gp.7
L	/	/	/	/	/	/	/	/	/	/	0	0	0	0	/	/	Gp.8
M	/	/	/	/	/	/	/	/	/	/	0	0	0	0	/	/	
P	/	/	/	/	/	/	/	/	/	/	0	0	0	0	/	/	
Q	/	/	/	/	/	/	/	/	/	/	0	0	0	0	/	/	
N	/	/	/	/	/	/	/	/	0	0	/	/	/	/	0	0	Gp.9
O	/	/	/	/	/	/	/	/	0	0	/	/	/	/	0	0	Gp.10

KEY 0 = incompatible pollination,
/ = compatible pollination.

Fig.22. <u>TO ILLUSTRATE THE WAY IN WHICH PROGENY DERIVED FROM A RECIPROCAL CROSS MAY HAVE A DIFFERENT NUMBER OF BREEDING GROUPS.</u>

Example taken from Table 10, sections d_4 and d_5.

$\underline{d_5}$ Gene relationships as d_4, see figure 21.

Parents $S_{3.4}Z_{6.7} \times S_{1.2}Z_{5.6}$

Compatible male gametes: (S_1Z_5),(S_2Z_5),(S_2Z_6).

Progeny

$S_{1.3}Z_{5.6}$ = A	$S_{1.4}Z_{5.7}$ = D	$S_{2.4}Z_{5.6}$ = G	$S_{2.3}Z_{6.7}$ = K
$S_{1.3}Z_{5.7}$ = B	$S_{2.3}Z_{5.6}$ = E	$S_{2.4}Z_{5.7}$ = H	$S_{2.4}Z_{6.6}$ = L
$S_{1.4}Z_{5.6}$ = C	$S_{2.3}Z_{5.7}$ = F	$S_{2.3}Z_{6.6}$ = J	$S_{2.4}Z_{6.7}$ = M

Intercrossing these progeny A - M inclusive.

	A	B	C	D	E	F	J	K	G	H	L	M	
A	0|	0	0	/	/	/	/	/	/	/	/	/	Gp.1
B	0	0|	/	0	/	/	/	/	/	/	/	/	Gp.2
C	/	/	0|	0	/	/	/	/	/	/	/	/	Gp.3
D	/	/	0	0|	/	/	/	/	/	/	/	/	Gp.4
E	/	/	/	/	0|	0	0	0	/	/	/	/	Gp.5
F	/	/	/	/	0	0|	0	0	/	/	/	/	
J	/	/	/	/	0	0	0|	0	/	/	/	/	
K	/	/	/	/	0	0	0	0|	/	/	/	/	
G	/	/	/	/	/	/	/	/	0|	0	0	0	Gp.6
H	/	/	/	/	/	/	/	/	0	0|	0	0	
L	/	/	/	/	/	/	/	/	0	0	0|	0	
M	/	/	/	/	/	/	/	/	0	0	0	0|	

KEY 0 = incompatible pollination,

/ = compatible pollination.

Fig.23. THEORETICAL CROSSING TABLE DERIVED FROM SELF $S_{1.2}Z_{3.4}$

All genes and alleles are equal, pollen behaviour is gametophytic, and incompatibility is present when both alleles of the pollen are matched in the style. Self-incompatibility is overcome in the parents only. Table 11, section a.

Parents $S_{1.2}Z_{3.4} \times S_{1.2}Z_{3.4}$

Gametes: $(S_1Z_3),(S_1Z_4),(S_2Z_3),(S_2Z_4) \times (S_1Z_3),(S_1Z_4),(S_2Z_3),(S_2Z_4)$

Progeny:

$S_{1.1}Z_{3.3}$ = A $S_{1.1}Z_{3.4}$ = B $S_{1.2}Z_{3.3}$ = C $S_{1.2}Z_{3.4}$ = D
$S_{1.1}Z_{3.4}$ = B $S_{1.1}Z_{4.4}$ = E $S_{1.2}Z_{3.4}$ = D $S_{1.2}Z_{4.4}$ = F
$S_{1.2}Z_{3.3}$ = C $S_{1.2}Z_{3.4}$ = D $S_{2.2}Z_{3.3}$ = G $S_{2.2}Z_{3.4}$ = H
$S_{1.2}Z_{3.4}$ = D $S_{1.2}Z_{4.4}$ = F $S_{2.2}Z_{3.4}$ = H $S_{2.2}Z_{4.4}$ = J

Intercrossing the nine different progeny A - J inclusive.

	A	B	C	D	E	F	G	H	J
A	0|	/	/	/	/	/	/	/	/
B	0	0|	/	/	0	/	/	/	/
C	0	/	0|	/	/	/	0	/	/
D	0	0	0	0|	0	0	0	0	0
E	/	/	/	/	0|	/	/	/	/
F	/	/	/	/	0	0|	/	/	0
G	/	/	/	/	/	/	0|	/	/
H	/	/	/	/	/	/	0	0|	0
J	/	/	/	/	/	/	/	/	0|

KEY 0 = incompatible pollination,

/ = compatible pollination.

Fig.24. TO SHOW HOW RECIPROCAL DIFFERENCES ARISE WHEN INTERCROSSING PLANTS OF A FAMILY. See Table 10, sections d_2 and d_3.

d_2 Parents $S_{1.2}Z_{5.6} \times S_{3.4}Z_{6.7}$.

Gene relationship. S_1 independent, S_2S_3 epistatic to Z_5Z_7 pollen
S_2 epistatic to Z_5Z_7 in style.

Compatible male gametes: (S_3Z_7), (S_4Z_7).
Progeny

$S_{1.3}Z_{5.7}$ = A $S_{2.3}Z_{5.7}$ = C $S_{1.4}Z_{5.7}$ = E $S_{2.4}Z_{5.7}$ = G
$S_{1.3}Z_{6.7}$ = B $S_{2.3}Z_{6.7}$ = D $S_{1.4}Z_{6.7}$ = F $S_{2.4}Z_{6.7}$ = H

	A	B	C	D	E	F	G	H	
A	0|	0	/	/	0	/	/	/	Gp. 1
B	0	0|	/	/	/	0	/	/	Gp. 2
C	/	/	0|	0	/	/	/	/	Gp. 3
D	/	/	0	0|	/	/	/	/	
E	/	/	/	/	0|	0	/	/	Gp. 4
F	/	/	/	/	0	0|	/	/	Gp. 5
G	/	/	/	/	/	/	0|	0	Gp. 6
H	/	/	/	/	/	/	0	0|	

d_3 reciprocal of d_2. Compatible male gametes: (S_1Z_5), (S_2Z_5), (S_2Z_7).

Progeny

$S_{1.3}Z_{5.6}$ = A $S_{1.4}Z_{5.7}$ = D $S_{2.4}Z_{5.6}$ = G $S_{2.3}Z_{7.7}$ = K
$S_{1.3}Z_{5.7}$ = B $S_{2.3}Z_{5.6}$ = E $S_{2.4}Z_{5.7}$ = H $S_{2.4}Z_{6.7}$ = L
$S_{1.4}Z_{5.6}$ = C $S_{2.3}Z_{5.7}$ = F $S_{2.3}Z_{6.7}$ = J $S_{2.4}Z_{7.7}$ = M

	A	B	C	D	E	F	J	K	G	H	L	M	
A	0|	0	0	/	/	/	/	/	/	/	/	/	Gp.1
B	0	0|	/	0	/	/	/	/	/	/	/	/	Gp.2
C	/	/	0|	0	/	/	/	/	/	/	/	/	Gp.3
D	/	/	0	0|	/	/	/	/	/	/	/	/	Gp.4
E	/	/	/	/	0|	0	0	0	/	/	/	/	Gp.5
F	/	/	/	/	0	0|	0	0	/	/	/	/	
J	/	/	/	/	0	0	0|	0	/	/	/	/	
K	/	/	/	/	0	0	0	0|	/	/	/	/	
G	/	/	/	/	/	/	/	/	0|	0	0	0	Gp.6
H	/	/	/	/	/	/	/	/	0	0|	0	0	
L	/	/	/	/	/	/	/	/	0	0	0|	0	
M	/	/	/	/	/	/	/	/	0	0	0	0|	

KEY / = compatible pollination, 0 = incompatible pollination.

Fig.25. BREEDING PATTERN PRODUCED BY INTERCROSSING PLANTS OF CAMPANULA THRYSOIDEA (166.1).

	1	2	3	4	5	6	7	8	9
1	00|	/	//	//	0/	00//	///	00	//
2	/	00|	//	//	/	//	//	//	//
3	//	//	00|	//	0/	//	//	//	//
4	0//	//	//	-|	//	/	/	/	/
5	//	00	//	//	00|	/	///	0/	0///
6	//	//	00	//	///	00|	//	//	//
7	/	//	0/	/	//0	/	00|	//	/
8	//	//	//	//	//	00	//	00|	///
9	//	//	//	//	-	/	///	//	00|

KEY

0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig.26. BREEDING PATTERN PRODUCED BY INTERCROSSING PLANTS OF CAMPANULA BARBATA (106.1).

	1	3	4	5	7	8	9	10	11
1	000|	/	/	-	0	-	0	/	-
3	0	00|	00	00	/	/	00	000	0
4	/	000	0000|	000	/	00	000	0	0
5	/	00	000	000|	0	00	/00	0	00
7	0	/	/	0	00|	-	/	0	-
8	-	0/	00	00	-	00|	00	00	0
9	00	00	00	000	/	-	000|	0	-
10	/	000	000	000	0	00	000	00|	00
11	-	-	-	-	-	-	-	-	00|

Fig.26a. SIMPLIFIED BREEDING PATTERN OF C.BARBATA (106.1) DERIVED FROM FIGURE 21

	3	4	9	10	5	11	8	1	7
3	0|	0	0	0	0	0	/	0	/
4	0	0|	0	0	0	0	0	/	/
9	0	0	0|	0	0	-	-	0	/
10	0	0	0	0|	0	0	0	/	0
5	0	0	/	0	0|	0	0	/	0
11	-	-	-	-	-	0|	-	-	-
8	/	0	0	0	0	0	0|	-	-
1	/	/	0	/	-	-	-	0|	0
7	/	/	/	0	0	-	-	0	0|

KEY 0 = incompatible pollination,
/ = compatible pollination,
- = pollination not made,

More than one symbol per square indicates the result of each repeated pollination.

Figs.27 & 27a.

Fig.27.

BREEDING PATTERN PRODUCED BY INTERCROSSING PLANTS OF CAMPANULA MEDIUM (137.1)

	1	2	3	4	5	6	7	8	9
1	00|	/	0	0	0	/	0	0	/
2	0	0|	/	/	-	-	-	-	-
3	0	/	0|	0	0	/	0	/	/
4	0	0	0	0|	0	0	/	0	0
5	/	/	0	/	0|	/	0	0	0
6	/	/	/	/	/	0|	0	/	/
7	0	0	0	0	0	0	0|	0	0
8	-	-	-	-	-	-	-	0|	-
9	/	0	0	0	0	/	0	0	0|

Fig.27a.

SIMPLIFIED BREEDING PATTERN OF C.MEDIUM DERIVED FROM FIGURE 22.

	4	7	1	3	9	5	6	2	8
4	0|	/	0	0	0	0	0	0	0
7	0	0|	0	0	0	0	0	0	0
1	0	0	0|	0	/	0	/	/	0
3	0	0	0	0|	/	0	/	/	/
9	0	0	/	0	0|	0	/	0	0
5	/	0	/	0	0	0|	/	/	0
6	/	0	/	/	/	/	0|	/	/
2	/	-	0	/	-	-	-	0|	-
8	-	-	-	-	-	-	-	-	0|

KEY 0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig.28.

BREEDING PATTERN PRODUCED BY INTERCROSSING PLANTS OF CAMPANULA LANATA (143.1).

	1	2	3	4	5	6	7	8	9	10
1	00|	0	-	/	/	0	0	0	00	0
2	0	00|	/	0	/	/	0/	/	0	0
3	0	/	/|	/	0	-	00	0	0	0
4	/	/	0	00|	0	0	0	0	/	/
5	0	/	0	0	0|	0	0	0	0	0
6	/	/	0	0	/	00|	/	0	/	0
7	0	0	0	0	0	0	0|	0	/	/
8	/	0	0	-	-	-	-	0|	-	-
9	0	0	0	-	0	//	/	0	0|	0
10	0	0	0	0	0	0	0	0	0	//|

Fig.28a.

SIMPLIFIED BREEDING PATTERN OF C.LANATA DERIVED FROM FIGURE 23.

	4	5	7	10	6	1	3	9	2	8
4	0|	0	0	/	0	/	0	/	/	0
5	0	0|	0	0	0	0	0	0	/	0
7	0	0	0|	/	0	0	0	/	0	0
10	0	0	0	/|	0	0	0	0	0	0
6	0	/	/	0	0|	/	0	/	/	0
1	/	/	0	0	0	0|	-	0	0	0
3	/	0	0	0	-	0	/|	0	/	0
9	-	0	/	0	/	0	0	0|	0	0
2	0	/	/	0	/	0	/	0	0|	/
8	-	-	-	-	-	/	0	-	0	0|

KEY

0 = incompatible pollination.

/ = compatible pollination.

- = pollination not made.

More than one symbol per square indicates the result of each repeated pollination.

Fig.29.

Fig.29.

BREEDING PATTERN PRODUCED BY INTERCROSSING PLANTS OF CAMPANULA GROSSEKII (190.2).

	1	2	3	4	5	6	7	8	9	10
1	00|	/	0	/	0	/	/	/	0	/
2	/	00|	0	0	/	/	0	0	0	0
3	0	/	00|	0	/	/	0	0	0	/
4	0	/	0	00|	0	/	0	0	0	0
5	/	0	/	0	0|	0	0	0	0	/
6	/	0	/	/	0	00|	0	0	0	/
7	/	/	/	/	/	/	00|	0	/	/
8	/	0	/	0	0	/	0	00|	0	0
9	0	0	0	0	/	/	/	/	000|	0
10	0	0	/	0	/	0	0	0	0	00|

KEY

0 = incompatible pollination,

/ = compatible pollination.

More than one symbol per square indicates the result of each repeated pollination.

Fig.30.

BREEDING PATTERN OF PROGENY OF CAMPANULA PATULA (141.1-14 /-12).

	0	43	80	114	8	
62	0	/	/	/	/	Group 1.
115	0	/	/	/	/	
10	0	/	/	/	/	
28	0	/	/	/	-	
92	0	/	/	/	-	
52	0	/	/	/	-	
75	0	/	/	-	-	
16	0	/	/	-	-	
63	0	0	/	/	/	Group 11
43	0	0	/	/	/	
67	0	0	0	/	/	Group 111
6	0	0	0	/	/	
61	0	/	0	/	/	Group 1V
80	0	/	0	/	/	
34	0	/	0	/	/	
103	0	/	0	/	/	
69	0	/	0	/	/	
40	0	/	0	/	-	
105	0	/	0	-	-	
113	0	/	0	-	-	
51	0	/	0	0	/	Group V
114	0	/	/	0	/	Group V1
8	0	/	/	/	0	Group V11
7	0	0	0	-	-	Group V111
21	/	/	/	/	0	Group 1X
23	/	/	/	-	-	
37	/	/	/	/	-	

KEY 0 = incompatible pollination.

/ = compatible pollination.

- = pollination not made.

Fig.31.

BREEDING PATTERN OF PROGENY OF CAMPANULA PATULA

(141.1-14 / -12)

	θ	43	80	㉕	114	8	㉙	
115	0	/	/	/	/	/	/	Gp.1
10	0	/	/	/	/	/	/	
62	0	/	/	/	/	/	-	
92	0	/	/	/	/	-	-	
52	0	/	/	/	/	-	-	
75	0	/	/	/	-	-	-	
28	0	/	/	/	-	-	-	
63	0	0	/	/	/	/	/	Gp.2
43	0	0	/	/	/	/	/	
67	0	0	0	/	/	/	-	Gp.3
6	0	0	0	/	/	/	-	
61	0	/	0	0	/	/	0	Gp.4
80	0	/	0	-	/	/	0	
34	0	/	0	0	/	/	/	Gp.5
103	0	/	0	0	/	/	-	
40	0	/	0	0	-	-	-	
105	0	/	0	0	-	-	-	
113	0	/	0	0	-	-	-	
51	0	/	0	/	0	/	0	Gp.6
69	0	/	0	/	/	/	0	Gp.7
16	0	/	0	/	-	-	-	
7	0	0	0	0	-	-	-	Gp.8
114	0	/	/	/	0	/	-	Gp.9
8	0	/	/	/	/	0	-	Gp.10

KEY
0 = incompatible pollination.
/ = compatible pollination.
- = pollination not made.

Fig.32. BREEDING PATTERN OF PROGENY OF CAMPANULA ROTUNDIFOLIA

CROSS 157.4-1 / 157.2-2.

	1	2	3	6	11	15	16	17	18	20	23
1	00|	////	00	/	//	0/	//	//	0/	//	////000
2	///00	00|	0/	0/	//	0	//	//	/	0	///0
3	//	00	/|	//	//	//	/0	/0	/0	//0	-
6	0Q	//	//	000|	/0//	//	////	//	//	0///	00
11	//0	///0	///	//	⑬/|	0///	//	00	///	//	0///
15	/	/	/	//	/	000|	/	///	/	/	/
16	//	//	///	00//	//	000	0|	///0	//	00	///0
17	///	//	//	//	0///	//	0	00|	00	00	///0
18	///	00	/00	//	0	//	00	00	00|	///0	00
20	////	//	//	//	///	00	//	0///	///	⑦/4|	//
23	///	000/	0/	//	///	0//	00	00	00	00	0|

Fig.32a. SIMPLIFIED BREEDING PATTERN OF C.ROTUNDIFOLIA (157.4-1/157.2-2)

DERIVED FROM FIGURE 32.

	15	6	1	⑪	2	③	⑳	16	17	23	18
15	0|	/	/	/	/	/	/	/	/	/	/
6	/	0|	0	/	/	/	/	/	/	0	/
1	/	/	0|	/	/	0	/	/	/	/	/
⑪	/	/	/	0|	/	/	/	/	0	/	/
2	0	/	/	/	0|	/	0	/	/	/	/
③	/	/	/	/	0	/|	/	/	/	-	/
⑳	0	/	/	/	/	/	/|	/	/	/	/
16	0	/	/	/	/	/	0	0|	/	/	/
17	/	/	/	/	/	/	0	0	0|	/	0
23	/	/	/	/	/	/	0	0	0	0|	0
18	/	/	/	0	0	/	/	0	0	0	0|

KEY. 0 = incompatible pollination, Ⓝ= number of times pollination failed.
/ = compatible pollination, /N= number of successful pollinations.
- = pollination not made. Ringed numbers indicate those plants which occasionally showed a low self-fertility.

SUMMARY

1. Incompatibility has been studied in the genus *Campanula*, by detailed investigation of the breeding behaviour of *C.persicifolia*, and by a survey of the extent of incompatibility within the genus.
2. Material of *C.persicifolia* was grown in an insect-proofed greenhouse, and incompatibility was measured both by seed set and by pollen tube growth.
3. Preliminary experiments in *C.persicifolia* showed that compatible and incompatible pollen could be distinguished in the pistil 48 hours after pollination, when compatible pollen tubes were at the style base.
4. *C.persicifolia* is self-incompatible, though a few plants were self-fertile under some undetermined conditions. Inhibition of incompatible pollen took place either in the stigma, or more rarely at the tip of the style.
5. When incompatibility was measured by pollen tube growth, pistils were removed 48 hours after pollination and were stored in a sub-zero refrigerator.
6. Pollen tubes were stained for scoring with an azo-dye, chlorazol paper brown.
7. *C.persicifolia* has binucleate pollen grains when mature.
8. No end-of-season effects or special pollination techniques were discovered to overcome self-incompatibility.
9. Intercrossing progeny of known parentage showed that the number of breeding groups obtained was larger than the number which could be explained on a one gene incompatibility system.

10. Crosses between the female parent and progeny were both compatible and incompatible, while results of those between the male parent and progeny could be identified with one of the groups formed within the progeny.
11. Reciprocal differences between crosses within the progeny were common.
12. Artificially produced tetraploids retained both cross and self-incompatibility.
13. In reciprocal crosses between tetraploids and related diploid progeny, both compatible and incompatible crosses were found.
14. The behaviour of the tetraploids with their parents was variable, but compatible crosses were present in both directions.
15. Material for a survey of the presence of incompatibility in other species of *Campanula* was grown in the garden. Incompatibility was measured by pollen tube growth, pollinations being made in the laboratory on cut flowers.
16. Out of 55 species considered 52 were self-incompatible.
17. Intercrossing plants of a species gave results in which cross-incompatible pollinations appeared to be at random. A statistical investigation of this, comparing the results of repeated pollinations and reciprocal pollinations, showed this to be spurious. It was concluded that there were insufficient incompatibility alleles in common in the plants considered for incompatibility to be apparent.

18. Investigations on a true family of C.*patula* (141.1 -14 / -12) showed the presence of 10 breeding groups. The breeding pattern obtained was compared to known two gene incompatibility systems.

19. Investigations on a true family of C.*rotundifolia* revealed low fertility in some plants on selfing. In one plant this might be considered to be an end-of-season effect. Eleven breeding groups were obtained within the progeny of the cross 157.4 -1 / 157.2 -2. This pattern was compared with two gene incompatibility systems.

20. The breeding patterns obtained in C.*persicifolia* were compared to other known incompatibility systems, and to theoretical two gene systems.

21. The complexity of incompatibility systems in general is discussed.

REFERENCES.

Allen, G.S. 1942. Parthenocarpy, parthenogenesis and self-sterility of Douglas Fir. J.For. 40; 642-644.

Anderson, E. & Sax, K. 1934. A cytological analysis of self-sterility in Tradescantia. Bot.Gaz. 95; 609-621.

Anderson, F. & de Winton, D. 1931. The genetic analysis of an unusual relationship between self-sterility and self-fertility in Nicotiana. Ann.Mo.bot.Gdn. 18; 97-116.

Archibald, J.F. & Pritchard, A.J. 1960. Coffee - Studies in physiology and breeding. Paper read at the Specialist Committee in Agricultural Botany, 7th meeting, Arusha, East Africa.

Atwood, S.S. 1944. Colchicine-induced polyploids in white clover. J.Amer.Soc.Agron. 36; 173-174.

Barlow, N. 1923. Inheritance of the three forms in trimorphic plants. J.Genet. 13; 133-146.

Bateman, A.J. 1943. Specific differences in Petunia. II. Pollen growth. J.Genet. 45; 236-242.

Bateman, A.J. 1952 a. Self-incompatibility systems in angiosperms. Heredity 6; 285-310.

Bateman, A.J. 1952 b. Trimorphism and self-incompatibility in Narcissus. Nature, Lond. 170; 496-497.

Bateson, W. & Gregory, R.P. 1905. On the inheritance of heterostylism in Primula. Proc.roy.Soc.B. 76; 581-586.

Beale, G.H. 1939. Further studies of pollen tube competition in Primula sinensis. Ann.Eugen., Lond. 9; 259-268.

Beddome, R.H. 1907. An annotated list of the species of _Campanula_. J.R.hort.Soc. 32; 196-221.

Brewbaker, J.L. 1957. Pollen cytology and self-incompatibility systems in plants. J.Hered. 48; 271-277.

Buchholz, J.T. 1931. The dissection, staining and mounting of styles in the study of pollen tube distribution. Stain Tech. 6; 13-24.

Burnett, J.H. 1956. The mating systems of Fungi. 1. New Phytol. 55; 50-90.

Burnett, J.H. & Boulter, M.E. 1963. The mating systems of Fungi. II. Mating systems of Gasteromycetes, _Mycocalia_ _denudata_, _M._ _duriaeana_. New Phytol. 62; 212-236.

Candolle, A. de. 1830. Monographie des Campanulées. Paris.

Chandler, C. 1931. A method for staining pollen tubes within the pistil. Stain Tech. 6; 25-26.

Chittenden, E.J. 1951. Dictionary of Gardening, Vol.1. The Royal Horticultural Society. Oxford. The Clarendon Press.

Clapper, R.B. 1954. Chestnut breeding, techniques and results. 1. Breeding material and pollination techniques. J.Hered. 45; 107-114.

Collins, O.R. 1962. Mating types in the slime mold _Physarum_ _pusillum_ (Abstr). Amer.J.Bot. 49; 659.

Collins, O.R. 1963. Multiple alleles at the incompatibility locus in the Myxomycete _Didymium_ _iridis_. Amer.J.Bot. 50; 477-480.

Conger, A.D. & Fairchild, L.M. 1953. A quick-freeze method for making smear slides permanent. Stain Tech. 28; 281-283.

Crook, H.C. 1937. Campanula I, II, III, IV. Bull.Alp.Gd.Soc. 5; 30-44, 107-117, 230-237, 361-367.

1938. Campanula V, VI, VII, VIII. Bull.Alp.Gdn.Soc. 6; 81-87, 180-184, 275-281, 307-318.

1940-41. Campanula IX, X, XI, XII. Bull.Alp.Gdn.Soc. 10; 78-87, 136-139, 275-282, 285-290.

Cope, F.W. 1962. The mechanism of pollen incompatibility in Theobroma cacao L. Heredity 17; 157-182.

Crane, M.B. & Lewis, D. 1942. Genetical studies in Pears. III. Incompatibility and sterility. J.Genet. 43; 31-43.

Crane, M.B. & Thomas, P.T. 1939. Genetical studies in Pears. I. The origin and behaviour of a new giant form. J.Genet. 37; 287-299.

Crosby, J.L. 1954. Populations and evolution. The significance of incompatibility. VIII Int.bot.Congr.; 163-169.

Crowe, L.K. 1955. The evolution of incompatibility in species of Oenothera. Heredity 9; 293-322.

Crowe, L.K. 1963. Competition between compatible nuclei in the establishment of a dikaryon in Schizophyllum commune. Heredity 18; 525-533.

Darlington, C.D. & Gairdner, A.E. 1938. The variation system in Campanula persicifolia. J.Genet. 35; 97-128.

Darlington, C.D. & La Cour, L.F. 1950. The handling of chromosomes. London, Allen & Unwin.

Darlington, C.D. & Wylie, A.P. 1955. Chromosome atlas of flowering plants. Second edition. London, Allen & Unwin.

Darwin, C. 1864. On the sexual relations of the three forms of Lythrum salicaria. J.Linn.Soc. 8; 169-196.

Darwin, C. 1876. The effects of cross and self-fertilisation in the vegetable kingdom. London. Murray.

Day, P.P. 1960. The structure of the A mating type locus in Coprinus lagopus. Genetics 45; 641-650.

Dee, J. 1960. A mating-type system in an acellular slime mould. Nature, Lond. 185; 780-781.

Denward, T. 1963. The function of incompatibility alleles in Red Clover, Trifolium pratense L. 1. The effect of grafting upon self-fertility. Hereditas Lund. 49; 189-202.

East, E.M. 1923. Genetical aspects of self and cross-sterility. Amer.J.Bot. 10; 468-473.

East, E.M. 1927. Inheritance of heterostyly in Lythrum salicaria. Genetics 12; 393-414.

East, E.M. 1929. Self-sterility. Bibliogr. genet. 5; 331-368.

East, E.M. 1932. Further observations on Lythrum salicaria. Genetics 17; 327-334.

East, E.M. 1940. The distribution of self-sterility in the flowering plants. Proc.Amer.phil.Soc. 82; 449-518.

East, E.M. & Mangelsdorf, A.J. 1925. A new interpretation of the hereditary behaviour of self-sterile plants. Proc.nat.Acad.Sci., Wash. 11; 166-183.

Emerson, S. 1940. Growth of incompatible pollen tubes in Oenothera organensis. Bot.Gaz. 101; 890-911.

Emsweller, S.L. & Stuart, N.W. 1948. Use of growth regulating substances to overcome incompatibility in *Lilium*. Proc.Amer.Soc.hort.Sci. 51; 581-590.

Ernst, A. 1936. Heterostylie-Forschung. Versuche zur genetischen. Analyse eines organisation - und 'Anpassungs' - Merkmales. Z.indukt.Abstamm.-u VererbLehre 71; 156-230.

Fincham, J.R.S. & Day, P.R. 1963. Fungal genetics. Botanical monographs, vol.4. Oxford, Blackwell Scientific Publications.

Fisher, R.A. & Martin, V.C. 1947. Spontaneous occurrence in *Lythrum salicaria* of plants duplex for the short-styled gene. Nature, Lond. 160; 541.

Fisher, R.A. & Mather, K. 1940. Non lethality of the Mid factor in *Lythrum salicaria*. Nature, Lond. 146;521.

Fisher, R.A. & Mather, K. 1942. Polyploid inheritance in *Lythrum salicaria*. Nature, Lond. 150; 430.

Fisher, R.A. & Mather, K. 1943. Inheritance of style length in *Lythrum salicaria*. Ann.Eugen., Lond. 12; 1-23.

Fulton, I.W. 1950. Unilateral nuclear migration and the interactions of haploid mycelia in the fungus *Cyathus stercoreus*. Proc.nat.Acad.Sci., Wash. 36; 306-312.

Fyfe, C.V. 1950. Genetics of tristyly in *Oxalis valdiviensis*. Heredity 4; 365-371.

Gairdner, A.E. 1926. *C.persicifolia* and its tetraploid form 'Telham Beauty'. J.Genet. 16; 341-351.

Grun, P. & Radlow, A. 1961. Evolution of barriers to crossing of self-incompatible with self-compatible species of *Solanum*. Heredity 16; 137-143.

Gustafsson, Å. Private communication to Bateman, appended note in Bateman, A.J. 1952. Evolution of incompatibility. Heredity 6; 285-310.

Habura, E.-Ch. 1957. Parasterilität bei Sonnenblumen. Z.Pflanzenz. 37; 280-298.

Haldane, J.B.S. 1934. Some natural populations of Lythrum salicaria. J.Genet. 32; 393-397.

Hayman, D.L. 1956. The genetical control of incompatibility in Phalaris coerulescens Desf. Austr.J.sci.Res.B., 9;321-331.

Henriques, J.A. 1887. Amaryllideas de Portugal. Bol.Soc.broteria 5; 159-174.

Holden, J.H.W. & Bond, D.A. 1960. Studies on the breeding systems of the field bean, Vicia faba L. Heredity 15; 175-192.

Hughes, M.B. & Babcock, E.B. 1950. Self-incompatibility in Crepis Foetida (L) subsp. Rhoeodofolia (Bieb.) Schinz et Keller. Genetics 35; 570-588.

Kakizaki, Y. 1930. Studies on the genetics and physiology of self and cross incompatibility in the common cabbage. Jap.J.Bot. 5; 133-208.

Kimura, K. 1958. Diploidisation in the Hymenomycetes. II. Nuclear behaviour in the Buller phenomenon. Biol.J.Okayama Univ. 4; 1-59.

Knuth, P.E.O.W. 1906-1909. Handbook of flower pollination based on Hermann Müller's work "The fertilisation of flowers by insects". Translated by J.R. Ainsworth Davis. Oxford, Clarendon Press.

Kölreuter, J.G. 1761-1766. Vorlaufige Nachricht von eini gendas Geschlecht der pflanzen lectreffenden Versuchen und Beobachtungen nebst Fortsetzungen 1, 2 & 3. In Ostwald's Klassiker, Nr.41 Leipzig: Engelmann.

Lange, M. 1952. Species concept in the genus *Coprinus*, a study on the significance of inter-sterility. Dansk.bot.Ark. 14; 1-164.

Lawrence, W.J.C. 1930. Incompatibility in polyploids. Genetica 12; 268-296.

Levan, A. 1940. Framställning av tetraploid rödclöver. Sverig. Utsädesfören. Tidskr. 50; 115-124.

Lewis, D. 1943. Physiology of incompatibility in plants. Ann.Bot., Lond. 7; 115-122.

Lewis, D. 1951. Structure of the incompatibility gene. III. Types of spontaneous and induced mutation. Heredity 5; 399-414.

Lewis, D. & Crowe, L.K. 1954. Structure of the incompatibility gene. IV. Types of mutation in *Prunus avium* L. Heredity 8; 357-363.

Lewis, D. & Crowe, L.K. 1958. Unilateral interspecific incompatibility. Heredity 12; 233-254.

Lewis, D. & Modlibowska, I. 1942. Genetical studies in Pears. IV. Pollen tube growth and incompatibility. J.Genet. 43; 211-222.

Loew, E. 1895. Einführung in die Blütenbiologie auf historischer Grundlage. Berlin.

Lundquist, A. 1954. Studies on self-sterility in rye, *Secale cereale* L. Hereditas, Lund. 40; 278-294.

Lundquist, A. 1955. Genetics of self-incompatibility in *Festuca pratensis* Huds. Hereditas, Lund. 41; 518-520.

Lundquist, A. 1961. Self-incompatibility in *Festuca pratensis* Huds. Hereditas, Lund. 47; 542-562.

Lundquist, A. 1962. Self-incompatibility in diploid *Hordeum bulbosum* L. Hereditas, Lund. 48; 138-152.

Maheshwari, P. 1949. The male gametophyte of Angiosperms. Bot.Rev. 15; 1-75.

Mather, K. 1943. Specific differences in *Petunia*. 1. Incompatibility. J.Genet. 45; 215-235.

Mather, K. 1950. The genetical architecture of heterostyly in *Primula sinensis*. Evolution 4; 340-352.

Mathieson, M.J. 1952. Ascospore dimorphism and mating type in *Chromocrea spinulosa* (Fuekel) Petch n. comb. Ann.Bot., Lond.N.S. 16; 449-460.

Muenscher, W.C. 1936. Quoted Godley, E.J. 1955. Monoecy and incompatibility. Nature, Lond. 176; 1176-1177.

Nebel, B.R. 1931. Lacmoid-Martius-yellow for staining pollen-tubes in the style. Stain Tech. 6; 27-29.

Pandey, K.K. 1956. Mutations of self-incompatibility alleles in *Trifolium pratense*. Genetics 41; 353-366.

Pandey, K.K. 1957. Genetics of self-incompatibility in *Physalis ixocarpa* Brot. - a new system. Amer.J.Bot. 44; 879-887.

Pandey, K.K. 1959. Mutations of the self-incompatibility gene (S) and pseudo-compatibility in angiosperms. Lloydia 21; 222-234.

Pandey, K.K. 1960 a. Evolution of gametophytic and sporophytic systems of self-incompatibility in Angiosperms. Evolution 14; 98-115.

Pandey, K.K. 1960 b. Incompatibility in Abutilon 'Hybridum'. Amer.J.Bot. 47; 877-883.

Pandey, K.K. 1962. The theory of S-gene structure. Nature, Lond. 196; 236-238.

Papazian, A.P. 1958. The genetics of Basidiomycetes. Advanc.Genet. 9; 41-70.

Parag, Y. 1962. Mutations in the B incompatibility factor of Schizophyllum commune. Proc.nat.Acad.Sci., Wash. 48; 743-750.

Pellew, C. 1917. Types of segregation. J.Genet. 6; 317-339.

Piatnitsky, S.S. 1934. Quoted Fryxell, P.A. 1957. Mode of reproduction of higher plants. Bot.Rev. 23; 135-233.

Raper, J.R.; Baxter, M.G.; & Ellingboe, A.H. 1960. The genetic structure of the incompatibility factors in Schizophyllum commune: the A factor. Proc. National Acad. Sci. (U.S.) 46; 833-842.

Raper, J.R.; Baxter, M.G.; & Middleton, R.B. 1958. The genetic structure of the incompatibility factors in Schizophyllum commune. Proc. National Acad. Sci. (U.S.) 44; 889-900.

Renner, O. 1917. Versuche uber die genetische konstitution der Oenotheren. Z. Vererbungslehre, 18; 121-294.

Savitsky, H. 1950. A method for determining self-sterility and self-fertility in sugar beets based upon the stage of ovule development shortly after flowering. Proc. Amer. Soc.Sug. Beet Tech.; 198-201.

Sears, E.R. 1937. Cytological phenomena connected with self-sterility in the flowering plants. Genetics 22; 130-181.

Snow, R. 1963. Alcohol hydrochloric acid-carmine as a stain for chromosomes in squash preparations. Stain Tech. 38;9-13.

Stebbins, G.L. 1950. Variation and evolution of plants. London, Oxford Univ. press.

Stout, A.B. 1917. Fertility in Cichorium intybus. The sporadic appearance of self-fertile plants among the progeny of self-sterile plants. Amer.J.Bot. 4; 375-395.

Sugiura, T. 1942. Studies on the chromosome numbers in Campanulaceae. 1. Campanuloideae-Campanuleae. Cytologia, Tokyo 12; 418-434.

Swiezynski, K.M. & Day, P.R. 1960. Heterokaryon formation in Coprinus lagopus. Genet.Res., Camb. 1; 114-128.

Takemaru, T. 1961. Genetical studies on Fungi. X. The mating system in Hymenomycetes and its genetical mechanism. Biol.J. Okayamo Univ. 7; 133-211.

Terakawa, H. 1960. The incompatibility factors in Pleurotus oesteatus. Sci. papers Coll.Gen.Educ.Univ., Tokyo 10; 65-71.

Transkowsky, D.A. 1931. Zyptologische Beobachtungen über die Entwicklung der Pollenschläuche einiger Angiospermen. Planta, 12; 1-18.

Ubisch, G. von. 1925. Genetisch physiologische analyse der heterostylie. Bibliog.genet. 2; 287-340.

Verdcourt, B. 1947. Chlorazol paper brown B as a stain for plant tissue. Stain Tech. 22; 155-156.

Whitehouse, H.L.K. 1949. Multiple allelomorph heterothallism in the fungi. New Phytol. 48; 212-244.

Whitehouse, H.L.K. 1950. Multiple-allelomorph incompatibility of pollen and style in the evolution of the Angiosperms. Ann.Bot.N.S. 14; 199-216.

Wilkie, D. 1956. Incompatibility in Bracken. Heredity 10; 247-256.

Yasuda, S. 1930. Physiological researches. VIII. On the self-fertilising ability of flowers in buds of the self-incompatible plants. Bot.Mag., Tokyo 44; 678-687.

APPENDIX 1.

ORIGIN OF SEED MATERIAL OF CAMPANULA SPECIES.

* Indicates names which are doubtful.

Code number.	Name.	Source.
101.2	C.alliariaefolia Willd.	Hortus Botanicus Univ., Uppsala, Sweden.
101.3	C.alliariaefolia Willd.	Botanisches Institut und Botanische Garten, Stuttgart, Germany.
103.1	*C.alpina Jacq.	Slovacia septentrionalis, Prage, Czechoslovakia.
193.1	C.aucheri	Royal Botanic Gardens, Edinburgh, Scotland.
105.1	C.autraniana	Northern Horticultural Society, Harrogate, England.
106.1	C.barbata	Northern Horticultural Society, Harrogate, England.
106.2	C.barbata	Hortus Botanicus Univ., Uppsala, Sweden.
204.1	*C.bellidifolia	St. Andrews Univ. Bot. Gdn., Scotland.
205.1	C.betulaefolia	St. Andrews Univ. Bot. Gdn., Scotland.
108.2	C.bononiensis	Bohemia centralis, Prage, Czechoslovakia.
114.4	C.cochlearifolia.Lam.	Hortus Botanicus Univ., Uppsala, Sweden.
115.1	C.collina. M.B.	Hortus Botanicus Univ., Uppsala, Sweden.
194.1	C.elatinoides	Royal Botanic Gardens, Edinburgh, Scotland.
120.1	C.erinus	Univ. of Leeds, England.
212.1	*C.fenestrellata Feer.	Botanicki urt. Univ., Yugoslavia.

Code number.	Name.	Source.
195.1	_C.filicaulis_	Royal Botanic Gardens, Edinburgh, Scotland.
123.1	_C.garganica_ Ten.	Liverpool Univ. Bot. Gdn., England.
124.3	_C.glomerata_ L.	Slovacia septentrionalis (montes Belanske Tatry: solo calcareo), Prage, Czechoslovakia.
124.5	_C.glomerata_ L.	Dept. of Biol. Univ. Coll. of North Staffs., Keele, England.
125.1	_C.grandis_	Hortus Botanicus Univ., Uppsala, Sweden.
190.2	_C.grossekii_ Henff.	Hortus Botanicus Univ., Uppsala, Sweden.
191.1	*_C.hondoensis_ Kitam	Hortus Botanicus Univ., Uppsala, Sweden.
-	_C.isophylla_ (actual plant)	Durham Univ. Bot. Gdn., England.
213.1	*_C.istriaca_ Feer.	Botanicki urt. Univ., Yugoslavia.
128.1	_C.kemulariae_	Northern Horticultural Society, Harrogate, England.
208.1	*_C.kolenatiana_	St. Andrews Univ. Bot. Gdn., Scotland.
130.2	_C.lactiflora_	Northern Horticultural Society, Harrogate, England.
143.1	_C.lanata_	Liverpool Univ. Bot. Gdn., England.
132.6	*_C.latifolia_ L.	Univ. of Leeds, England.
197.1	_C.linifolia_	Royal Botanic Gardens, Edinburgh, Scotland.
137.1	_C.medium L._ var. _annua_.	Hortus Botanicus Univ., Uppsala, Sweden.
199.1	*_C.mollis_	Royal Botanic Gardens, Edinburgh, Scotland.

Code number.	Name.	Source.
200.1	*C.nobili-macrantha.	Royal Botanic Gardens, Edinburgh, Scotland.
192.1	*C.ochroleuca Kem.-Nath.	Hortus Botanicus Univ., Uppsala, Sweden.
141.1	C.patula L.	Hortus Botanicus Univ., Uppsala, Sweden.
177	C.persicifolia var.Telham Beauty.	Univ. coll., London, England.
178	C.persicifolia	Leicester Univ. Bot. Gdn., England.
179	C.persicifolia var. alba.	Leicester Univ.Bot.Gdn., England.
180	C.persicifolia	Royal Botanic Gardens, Edinburgh, Scotland.
181	C.persicifolia	Jardin Alpine Champix, Switzerland.
183	C.persicifolia	Univ. of Lund, Sweden.
184	C.persicifolia	Botanic Gdn., Prage, Czechoslovakia.
185	C.persicifolia	Botanicki urt. Univ., Yugoslavia.
201.1	*C.phyctidocalyx	Royal Botanic Gardens, Edinburgh, Scotland.
209.1	C.pilosa	St. Andrews Univ. Bot. Gdn., Scotland.
198.1	C.piperi	Liverpool Univ. Bot. Gdn., England.
145.1	C.portenschlagiana	Royal Botanic Gardens, Edinburgh, Scotland.
202.1	*C.pseudo-raineri	Royal Botanic Gardens, Edinburgh, Scotland.
148.1	C.punctata	Hortus Botanicus Univ., Uppsala, Sweden.
151.1	C.raddeana	Cambridge Bot. Gdn., England.
154.1	C.rapunculoides L.	Bot. Institut und Bot. Garten, Stuttgart, Germany.
155.1	C.rapunculus L.	Leicester Bot. Gdn., England.
157.1	C.rotundifolia	Lund, Scania Maglarp Stavsten coast 5 m., Sweden.
157.2	C.rotundifolia L.	Bot. Institut und Bot. Garten, Stuttgart, Germany.
157.3	C.rotundifolia	Hortus Botanicus Univ., Bergensis, Norway.

Code number.	Name.	Source.
157.4	*C.rotundifolia* (actual plant collected)	Top of Cronkley Fell, Teesdale, Co.Durham, England.
157.5	*C.rotundifolia* (actual plants collected)	Glen Coe, Argyll, Scotland.
157.6	*C.rotundifolia* (actual plant collected)	St. David's Island, Pembrokeshire, Wales.
159.1	*C.saxatilis*	St. Andrews Univ. Bot. Gdn., Scotland.
160.1	*C.scheuchzeri*	Liverpool Univ. Bot. Gdn., England.
160.2	*C.scheuchzeri*	Bot. Institut und Bot. Garten, Stuttgart, Germany.
162.1	*C.sibirica* L.	Bot. Gdn., Prage, Czechoslovakia.
163.1	*C.speciosa*	St. Andrews Univ. Bot. Gdn., Scotland.
164.1	*C.spicata* L.	Hortus Botanicus Univ., Uppsala, Sweden.
210.1	*C.spruneriana*	St. Andrews Univ. Bot. Gdn., Scotland.
166.1	*C.thrysoidea* L.	Hortus Botanicus Univ., Uppsala, Sweden.
168.1	*C.trachelium* L.	Scania Ivetofta 30 m Lund, Sweden.
203.1	**C.tridentata*	Royal Botanic Gardens, Edinburgh, Scotland.
214.1	*C.vanhoutteri* Carr.	Botanicki urt. Univ., Yugoslavia.
170.1	*C.versicolor* Andr.	Cambridge Bot. Gdn., England.
172.1	*C.waldsteiniana*	Liverpool Univ. Bot. Gdn., England.
172.2	*C.waldsteiniana* Roem.and Schult.	Liverpool Univ. Bot. Gdn., England.
Z 2	*C.* X E.K. Toogood	Northern Horticultural Society, Harrogate, England.