SPECIES CROSSES IN *ANTIRRHINUM*

II. CLEISTOGAMY IN THE DERIVATIVES OF
A. MAJUS × *A. GLUTINOSUM*

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I. THE ORIGIN OF CLEISTOGAMY

THE flowers of *Antirrhinum majus* are too well known to require description, and while those of *A. glutinosum* differ in size and in pigmentation, they are of the same general shape, structure and development. The F_1 hybrid between those species has its own typical size of flower, intermediate between those of the parents though nearer to *A. majus*, but once again the shape, structure and development is of the same general kind (see Mather, 1947, plate I).

At the bud stage in both the species and their hybrid the upper lobes of the corolla limb are folded over the lower so that the mouth of the corolla tube is completely sealed. When the flower opens both the upper and lower parts turn back, so that although the entrance to the corolla tube is still no more than a slit, visiting bees can alight on the lower lobes and force their way into the tube. The reproductive organs are protected from the weather, but bees can gain access to them and effect pollination.

Visits by bees are not essential for pollination in at least most strains of *A. majus*. Even when the insects are excluded by bags the flowers will set a crop of seed. Evidently the pollen can reach the stigma of the same flower without the intervention of any external agency. Nevertheless, when bees are allowed free access to the flowers, interchange of pollen does occur between the flowers of different plants and also, one must presume, between flowers of the same plant. In a mixed stand of approximately equal numbers of two strains of *A. majus*, distinguished by marker genes whose effect on flower colour enabled hybrids to be recognised, Mather (1947) found that natural pollination resulted in 12 per cent. inter-strain hybrids. Assuming that transference of pollen from one plant to another of the same strain was as likely as transference to a plant of opposite strain, we can estimate that on the average 24 per cent. of the seed set by each flower was from fertilisation by pollen from a different plant. The remaining 76 per cent. of the seed must have been the outcome of self-pollination within the flower, or of its genetical equivalent, the interchange of pollen between flowers of the same plant.

In *A. glutinosum* cross-pollination between plants not merely occurs, but is almost essential for seed setting. The species possesses an incompatibility mechanism of the type found for example in *Nicotiana*, sweet cherries and clover (see Lewis, 1949). The incompatibility is not quite complete ; when sufficient flowers are self-pollinated out of doors some seed is obtained, and in the greenhouse capsules have been obtained from as many as 38 per cent. of the flowers so treated. Under these special conditions, self-pollination, and with it, of course, cross-pollination between plants having the same genotype in respect of the incompatibility, can lead to some seed setting ; but under the conditions of natural pollination, when the incompatible pollen will be in competition in the style with the compatible, the proportion of seed resulting from self-pollination must be negligible.

Thus both the species *A. majus* and *A. glutinosum* show cross-pollination as a result of visits by bees ; in one of them a quarter of the seed comes from this source and virtually all of it in the other. One is a facultative and the other an obligatory cross-breeder.

Nine different inbred strains of *A. majus* have been crossed with *A. glutinosum*. During the seven years 1943 to 1949, a total of 786 plants have been raised by selfing within these strains taking all of them together. None of these plants has shown any indication of abnormality in the opening of its flowers though some abnormality of the shape of the corolla, and distortion of the anthers has been seen at times in two of the strains, and petaloidy of the anthers in a third. These abnormalities in no way resemble the cleistogamy which is described below. Of some thousands of non-inbred *A. majus* plants raised during the past nineteen years none has been observed to show any abnormality resembling this cleistogamy.

Over the same period of seven years 293 plants have been raised of normally cross-bred *A. glutinosum*, excluding inbred lines. The plants have all had normal flowers. In inbred lines some plants have tended to have abnormal corollas somewhat reminiscent of the cleistogamous type. These would, however, never be confused with the true cleistogamous type nor even with the partially cleistogamous which will be described. They are abnormal mainly in having the limb of the corolla much reduced.

The nine F_1's of the species cross have included between them 679 plants, all of which have had perfectly normal flowers. The F_2's, however, have included in their total of 1397 plants, 6 with flowers of the cleistogamous type. These 6 plants occurred in three different F_2's as shown in table 1. There is nothing to indicate that the remaining six crosses were incapable of giving cleistogamous plants in F_2. Indeed, very large families would be required to demonstrate that the nine F_2's differed in this respect. We have therefore pooled all the crosses in considering the frequency of occurrence of cleistogamy. Should differences exist between the families we shall have underestimated the frequency of cleistogamy in the families where it can occur.

Plate I

Fig. 1.—Vertical section through the full thickness of cow's skin from the withers area to illustrate its general anatomy (see text). See fig. 2 and contrast fig. 3. ×20.

Fig. 2.—A view in higher power of the preparation illustrated by fig. 1. The superficial epidermis is rather thinner and more irregular than that of ear skin (fig. 3). ×70.

Fig. 3.—Vertical section through the full thickness of the skin of the dorsum of the cow's ear. Contrast fig. 2. ×70.

Fig. 4.—Recipient : DZ 11A. Showing 4 homografts of 69 days' standing, and one control autograft, transplanted to the outer aspect of the foreleg. Although all grafts are surrounded by rings of " pigment spread," the homografts are depigmented, scabbed and inflamed (see text).

Figs. 5 and 6.—Recipient : HZ 9A. A single autograft of 153 days' standing photographed before (fig. 5) and after (fig. 6) shaving, to show that the white hairs of the operation field are unaffected by the pigment spreading from the graft centre, which is clearly seen in fig. 5.

Fig. 8.—Illustrating the hessian truss that is used to maintain a perpendicular pressure upon grafts transplanted to the withers region (cf. figs. 7 and 9).

Fig. 7.—Recipient : DZ 16B. To illustrate the now preferred operation field in the wither area : a group of 24 " third-set " pigmented homografts from DZ 16A, here shown 38 days after transplantation to an area of white skin.

Fig. 9.—Recipient : DZ 11A. Photograph taken 36 days after transplanting a second set of 12 pigmented ear skin homografts from DZ 11B. Pigment spread is in progress, but the grafts themselves are grossly inflamed and scabbed (see text).
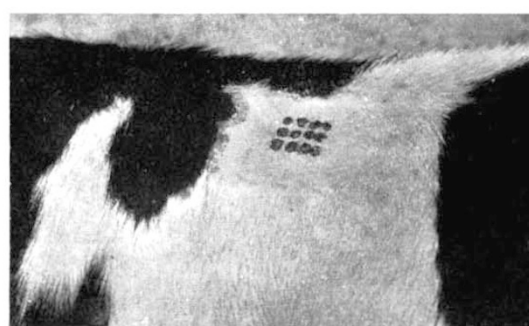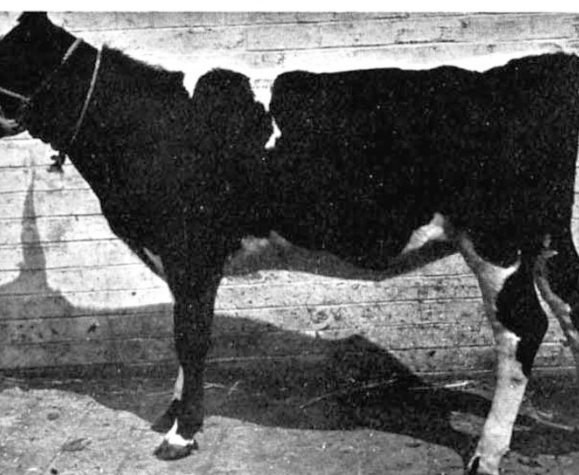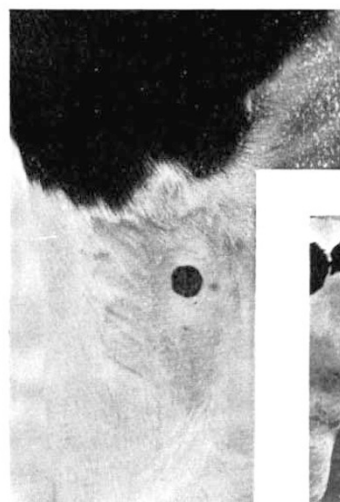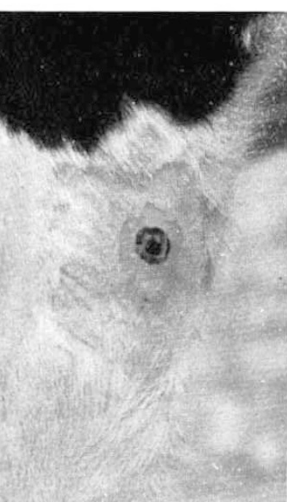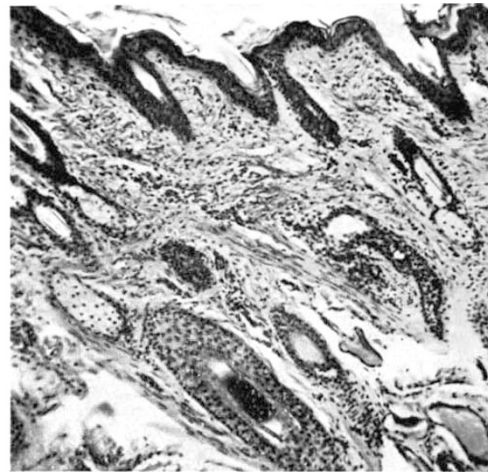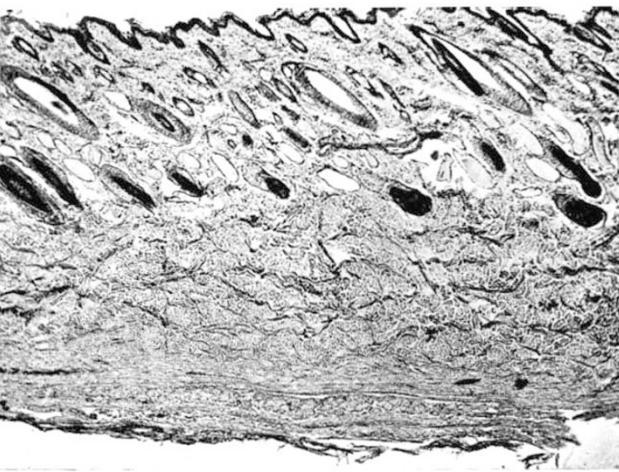
Plate II

Fig. 10.—Recipient : MZ 13B. Transverse section through a homograft 14 days after transplantation from a younger full sister, MZ 13s, showing complete necrosis of the graft, which has been riddled by round cells and now forms a dried " mushroom " scab in the process of being undermined by native epithelium. See also fig. 11. ×18.

Fig. 11.—Recipient : MZ 13s. Transverse section through a homograft 14 days after transplantation from an older full sister, MZ 13B. The homograft reaction is far advanced, but not quite complete. The dermal papillæ have been densely infiltrated with round cells and the basal layer of the epidermis is already disintegrating. See also fig. 10. ×120.

Figs. 14 and 15.—Recipients : BH 3B and BH 3A, showing autografts and reciprocally exchanged homografts of 109 days' standing.

In fig. 14 (recipient : BH 3B) the top row of grafts are autografts, showing blotchy depigmentation : compare with their appearance as homografts in the bottom row of fig. 15 (recipient : BH 3A). The bottom row of grafts in fig. 14 are 6 homografts transplanted from BH 3A ; compare with their appearance as autografts in the top row of fig. 15.

There has been complete survival of the homografts on both animals : the differences between the autografts on the one and the homografts on the other are presumably due in part to differences between the genotypes of the hosts. Natural size.

Figs. 12 and 13.—Recipient : DZ 2A. Transverse section through the edge of one of a second set of homografts 60 days after transplantation from DZ 2B, illustrating a violent reaction of delayed onset. The graft is now totally necrotic : notice the incursion of native skin epithelium on either side, and the still active round cell reaction in the dermis. Fig. 12 × 56, fig. 13 × 100.

Figs 16. and 17.—Recipient : BH 1A, showing sections through ear skin grafts of 41 days' standing. Fig. 16 shows an autograft and fig. 17 a homograft transplanted from BH 1B. The latter shows a very minor perivascular round cell reaction (see text). ×100.

Figs. 18 and 19.—Recipient : BH 1B, showing sections through ear skin grafts of 41 days' standing. Fig. 18 shows an autograft and fig. 19 a homograft transplanted from BH 1A. The two are virtually indistinguishable (see text). ×100.

All sections were stained with Ehrlich's hæmatoxylin and eosin.
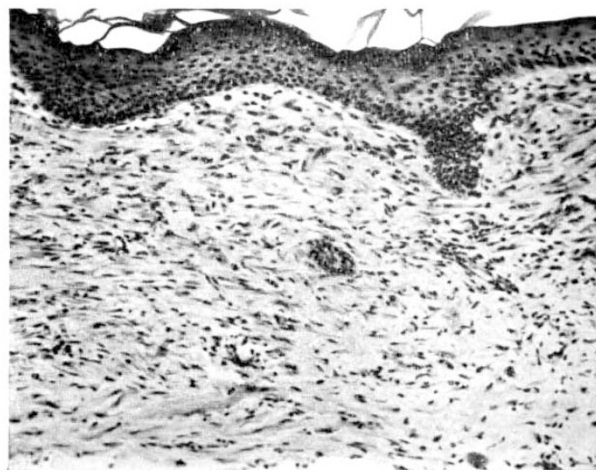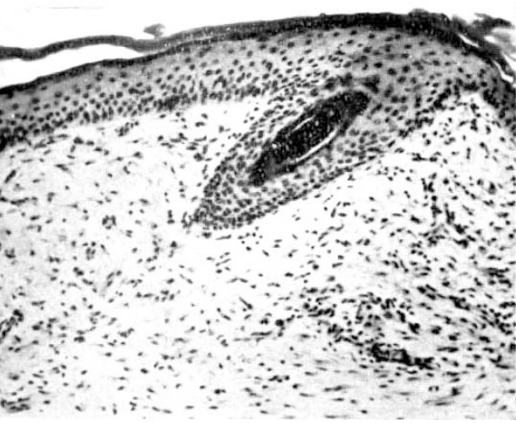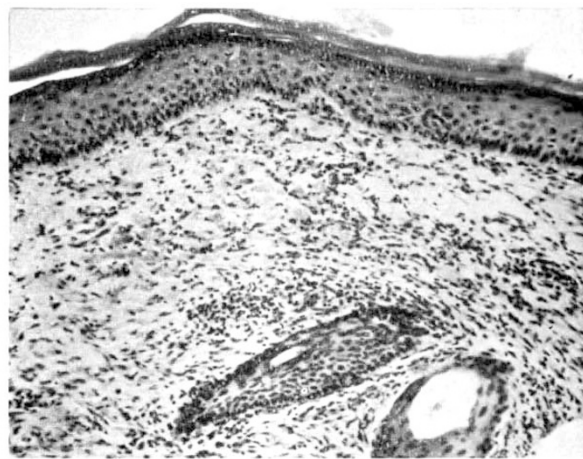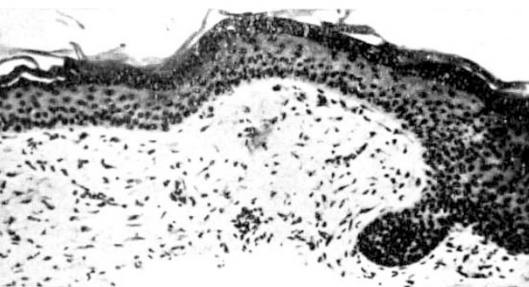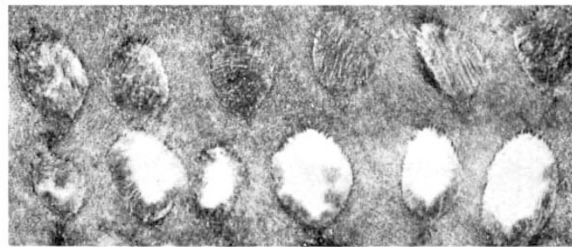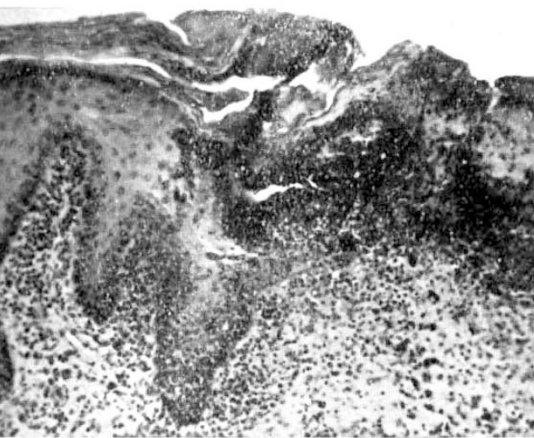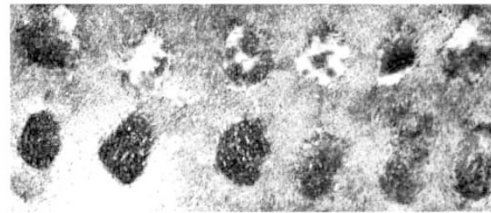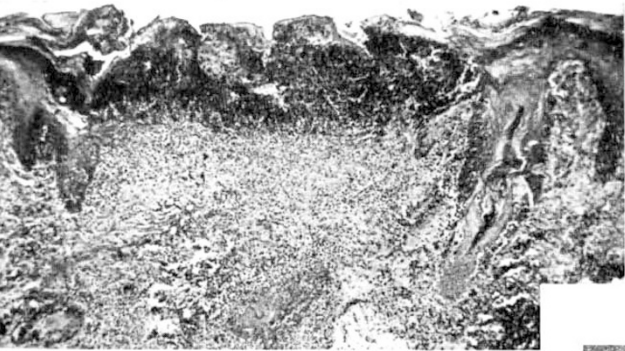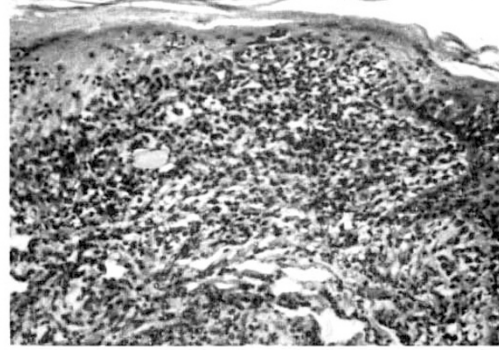
With six cleistogamous plants out of 1397, the frequency is 0·43 per cent. If cleistogamy had been as likely in the parents and F_1's as in F_2, we should expect to have found 3·38 such plants among

TABLE 1

The incidence of cleistogamy in A. majus, A. glutinosum *and their cross*
(1943-49 *inclusive*)

A. glutinosum—293 plants, all normal

	A. majus Plants all normal	F_1 with A. glutinosum Plants all normal	F_2	
			Normal	Cleistogamous
Line 1	81	34	155	0
2	80	83	222	2
3	90	81	148	0
4	81	52	115	1
5	76	87	171	0
6	94	76	170	3
7	96	72	115	0
9	80	94	137	0
10	108	100	158	0
Total . .	786	679	1391	6

the 786 individuals of *A. majus*, 1·26 among the 293 *A. glutinosum*, and 2·92 among the 679 F_1's. No such plant was in fact found. The differences in frequency of cleistogamy between parents, F_1 and F_2 are not, however, significant when the two parents and F_1 are compared separately with F_2 by contingency χ^2's using Yates' correction for continuity. If parents and F_1 are pooled, to give a total of 1758 plants none of which was cleistogamous, the difference between the pool and the F_2 is just significant $(\chi^2_{[1]} = 5·471, P = 0·02)$. It may be noted that even if the three segregating F_2's are compared, as a group, with their three parental strains of *A. majus*, or with their corresponding three F_1's, the differences in frequency are not significant.

There is thus no final statistical evidence that the frequency of cleistogamy in the species F_2 is higher than in the parental species. The difference between F_2 on the one hand and the pool of parents and F_1 on the other is admittedly significant, but the validity of such pooling may well be questioned. It should, however, be remembered that the 786 plants of the parental strains of *A. majus* are only a small fraction of the many thousands of individuals of this species which have been grown during the past nineteen years, and no plant with cleistogamous flowers has been recorded. It is therefore unlikely that this cleistogamy in the species F_2 is merely a reflection of a similar phenomenon in the parent strains of *A. majus* which has escaped detection by the hazards of sampling.

The evidence in respect of *A. glutinosum* is less good. Though some inbreds have been grown additional to the 293 normally cross-

bred plants, our experience with this species is much smaller than that with *A. majus*. The most that can be said is that although some floral abnormalities have been observed in inbred material, nothing which could properly be termed cleistogamy has been seen in this species up to the present.

2. GRADES OF CLEISTOGAMY

Fully cleistogamous flowers differ in two chief ways from those which are normal for the parent species and F_1. Both upper and lower lobes of the corolla limb are much reduced in size (a character which is shared with some inbred plants of *A. glutinosum*), and the upper lobes never turn back, so that even when fully developed the entrance to the corolla tube is closed by the limb (which has not been seen even in inbred *A. glutinosum*). Cleistogamous flowers are also often smaller in overall size than even the *A. glutinosum* parent. The calyx and the internal parts, stamens and pistil, seem, however, to be as well formed as those of the normal flower. So far as can be judged from simple inspection, the pollen of a cleistogamous flower is good. A cleistogamous flower is illustrated on the right of plate I.

Plants which bear cleistogamous flowers also differ from the normal in their foliage. Growth is very compact and bushy and the leaves are small, convex and closely set on the stems, especially near the tips. This " pin-cushion " habit, reminiscent of many rock plants, is especially noticeable during the first year's growth of the plant. If kept for a second year there is a tendency to show a more normal habit. Pin-cushion growth is shown in the lower part of plate III.

Grades exist of vegetative, as well as of floral, differences from normal. In regard to the flowers we have found it convenient to recognise five grades of abnormality. In two of these (1 and 2) the flowers can be described as near normal, their main departure being some reduction in size of the corolla limb whose edges are also curled more than usual. Grade 3 we have described as partial cleistogamy. In it the upper limb of the corolla fails to turn back properly so that the mouth of the corolla tube is partly blocked ; but the blockage is not so complete, nor presumably so effective as in the fully cleisto-gamous flower. In grade 4, which we have called near cleistogamy, the flowers of one plant may include some which are fully cleistogamous (grade 5), but will also include others which are nearer to grade 3. Some of the types of flowers and plants are shown in plates I and III, which will illustrate the classification adopted better than a verbal description.

It will be seen from the description of grade 4 that the flowers of one plant may vary amongst themselves at one time. There may also be some variation with the progress of the season, the earlier flowers tending if anything to be a little more cleistogamous than the later ones. This variation has at times led to revision of the classification

of a plant, but never by more than one grade. The classification in one year can thus be regarded as reliable to within one grade. When kept for more than one year, the second season's flowers of a cleisto-gamous plant have been noticeably less cleistogamous than the first season's.

Six grades of leaf type have been recognised. Three of these are the two parental (which of course differ) and the F_1 types (plate II and plate I in Mather, 1947). Our grade D is similar to *A. majus* but with the leaves reduced and narrower though without the dense pin-cushion type of growth. Grade E has much reduced leaves and a more nearly pin-cushion habit, while grade F is the type which characteristically can bear fully cleistogamous flowers (plate III).

There is not a complete correlation between the grades of floral and vegetative variation. Fully cleistogamous plants (grade 5) have never been seen to be other than grade F vegetatively. Partial cleistogamy and even near cleistogamy (grades 3 and 4) may occur with vegetative development of types D and E as well as F and even at times with grade C, similar to the species F_1. Near normal flowers may be associated with any type of foliage, and normal flowers have been seen on plants with all types of vegetative development except grade F. These observations are summarised in table 2. It should

TABLE 2

Flowers / Leaves	Normal	1 and 2	3	4	5
A and B	+	+	—	—	—
C	+	+	+	—	—
D	+	+	+	+	—
E	+	+	+	+	—
F	—	+	+	+	+

+ = combination observed
— = combination not observed

be noted, of course, that in the case of full cleistogamy, inspection of more plants than we have been able to raise up to the present might reveal the possibility of grade 5 flowers being associated with less extreme pin-cushion foliage. No such observation has, however, yet been made.

3. THE CONSEQUENCES OF CLEISTOGAMY

Pollination is normally effected by bees in both *A. majus* and *A. glutinosum*. The bee forces its way in through the mouth of the corolla tube and, when inside, its dorsal surface must come into contact with both anthers and stigma. Self-pollination seems inevitable from the positions of anthers and stigma, which mature at the same time ;

and, of course, cross-pollination must also occur as the bees move from the flowers of one plant to those of another. In *A. glutinosum*, incompatibility places the pollen from the same plant at a disadvantage on the stigma and the seed is preponderantly, if not entirely, from cross-pollination. In *A. majus* on the other hand, incompatibility plays no part, with the result that most of the seed is from selfing, about a quarter coming from crossing (see above). In *A. majus* selfing can, and indeed commonly does, occur without the intervention of bees, as when these are excluded by protective bags.

Full cleistogamy appears to be effective in denying bees entry into the corolla tube. Bees have been seen endeavouring to gain entry into such flowers on a number of occasions, but never successfully. Thus any seed set naturally by a fully cleistogamous flower will be by self-pollination without an insect vector. This is perhaps less regularly true for a partially cleistogamous flower, or even possibly for a near cleistogamous. Nevertheless full cleistogamy, even if not partial cleistogamy, must be regarded as a means of securing regular inbreeding.

Granted, however, that seed from fully cleistogamous flowers must be by self-pollination, two further questions arise in considering cleistogamy as an inbreeding mechanism :—(i) can such flowers pollinate themselves without outside intervention, and (ii) would such pollination be effective, or would it on the other hand be vitiated by incompatibility or sterility ? Experimental evidence is available on these points.

Tests were conducted in 1947, 1948 and 1949 of the ability of plants having cleistogamous flowers of various grades, to set seed when insects were excluded. Self-pollination was ensured by artificial means in some flowers, while others were left to pollinate themselves naturally. The tests of 1947 were not comparable with those of the later years in that observations were not made on individual flowers but were confined to recording whether whole inflorescences set one or more capsules as opposed to failing entirely to give seed. In 1948 and 1949 the numbers of flowers were observed which set capsules after treatment in the one way or the other.

In 1947 four plants, two fully and two partially cleistogamous, were covered by cages. The flowers on some inflorescences of each plant were self-pollinated by hand while those of others were left to self-pollinate themselves. No consistent difference was observed in the proportion of inflorescences setting at least one capsule following the two treatments (table 3A). A difference appeared, however, between the cleistogamous and partially cleistogamous plants. Of 18 inflorescences on the former, 7 bore at least one capsule. Of 19 inflorescences in the partially cleistogamous plants, 16 bore at least one capsule. The cleistogamous plants were less successful than the partially cleistogamous in setting seed whether artificially self-pollinated or not (Mather, 1948).

The results of the tests made on individual flowers in 1948 and 1949 are shown in table 3B.

TABLE 3A

Seed setting following artificial self-pollination (A) and undisturbed natural self-pollination (N) in cleistogamous plants guarded from insects

Type of plant	Inflorescences		Total
	A	N	
Partially cleistogamous . . .	10/11	6/8	16/19
Cleistogamous	2/8	5/10	7/18
Total . . .	12/19	11/18	

TABLE 3B

Grade of cleistogamy	3		4		5	
Type of pollination	A	N	A	N	A	N
1948	2/37	1/24	0/1	0/1	1/15	0/16
1949	1/8	1/9	0/3	0/2	0/1	...
Total . . .	3/35	2/33	0/4	0/3	1/16	0/16

The figures show (in table 3B) the number of capsules set over the number tested, and (in table 3A) the number of inflorescences setting at least one capsule over the number of inflorescences tested.

Again there is no good evidence that natural pollination is less successful than artificial. It might be said that there is a hint of such a difference, but any inadequacy of natural pollination is certainly small as a cause of reduction in seed setting when compared with the other causes of sterility so obviously at work in these plants. As we have seen, the results from the cleistogamous plants of 1947 lend no support to such a difference.

It will be noted that, as in 1947, the partially cleistogamous flowers set more capsules than the cleistogamous. Taking all pollinations on grade 3 plants, 5 capsules set out of 68 flowers, while grades 4 and 5 pooled gave 1 capsule out of 39 flowers. These results are not significant by themselves, but when combined with those of 1947 the trend seems reasonably clear.

There is thus no evidence that the mechanism of natural self-pollination is badly inadequate. The cleistogamous plants set few seeds but not on account of failure of self-pollination by natural means. Other causes must be at work and these might spring from self-incompatibility inherited from the *A. glutinosum* parent, or from

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genuine sterility due to genic unbalance. Certain crosses, made particularly in 1948, throw some light on the position. The results of these crosses are set out in table 4.

TABLE 4

Results of crosses with the cleistogamous types

Parent / Male / Female	Cleist. of grade			A. majus	A. glutinosum
	3	4	5		
Cleist. of grade {3	5/11	0/1	0/1	2/4	1/1
4	...	...	...	...	...
5	3/6		0/1	1/1	...
A. majus . .	2/3	0/1	1/1	...	...
A. glutinosum .	4/9	...	...	...	...

All crosses among cleistogamous parents 8/20.
All crosses of cleistogamous and species 11/20.
The results are given as number of capsules set over number of flowers pollinated.

There is no good indication in the table that the seed setting of the crosses with the parent species differed according to whether the species was male or female or that crosses with one parent species were more successful than those with the other. We can thus pool all the crosses of cleistogamous plants with the two parental species to find 11 successful pollinations out of 20 made. This is a markedly higher proportion of successes than that obtained when the cleisto-gamous plants were self-pollinated. Furthermore, the result of intercrossing cleistogamous plants, as shown by table 4, was 8 successes out of 20 pollinations. This proportion of successes is nearly as high as that from the species crosses, and is significantly higher than the proportion of successes, 6 out of 107, achieved by selfing the cleisto-gamous plants as shown in table 3B ($\chi^2_{[1]} = 16 \cdot 97$). Even if, in order to make a more rigorous comparison we confine our attention to the behaviour of grade 3 plants, we find 5 successes out of 11 intercross pollinations between such plants while there were only 5 successes out of 68 flowers self pollinated. This gives the highly significant $\chi^2_{[1]} = 9 \cdot 23$ after applying Yates' correction.

Most of these tests of the behaviour of cleistogamous plants were made in 1948. In that year, 8 grade 3 plants were tested for seed setting ability following artificial and natural self-pollination. Eight were also used in the tests with cross-pollination, 6 of the plants being common to the self and cross-pollination tests. There is thus no great likelihood that the difference in success of self and cross- pollination is to be traced to the individual vagaries of behaviour in the plants used. It seems that cross-pollination, whether with other cleistogamous plants or with the parent species, is more successful in setting seed than is self-pollination in the cleistogamous types.

About 50 per cent. of the crosses made were successful in that the flower set a capsule. Mather (1947) records that in an extensive test carried out during 1943 the success of crosses within and between the parental species was as shown in table 5.

TABLE 5

Percentage of successful cross-pollinations within and between the parent species (Mather, 1947)

Parent Male / Female	A. majus	A. glutinosum
A. majus . . .	93 per cent. (15)	72 per cent. (96)
A. glutinosum . . .	47 ,, (32)	35 ,, (20)

The figures in brackets after the percentages are the numbers of flowers pollinated.

The percentage success is lower in the present tests of cleistogamous flowers than in these earlier tests of *A. majus* females, whether with *A. majus* or *A. glutinosum* pollen ; but the results from *A. glutinosum* mothers are very similar to those from the cleistogamous plants. Further, *A. glutinosum*, being self-incompatible set capsules from only 4 per cent. of its flowers self-pollinated in 1947. This compares with 7 per cent. after self-pollination of grade 3 plants and 2·5 per cent. after self-pollination of grade 4 and 5 plants (table 3). The grade 3 plants are thus the equal of *A. glutinosum* in fertility whether crossed or selfed, and even the grade 5 plants are only little inferior.

We can now give an answer to the question whether the poor set of capsules following the self-pollination of cleistogamous plants is due to incompatibility or true sterility. The grade 3 plants are no less successful in capsule setting than is the parent species *A. glutinosum*. Like *A. glutinosum* the grade 3 plants set fewer capsules after selfing than after crossing and this, we presume, must be due to incompatibility inherited from this parent species. Grade 3 plants are not so successful in crosses as *A. majus*, but even if this should be taken as indicating some sterility (at least under London conditions) the degree is no greater than that shown by *A. glutinosum*.

The fully cleistogamous plants of type 5 are less successful after self-pollination than are those of grade 3, and possibly also a little less successful after crossing. Thus they show the same incompatibility as *A. glutinosum* and grade 3 and they may well also suffer the handicap of some genuine sterility.

4. THE INHERITANCE OF CLEISTOGAMY

The cleistogamous plants whose breeding behaviour was followed arose in the F_2 of the cross of *A. glutinosum* with *A. majus* strain 6.

Three such plants appeared in 1945, two being fully, or at least near cleistogamous, and the third a partial cleistogamous (it was described at the time—before the numerical grades were introduced—as half way between normal and cleistogamous). This partially cleistogamous plant was not bred. The other two fully cleistogamous plants were self pollinated. One gave 6 offspring, all cleistogamous like the parent, which were bred no further, or at least gave no seed. The other gave 8 offspring, all cleistogamous. One of these was selfed and gave three offspring, two of which failed to flower, the third being cleistogamous like its parent. On selfing, this third plant gave some seed which, however, failed to germinate when sown in 1948. All these cleistogamous plants, both the two parents and their descendants, had pin-cushion foliage.

At the same time as the two original cleistogamous plants of 1945 were self-pollinated, 7 of their normal sibs in the F_2 were also selfed. Of these 5 gave respectively 10, 10, 9, 9 and 2 normal offspring. A sixth plant gave 2 normals, 1 cleistogamous and 1 which did not flower; and the last gave 7 normals and 1 cleistogamous. None of these was bred further. These breeding results from the F_2 plants are summarised in table 6.

TABLE 6

Breeding behaviour (by self-pollination) of normal and cleistogamous plants in F_2 and F_3 of the species cross

Parent plants	Offspring		
	Normal	Cleistogamous	Not flowering
Cleistogamous	0	8	0
Cleistogamous	0	6	0
1945 { Five normal (pooled)	40	0	0
Normal .	2	1	1
Normal .	9	1	0
1946 Cleistogamous (ex 1st family above)	0	1	2

The last of the selfed cleistogamous lines, which had given nothing but cleistogamous and non-flowering plants, failed after 1947. The plants grown since that date mostly trace back to a cross made between the two original cleistogamous plants in the F_2 of 1945. This family, 79/1946, contained 10 plants, consisting of 2 normal, or nearly so, 3 partially cleistogamous and 5 fully cleistogamous. The breeding behaviour of this line is shown in table 7.

Several remarkable things appear from this table. In the first place no member of this line bred true for full cleistogamy, even though families were grown from a fully cleistogamous individual in 1947, 1948 and 1949, as well as from partially cleistogamous individuals.

Indeed there is little evidence that the fully cleistogamous parent produced families (164/47, 166/48 and 43/49) more cleistogamous

TABLE 7

Breeding behaviour in the line descended from the cross between the cleistogamous plants in the F_2 of 1945

Family	Parent			Offspring					
				N	Grade				Not flowering
	Origin	Grade	Pollination		1 and 2	3	4	5	
79/46	Original cross				2	3	5		...
164/47	79/46	Cl	S	3		7	1		...
166/48	164/47	Cl	S	...	2	11	4	...	2
43/49				...	...	1	...	...	...
163/48	164/47	P. Cl	S	...	5	1	1	1	...
164/48	164/47	P. Cl	S	...	4	14	3	2	7
49/49				...	8	2	...	...	7
152/49	163/48	2	O	13	5	...	...	...	...
78/49	,,	4	O	15	3	...	...	...	...
53/49	164/48	3	S	12	8	...	...	...	...
54/49	,,	3	S	3	5	...	...	...	4
79/49	,,	3	O	1	9	4	...	...	1
80/49	,,	3	O	19	1	...	...	...	...
81/49	,,	3	O	16	1	...	...	...	...
82/49	,,	3	O	19	...	...	...	...	...
83/49	,,	3	O	13	5	...	1	...	...
84/49	,,	2	O	14	6	...	...	...	...
85/49	,,	1	O	16	4	...	...	...	...
86/49	,,	3	O	16	4	...	...	...	...
87/49	,,	2	O	1	1	...	...	...	...
48/49	166B/48	3	S	7	3	2	...	...	1
77/49	,,	3	O	3	1	...	...	...	...

Cl = fully cleistogamous (= later grades 4 or 5)
P. Cl = partially cleistogamous (= later grade 3)
S = self pollination
O = open pollination

on the average than the partially cleistogamous parents (families 163/48, 164/48, 49/49). It does appear, however, that before 1949 the line bred true for the occurrence of cleistogamy of greater or lesser grade.

In 1949 there was less cleistogamy than in earlier years and the grade of such cleistogamy as occurred was generally lower. Many of the families grown in 1949 were admittedly from open pollination of cleistogamous plants in the previous year. This seed was used to ensure the maintenance of cleistogamy following the poor success of self-pollination in 1948. On the whole, normal offspring are

more frequent (as compared with grades 1 and 2) in these families from open pollination than in those from selfing. This is fairly attributable to the natural crossing that would presumably occur even in grade 3 plants (as these have flowers which are not completely closed at maturity) and which would be at an advantage as compared with self-pollination. Even the families from known self-pollination contained, however, a great proportion of plants with normal flowers and a low proportion of grades 3, 4 and 5. Evidently cleistogamy manifested itself less clearly in 1949 than in earlier years, a difference which we may perhaps relate to the change of location of the crop from London (where it was grown in 1948 and earlier) to Birmingham (where it was grown in 1949). The duplicate families 164/48 and 49/49 confirm this difference. The environmental effect is in fact greater than the genetical difference between the fully and partially cleistogamous parents of 1947.

The line just described was from a cross between the two cleisto-gamous plants in the F_2 of 1945. The reciprocal of this cross was allegedly made, but the seed so obtained gave, in 1946, a family of 10 plants all like *A. glutinosum*. One of these plants on selfing gave 10 offspring in 1947, again all like *A. glutinosum*. A second plant of the 1946 family was crossed with the single fully cleistogamous plant of 1946 recorded in table 6. The offspring of the cross included 7 plants with normal, 3 with partially cleistogamous and 1 with fully cleistogamous flowers. These results suggest strongly that as a result of some slip the original cross of 1945, from which all the plants described in this paragraph were descended, was not cleistogamous × cleistogamous as supposed, but cleistogamous × *A. glutinosum*.

However this may be, the single grade 5 plant of 1947 was selfed and gave in 1948 1 grade (3), 3 grade (4), 5 grade (5) and 5 not flowering. A duplicate of this family, grown in 1949 from stored seed, gave 2 grade (2), 3 grade (4), 2 grade (5) and 3 not flowering, again suggesting less cleistogamy in 1949 than in 1948. The grade (5) plant of 1947 seems on this evidence to have been breeding true to cleistogamy even if not to a particular grade. A further family from the self-pollination of a 1948 grade (4) plant which gave in 1949, 14 normal and 5 grade (1) plants. As in the other crossed line, described earlier, the semblance of breeding true for cleistogamy in 1948, vanished in 1949. The environmental effect obscures the genetical situation and makes confident interpretation impossible.

Crosses were made between cleistogamous plants and the parental species in the hope of learning something of the inheritance of cleisto-gamy. The results are summarised in table 8. It will be seen that the cross allegedly between the two cleistogamous plants of 1945, but now believed to have been of the cleistogamous mother by *A. glutinosum* has been included as of this latter kind but marked with an asterisk. With a few exceptions all the F_1's of cleistogamous by both species had normal flowers, close in type to those of the species

used in the cross. A single plant out of 80 grown in 1949 from cleistogamous × *A. majus* was of grade 1, *i.e.* just not quite normal. A further family in 1949 from cleistogamous × *A. glutinosum* was recorded as

TABLE 8

Crosses of cleistogamous (cl) *with the parental species* A. majus (m) *and* A. glutinosum (gl)

Cross	Family	Offspring					
		Grade					Not flowering
		Normal	1 and 2	3	4	5	
cl × *m*							
F₁ 〔162/48	26	...	...	...	...	...
	56/49	20	...	...	...	...	...
	57/49	20	...	...	...	...	...
	58/49	19	1	...	...	...	...
	60/49	20	...	...	...	...	...
F₂ (from 162/48)	74/49	30	1	...	...	...	...
F₁ (from 162/48) × *m*	63/49	19	...	...	...	...	...
F₁ (from 162/48) × *cl* { 64/49	14	5	...	...	...	...
	65/49	11	6	2	1	...	...
cl × *gl* (normal)							
F₁ 〔 81/46*	10	...	...	...	...	...
	165/48	30	...	...	...	...	...
	167/48	10	...	...	...	...	...
F₂ (from 81/46)	162/47*	10	...	...	...	...	...
F₁ (from 165/48) × *gl*	66/49	[19	1]	...	...	...	...
F₁ (from 167/48) × *gl*	88/49	20	...	...	...	...	...
gl × F₁ (from 167/48) { 68/49	[18	2]	...	...	...	...
	69/49	[15	5]	...	...	...	...
F₁ (from 81/46) × *cl*	163/47*	7		3	1		...
gl (inbred) × *cl*							
F₁	159/48	10	8	...	...	...	...
	62/49	[9	11]	...	...	...	...
F₂ (from 159/48) { 71/49	2	24	4	...	...	...
	72/49	6	7	3	1	...	1

* From cross in 1945 inferred to be *cl* × *gl*.

[ ] Grade 1 plants so near normal that validity of distinction doubtful.

having 9 normally flowered and 11 grade (1) flowered plants; but the two kinds were so similar that the distinction between the two classes was very difficult to draw. This family qualifies but little the general statement that the F₁'s with the species have normal flowers. The genes associated with cleistogamy are recessive to, or of low joint potence as compared with, those brought in from the parents. Indeed, only one F₁ family requires special mention, viz. 159/48. This definitely included plants with non-normal flowers. It was a cross between a cleistogamous and an inbred line of *A. glutinosum* and as

we recorded in Section 1, our inbred lines of this species departed from the type by often having a reduced corolla. It may be, therefore, that this exceptional behaviour is also reflected in the F_1 with cleistogamy, the genes coming in from the inbred line being more similar to the genes of the cleistogamous line.

In the only F_2 grown from the cross with *A. majus* 1 plant out of 31 departed slightly from the normal type of flower. There is thus little evidence of segregation since a single departure was also observed in F_1. The backcross of the F_1, from $cl \times m$ to *A. majus* gave, as might be expected, nothing but normally flowered plants in the family of 19 which was grown. The backcross of this same F_1 to cleistogamous showed segregation. Of 19 plants, 5 were not normal and 3 of these at least departed sufficiently from the normal flower type of the species for there to be no doubt that cleistogamy was manifesting itself once again. In the case of a second family from backcrossing to *cl* the evidence is even stronger. Only 11 plants of 20 had normal flowers and 3 fell into grades 3 and 4. It is worth while noting too that these families were grown in 1949 when the manifestation of cleistogamy was, in general, poor.

The later generations of the cross with *A. glutinosum* require consideration in two parts. The F_2 of the cross made in 1945, and now believed to be with *A. glutinosum*, was of 10 plants, all like the species. No segregation was apparent. Equally the four backcrosses of F_1's from later crosses between *cl* and standard *A. glutinosum* (66, 68, 69 and 88/49) gave little evidence of segregation for cleistogamy. Of 80 plants, 8 were regarded as having not quite the normal type of flower for the species, but they departed so slightly from the species type as to leave considerable doubt whether they should be regarded as a distinct class. Only one backcross to *cl* has been grown and that from the putative F_1 in 1946. This gave 7 normal and 4 partially or fully cleistogamous plants.

The second group of descendants were true F_2 families for the cross with inbred *A. glutinosum* to whose F_1 (159/48) special attention has already been drawn. This F_1 contained non-normally as well as normally flowered plants. The F_2's were from two non-normal individuals and 47 plants which flowered gave 8 in grades 3 and 4 and only 8 classified as normal. This is in clear contrast to the other descendants of the cross with *A. glutinosum* and shows, together with the F_1 data that the inbred line of *A. glutinosum* differed from the standard lines in its content of the genes related to cleistogamy. The greater proportion of cleistogamous individuals in the descendants of the inbred line suggest fewer gene differences between this line and cleistogamous than between type *A. glutinosum* and cleistogamous.

Confident interpretation of these various pieces of evidence on the inheritance of cleistogamy is made difficult by the non-heritable modification of the expression of cleistogamy. It is particularly difficult to relate the observations made in 1949 to those of earlier

years. If we neglect the 1949 results the data of tables 6 and 7 suggest strongly that the early cleistogamous lines bred true to cleistogamy of one grade or other, even if not to a particular grade. The 1949 results may be accommodated with this view if we postulate that conditions in Birmingham during 1949 did not consistently lead to the development of cleistogamy from genotypes which would consistently have given this flower form (in greater or lesser degree) had they developed under the London condition of earlier years. And we should remember that cleistogamy was, in fact, detected and selected under the earlier conditions, so that had the original species F_2's been first produced in Birmingham in 1949 we might well never have recognised the phenomenon. The lines bred true under the conditions in which cleistogamy was detected : their phenotypes failed in consistency under new conditions.

The variation of grade in the crossed line in its early years also seems to have been largely non-heritable, for such partially and fully cleistogamous plants as were tested bred alike. There would seem, however, to have been some genetical modification of grade, for the families of table 6 (in the lines descended by self-pollination from the first cleistogamous plants) contained only fully cleistogamous individuals, while those of table 7 (from the crossed line) contained more partially than fully cleistogamous plants. This difference is not to be attributed wholly to different conditions or finer classification arising with increasing familiarity with the phenomenon, since the first crossed family was grown side by side with progenies obtained by selfing its parents, the difference in grade between them being observed and recorded at that time. There must be a genetic element in the determination of grade, though no precise statement about it is possible. There is even a suggestion that the cleistogamous families segregated (in 1947 and 1948) from the probable cross of one of the first such plants with *A. glutinosum* had their own characteristic balance of grade, with more of grade 4 and 5 than the crossed line but less than the original selfed lines.

Assuming that the results of 1949 are obscured by the changed conditions and that the lines bred true to a basic genotype giving consistent cleistogamy (though of genetically modifiable grade) under the conditions of earlier years, we must next enquire as to the nature of this basic genotype, leaving aside for this purpose consideration of the grade modifiers. Here our difficulties increase because most of the segregating families from the crosses with the parental species —and it is these crosses which would tell us most—were grown in 1949.

The crosses with both the parental species show segregation in backcrosses to cleistogamous though not in backcrosses to the species. A minimum of two recessive genes must thus be involved in the primary genotype of cleistogamy, one differentiating it from each species. The backcross ratios are not wholly incompatible with this minimal postulate, though in general more normals are found in

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the backcrosses than the simple half which would be predicted on this view. The original segregation of cleistogamous individuals in the F_2 of the species cross also throws doubt on the adequacy of the two gene hypothesis for, even excluding F_2's in which no cleistogamous plants were found, the ratio of normal : cleistogamous was 507 : 6 or 84·5 : 1—a ratio hard to reconcile with the 15 : 1 expected. Even allowing for the effects of grade modifiers and the possibility that very low grade cleistogamy was overlooked in the early years, it is difficult to believe that only two primary genes are involved. Three, however, would be sufficient to account for the segregation observed.

The crosses of cleistogamous to inbred *A. glutinosum* suggest that this latter line may differ from cleistogamous in fewer genes than does normal *glutinosum*. If we are prepared to admit three primary genes, normal *A. glutinosum* might differ from cleistogamous in two (of which one might be heterozygous) and inbred *glutinosum* in one. It would be necessary to suppose that the gene by which the inbred line differed from the type *A. glutinosum* had a reducing effect on the corolla, for the inbred line itself shows this departure from type. The postulation of this effect would serve further to explain the low number of normals in the F_2 of cleistogamous $\times$ inbred *A. glutinosum*, for such a phenotype would easily be confused with low-grade cleistogamy. It would indeed, in a sense, be low-grade cleistogamy requiring further supplementary genes to enhance it.

Thus the genetical architecture of cleistogamy, though not clearly established, must be of some complexity. A number of genes must be involved, some supplementary in action and primarily concerned in giving cleistogamy as opposed to normal flower type : others perhaps modifying the grade of cleistogamy, where the primary genotype gives this departure from normality. Furthermore, none of the genes has an effect which is great as compared with the environmental and developmental differences observed in the experiments. The system involved is not a polygenic system in the strict sense, but it has some of the latter's properties, and in particular it is one which can give rise to grades of expression of the character.

5. CLEISTOGAMY AS AN INBREEDING MECHANISM IN *ANTIRRHINUM*

It is clear that seed set sexually by fully cleistogamous flowers in any species of flowering plants, must be the result of self-pollination. Cleistogamy is therefore an inbreeding device. While our lower-grade cleistogamous flowers in *Antirrhinum* may well permit the ingress of bees and so give some seed by cross-pollination, the grade 5 and even grade 4 plants must be regarded as showing this inbreeding mechanism. Thus the two parental species, one an obligatory cross-breeder and the other regularly setting a considerable fraction of its seed by cross-pollination, contain between them all the genetical

materials necessary for the production of an inbreeding type of plant, *i.e.* a type of plant with a breeding system distinct from, and even opposed to, those of its parents.

A successful change from cross-breeding to inbreeding must, however, involve more than the mere development of a device, such as cleisto-gamy, which precludes cross-pollination.   The plants must be capable of setting as good a crop of seed by inbreeding as their predecessors did by cross-pollination and these inbred offspring must not be so genetically incapacitated as to be at a disadvantage in competition with the hitherto normal cross-bred types.   Indeed, they must be at a slight advantage in the particular environment if the inbreeding mechanism is to spread and become more than a chance, or floating, novelty.

That these conditions can be fulfilled is attested by the rise of inbreeding devices that must be inferred in many species of plants—wheat, peas, chickweed and so on.   Even *A. majus* itself seems to have moved towards inbreeding as compared with its relatives such as *A. glutinosum* and *A. molle*, though it is by no means a complete inbreeder.   The extreme cleistogamous type which has arisen in our experiments is mechanically such a complete inbreeder, but it could not in its present form be regarded as representing a biologically successful move towards complete inbreeding.

The pollination mechanism of our cleistogamous plants is mechanic-ally successful, in that natural pollination gives rise to as many capsules as does deliberate selfing.   Biologically, however, it is not successful. The cleistogamous plants appear to have inherited the self-incom-patibility of their *A. glutinosum* parent, and the cross-breeding device is not overcome by the cleistogamy.   Indeed, the combination of the two results essentially is a great reduction of seed set, since one device precludes cross-breeding and the other precludes, or at least materially reduces, inbreeding.

The incompatibility mechanism is not, in itself, a final obstacle. Incompatibility mechanisms can be broken down (Mather, 1943*a*). Indeed self-compatibility does exist in the genus and, in the case of *A. majus*, may well have supervened on incompatibility.   It is, however, clear that if cleistogamy were to become a workable mechanism for regular inbreeding, its rise must be accompanied, or preceded, by the destruction of self-incompatibility.   So far as the physiological relations of pollen and style are concerned, cleistogamy could be successful in *A. majus* but not in *A. glutinosum* as at present constituted.

In addition to this need for a physiological readjustment of pollen and style coordinated with the mechanical change in pollination—a need which, though widespread, would not be encountered in all cases of movement from cross-breeding to inbreeding—there is another necessary adjustment and one which will always be concerned in such a change of breeding system.   This is the genetical readjustment necessary for the avoidance (or material reduction) of the inbreeding

depression which is a universal feature of cross-breeding species. The property of poor internal balance is one which must be shown by the combinations of virtually all the systems of genes in a cross-breeding species (see Darlington and Mather, 1949). The readjustment towards that good internal balance of the genic combinations, by which inbreeding is permitted without depression of vigour and fertility, must thus be complex and therefore slow ; and if the rise of an inbreeding mechanism should outstrip the rise of this general genetical readjustment, the mechanism will be to that extent put at a disadvantage, by the poor capacity of the offspring it produces. On the other hand, good internal balance will not be achieved without inbreeding, for only by inbreeding will the homozygous types be exposed regularly to the natural selection which will pick out the balanced combinations of genes. Only where breeding mechanisms and internal genetic balance rise together in a coordinated way can success be achieved. The high fitness, which inbreeding can give and which is the advantage of inbreeding (Mather, 1943b), requires not merely uniformity, but uniformity at a highly adjusted genetical level, and it is this which will govern the pace of the change from outbreeding to inbreeding. The changes in breeding system and balance must be kept in step and the steps must therefore be small.

A change towards inbreeding in the species of *Antirrhinum* with which we are concerned, would constitute no exception to this rule. The effects of inbreeding in *A. glutinosum* are as clear and drastic as is expected in a crossbreeder. Even in *A. majus*, where cross-breeding is neither obligatory nor predominant, inbred lines are generally less vigorous and more prone to developmental upset than normally open-bred populations, with which they could not, therefore, compete on even terms. They are not, however, so much incapacitated as in *A. glutinosum*, and this, together with the absence of incompatibility, suggests that *A. majus* may have changed and indeed be in the process of changing, towards inbreeding. Incompatibility and poor internal balance are not invariable with the genus and cannot therefore be final obstacles to successful inbreeding within it.

Thus in *Antirrhinum* as elsewhere the rise of an inbreeding mechanism would be contingent on a coincidental readjustment of the general genetic balance, as well as the genetical abolition of any initial self-incompatibility. That these changes are to be regarded as practical possibilities is attested by behaviour within as well as outside the genus. Our observations show that the genic materials exist, at least within the two species jointly, for the production of the cleistogamous type which would mechanically enforce inbreeding. Cleistogamy as we have observed it could not be a workable inbreeding mechanism, since sudden appearance has precluded its accompaniment by the necessary correlated adjustment. Its complexity of inheritance is, however, such as to leave little doubt about the possibility of it

developing slowly and with increasing efficiency, as would be required if it were to be accompanied and matched by these other adjustments.

These two species of *Antirrhinum*, obligatory and partial cross-breeders though they are respectively, have between them the latent genetical possibility of cleistogamy. In other words crossbreeders can carry, and carry in a realisable form, the latent possibility of inbreeding, just as must indeed be the case if, as has been supposed (Mather 1943*b*, Darlington and Mather, 1949) inbreeding species are constantly evolving from cross-breeding ancestors and as constantly being extinguished by their incapability, due to genetical rigidity, of accommodating themselves to changing circumstances.

6. SUMMARY

A. glutinosum is an obligatory cross-breeder and *A. majus* regularly shows some cross-breeding. In F_2's between these species plants occurred with cleistogamous flowers, such that bees cannot enter and which must therefore set seed by self-pollination. Cleistogamy has not been seen in the parental species nor in the species F_1, though some floral abnormality is found, especially in inbred lines of *A. glutinosum*.

Cleistogamy is variable in expression, some of the variation being non-genetic in origin. The more extreme forms of cleistogamy are regularly associated with an unusual type of vegetative development. Self-pollination regularly occurs in cleistogamous flowers, but is not effective in giving a good set of seed, the cleistogamous plants appearing to have inherited self-incompatibility from *A. glutinosum*. They show little, if any, more true sterility than does *A. glutinosum*.

The inheritance of cleistogamy is complex. Several genes of supplementary effect are apparently necessary for cleistogamous development of the flowers, and others modify the grade (and hence the efficacy) of the cleistogamy which is developed. With such a complexity of inheritance, a gradual change should be possible towards cleistogamy, even though in these experiments it arose suddenly. Cleistogamy is mechanically sufficient to ensure inbreeding, but a successful move towards inbreeding would require simultaneously the abolition of the genetical mechanism which determines incompatibility and also a general re-balancing of the genotype such as would remove, or almost remove, inbreeding depression. The need for this readjustment of genic balance must enforce a gradual rise of any inbreeding mechanism, with which a gradual rebalancing would keep in step. These coordinated adjustments must be regarded not only as necessary, but also as possible in *Antirrhinum*. Thus these species of *Antirrhinum*, cross-breeders though they are, would appear jointly to have already existing within them the genetic materials for the gradual development of inbreeding through cleistogamy. Cleistogamy, which is an established inbreeding mechanism in some

plant species, and indeed inbreeding devices in general, can well be possibilities already latent in the genetical constitutions of cross-breeding species.

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Plate I
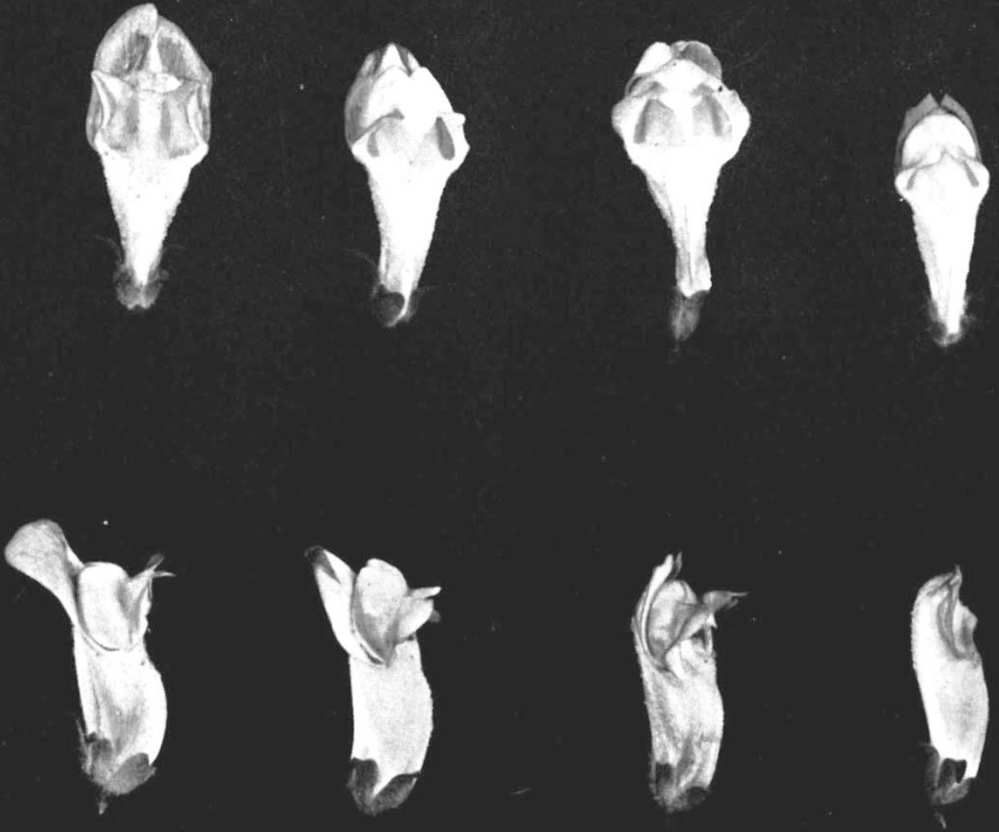
Ventral and lateral views of single flowers of types (left to right) (i) near normal (grade 1),
(ii) near normal (grade 2), (iii) partially cleistogamous (grade 3-4), (iv) cleistogamous
(grade 5). × 3/2.