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HYBRIDS OF *DIGITALIS AMBIGUA* AND *DIGITALIS PURPUREA*, THEIR FERTILITY AND CYTOLOGY.

BY B. H. BUXTON AND W. C. F. NEWTON.

(With Two Plates (one coloured) and Two Text-figures.)

THE Hybrid between *Digitalis purpurea* L. and *D. ambigua* Murr. was got by Gärtner (1849) who succeeded in making the cross reciprocally, though he only obtained one plant of *D. ambigua* × *purpurea* after repeated attempts. He found both hybrids sterile, and this was also the experience of Haase-Bessell (1916, 1921) and Hill (1925). Warren (1924) however describes a hybrid between *D. gloxiniflora* (*purpurea*) and *D.* "*lutea*" which was partially fertile. He obtained back-crosses to both parents, and one F_2 plant which died without flowering. The plant described by Warren as *D. lutea* was evidently not *lutea*, and the figures and description both indicate that *ambigua* was the species used. The name *gloxiniflora* is used horticulturally for many colour forms of *purpurea*. Warren's cross was certainly *D. purpurea* × *D. ambigua*.

There are also in existence horticulturally *Digitalis* hybrids offered as Lutz hybrids, etc., which are probably back-crossed derivatives of *purpurea* and *ambigua*.

The chromosome numbers of the species in question have been published by Haase-Bessell as 24 in each case, in agreement with the numbers obtained by her in other species of *Digitalis*. Warren however gives eight as the number of his "*lutea*" (*ambigua*) and 12 as that of *gloxiniflora*.

In the present research a number of forms of *D. purpurea* and *D. ambigua*, as well as the species *D. viridiflora*, have been examined, and the haploid number has always been found to be 28. This number has been confirmed by somatic counts, which are very easy on account of the shortness of the chromosomes.

Haase-Bessell's error in regard to these two species may perhaps be due to the occurrence of secondary pairing, which on occasional plates might give the appearance of a lower number of bivalents than 28. Undoubtedly Warren's numbers are also erroneous, as indeed might be expected from the fixatives he used, which, though suitable for chondriosome work, penetrate very slowly indeed through anther tissue and cause excessive contraction.

PART I. THE HYBRIDS AND THEIR PARENTS.

By B. H. BUXTON.

The *purpurea* plants used for the crosses were of garden origin. They were purple in colour, but it is not certain that they were homozygous for the factors affecting colour, for there was a slight variation in this respect in the F_1 flowers, though perhaps not more than could be accounted for by fluctuation around a mean. The plants were selected as typical examples of *D. purpurea*.

The *ambigua* plants were raised from seed obtained from the R.H.S. Gardens at Wisley, all of which came true to type.

The flowers used for crossing were emasculated and protected in muslin cages.

Cross 1. *D. ambigua* ♀ × *D. purpurea* ♂. No seeds germinated.

Cross 2. *D. purpurea* ♀ × *D. ambigua* ♂. Fifty seedlings raised.

Two controls. One of each species. Not pollinated. No seeds set.

The crosses were made in the summer of 1924, the seeds sown as soon as ripe, and the seedlings kept from frost until early in January, 1925, when they were planted out in the open. In the summer of 1925 the 50 hybrid plants made vigorous growth and eight of them flowered, though rather late, as was to be expected from plants barely a year old.

The first four plants to flower were pollinated artificially among themselves, until about one-half of the flowers had been so treated. At this stage the foxgloves in the garden and vicinity were all over, so the muslin was taken off, and the remaining flowers allowed to pollinate normally. Two plants did not flower till all the foxgloves were over, so they were not protected but allowed to pollinate normally.

Some of the capsules after pollination quickly turned brown and shrivelled, and were found to be completely empty. Other capsules swelled a little and produced only minute seeds, little more than dust, none of which germinated.

Several capsules on one plant, and a few on another plant, set a few well-formed seeds intermediate in size between the larger *ambigua* and the smaller *purpurea* seeds.

From these seeds about 200 F_2 plants were obtained which will be dealt with later.

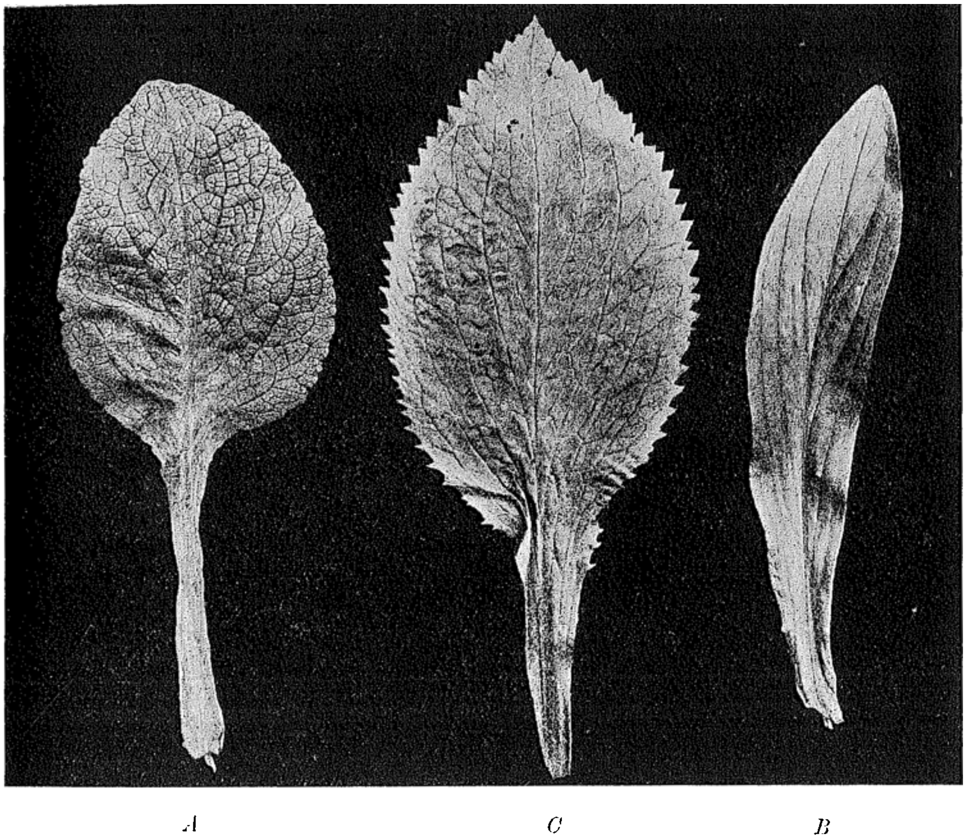
By the end of the summer of 1926, 43 F_1 plants in all had flowered, of which only two showed some degree of fertility, though a few of the others set occasional stray seeds here and there.

Remarks on the Characters of the F_1.

1. *The leaves* (see Text-fig. 1).

A simple method of comparing the leaves is to take a phyllic index on the same principle as the cephalic index of the anthropologists

$$\frac{\text{breadth} \times 100}{\text{length}} = \text{index}.$$



Text-fig. 1. Rosette leaves of (*A*) *D. purpurea*, (*B*) *D. ambigua*, and (*C*) *D. purpurea* × *D. ambigua* (× about ⅓).

Worked out in this way the leaves of the rosette give approximately on the average

Phyllic index	*D. purpurea*	41
Phyllic index	F_1 *hybrids*	28
Phyllic index	*D. ambigua*	19

In other words the leaves of *D. purpurea* are comparatively broad, those of *D. ambigua* narrow, and the F_1 intermediate. In *D. purpurea* the lamina is narrowed abruptly into a long petiole. The leaf of *D. ambigua* is sessile, broadest towards the middle and gradually attenuate towards both ends. The leaves of the hybrid are shortly stalked and intermediate in shape. The margins of the *D. purpurea* leaves are crenate, those of *D. ambigua* have distinct but fine serrations, the F_1 leaves are intermediate (cf. Text-fig. 1).

2. *The flowers.*

A. Colour. The purple colour of *D. purpurea* (Plate XIX, fig. 1) is due to an anthocyanin which dissolves out in water. The spots on the lip are due to concentration of the anthocyanin in clumps of cells, leaving the surrounding cells without any pigment, and the spots appear as deep purple on a white ground. The yellow of *D. ambigua* flowers (Plate XIX, fig. 2) is a plastid pigment, insoluble in water. There are no spots, but the lip shows a network of darker brownish pigment among the veins. The flowers of the F_1 hybrids (Plate XIX, fig. 3) are pink, the shade of pink varying slightly in different plants, but in all of them is an undertone of yellow. There are spots on the lip as in *purpurea*, but the spots are less numerous and much smaller. Sometimes there are traces of the network of *ambigua*, but not on all the flowers. On examining the spots with a pocket lens or under a low power of the microscope, they are found to be due, as in *purpurea*, to a concentration of anthocyanin in a group of cells surrounded by a zone of cells without anthocyanin, but the cells in the outer zone are yellow on account of plastid pigment; the general effect being one of pink on a yellow ground instead of pink or purple on a white ground, as in the normal *purpurea*.

A very distinctive feature in the *ambigua* flowers is the presence of two reddish brown spots on the outer surface of the upper lip near its base. In the hybrid these spots are usually clearly marked, though in many of the flowers they are absent or only traces of them can be detected. The presence of these spots may be taken as a certain indication of an *ambigua* strain, as there is no sign of them in *purpurea* flowers.

B. Shape of the flowers. The flowers of *D. purpurea* are longer than those of *ambigua*, but relatively narrower, and the hybrid is intermediate, being somewhat shorter than *purpurea*, but even broader than *ambigua*. Enough measurements have not yet been made for accuracy, but if a floral index is taken, it will be found approximately as follows:

	Length (cm.)	Breadth (cm.)	Index
Purpurea	4·75	2·00	40
Ambigua	3·00	1·80	60
F_1 Hybrids	4·25	2·40	56

The flowers of the hybrid then are somewhat shorter than those of *purpurea*, but there is a marked increase in breadth. The length is measured from the base of the corolla to the tip of the upper lip, and the breadth across the upper lip at the point where it separates from the lower.

In addition to the cross of which particulars have been given, two other crosses have been made:

D. purpurea (yellow var. Sutton's strain) × *ambigua*.

D. purpurea (white var. no spots) × *ambigua*.

Only a few plants have been raised from these crosses, and those that flowered have proved so far to be sterile, so nothing further has been done with them. The leaves (as one would expect) are intermediate and resemble the F_1 of the cross described in detail, but it is rather surprising to find that in each case the flowers have a pink tinge. A specimen from each of these two crosses is figured on Plate XIX, figs. 5 and 6.

The F_2 *Generation.*

As already mentioned about 200 seedlings were raised; approximately 110 from controlled pollination (one plant), and 90 from open pollination (two plants).

Of the seedlings raised from controlled pollination 96 reached the flowering stage. There was no apparent segregation, the leaves being intermediate and resembling those of the F_1 throughout. The flowers also resembled those of the F_1 plants, though they were distinctly larger, running to about 5 cm. in length by 3 cm. in breadth (Plate XIX, fig. 4). Of 58 which flowered in the second year after sowing, 44 (about 75 per cent.) set some well filled capsules, while the remaining 14 (about 25 per cent.) were either sterile or very nearly so.

Besides this definite F_2 generation there were the 90 seedlings raised from F_1 plants exposed to open pollination, of which 72 reached the flowering stage. Here again segregation was not evident, though about half of the plants showed some tendency to revert to the *purpurea* type of leaf, which however did not become noticeable until the second year. The flowers on the other hand showed no such reversion, and were indistinguishable from those of the controlled pollination.

With regard to fertility not a single one of these plants was found with any well filled capsules, though a number, perhaps 25–30 per cent., set a little seed in more or less shrivelled ones. In view of the chromosomal differences found between these plants and those of the definite F_2 generation (cf. p. 276) it may be assumed provisionally that they resulted from a back-cross with *D. purpurea*, although it is difficult to understand how this can have occurred.

Bumble Bees visited the F_1 flowers in large numbers, and it does not seem possible that there was any cross pollination except among the hybrids themselves, since great care was taken to cut down any belated foxglove flowers. There were certainly none within half a mile or more of the hybrids after removal of the muslin.

An F_3 generation of about 80 plants has been raised from the F_2 individuals, but as only a few have hitherto flowered a detailed description must be postponed, as must also that of certain back-crosses which have been made.

PART II. CYTOLOGY.

By W. C. F. NEWTON.

Material was fixed in modified Flemming solution:

14 c.c. 1 per cent. Chromic acid;
4 c.c. 2 per cent. Osmium tetroxide;
·5 c.c. Glacial acetic acid;
16 c.c. Water,

also in Zenker's fluid or Carnoy's fluid (2 min.) followed by modified Flemming, a method for which I am indebted to Dr Kihara.

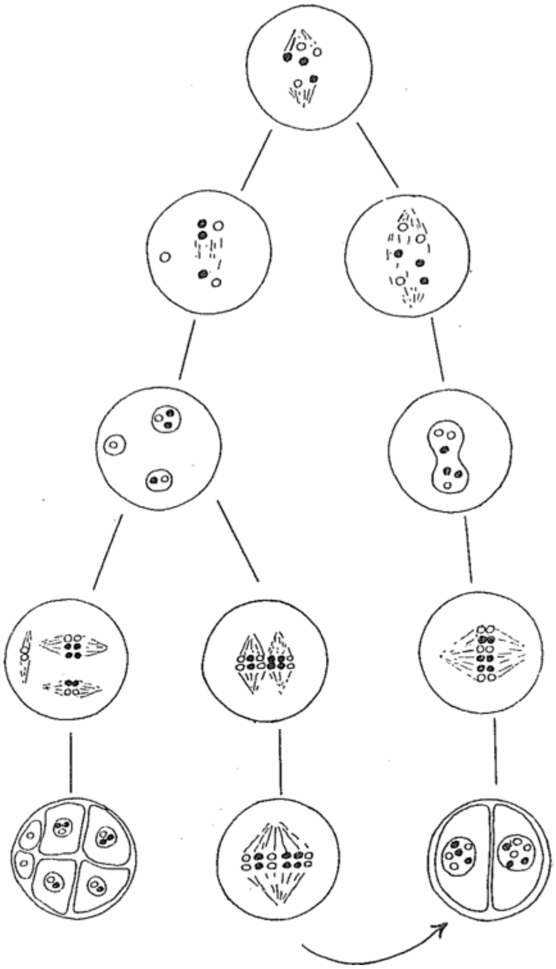
Digitalis purpurea. Root-tip counts gave 56 chromosomes showing little difference in size. The reduction divisions show 28 bivalents (Plate XX, fig. 1). Often a small number of bivalents are secondarily associated in pairs as shown by Ishikawa (1911) in *Dahlia* and more recently described in detail by Darlington (1928) in *Prunus.*

Digitalis ambigua. The somatic number was 56, the haploid number 28 (Plate XX, fig. 3). Again a certain amount of secondary pairing was to be observed.

The F_1 *Hybrid.* The somatic number was determined as 56 (Plate XX, fig. 2).

The reduction division. In diakinesis a small number of paired chromosomes is always to be seen, but the number is inconstant ranging between five and twelve. It is impossible to determine whether the members of these pairs are derived from the same or different species.

The first meiotic division is exceedingly irregular. A maximum of 11–12 bivalents divide, the remainder are distributed over the spindle in the most irregular manner (Plate XX, fig. 4). Frequently the whole of the chromosomes are included in a single "restitution" nucleus (Plate XX, figs. 5 and 6) at the end of this "division," but every stage between this and an approximately equal distribution of the chromosomes between two daughter nuclei is to be seen.



Text-fig. 2. Diagram of the production of diploid gametes in F_1 hybrid between *D. purpurea* L. and *D. ambigua* Murr.

In the former case the second division is regular and the pseudoreduction results in the formation of dyads instead of tetrads. In the latter cases there may be numerous or only two spindles formed in the second division, and the number of spores produced may be as few as two or as many as eleven. Dyads arise in two ways, (1) from failure of the first division, leading to the incorporation of the whole of the chromosomes in one interphase nucleus (Plate XX, figs. 5–7), (2) from combination of two second-division plates (Plate XX, fig. 9). The latter method is rarer, and as the union follows on two irregular divisions the chance of obtaining a spore with the full 56 chromosomes is much lessened. The univalents have not been observed to divide at the first division.

F_2 *plants and back-crosses.* The progeny of the F_1 hybrids examined included a number resulting from natural seed as well as those from controlled pollinations. Those from artificial self-fertilisation had 111–112 chromosomes, with the exception of one plant which had about 102. The naturally pollinated plants examined had, without exception, 84 chromosomes. The numbers examined were ten F_2 plants and eight from natural pollinations.

In the plants with 112 chromosomes the divisions were as a rule regular, though occasional univalents have been seen in all plants examined, and the aggregation of bivalents into groups of two, three and four is not uncommon (Plate XX, fig. 11).

In the triploid plants there are usually 28 bivalents, but slightly larger numbers, indicating the presence of autosyndesis in the complement of one of the parent species, have also been seen. The triploid plants examined, assuming them to have been back-crosses with *purpurea*, had probably two sets of *purpurea* and one of *ambigua* chromosomes. The behaviour in triploids will be described fully when reciprocal back-crosses from artificial pollination are available.

Discussion.

It is clear that the great majority of the viable spores formed by the F_1 hybrid contain the unreduced number of chromosomes, but the presence of F_2 plants with a reduced number of chromosomes, 102 in the one counted, shows that the absence of a certain number of chromosomes does not necessarily lead to non-viability of the spore. Moreover, there seems to be little doubt that these unreduced spores are formed as a result of the failure of the first division with the ultimate formation of dyads instead of tetrads, a method indicated by Federley in *Pygaera* (1913) and since by Rosenberg (1926), Karpechenko (1927) and others.

It is possible that the modification of this process involving a partial reduction followed by a union of the anaphasic groups of the second division also plays a part in the production of fertile spores, especially those with rather less than 56 chromosomes.

No syndiploid nuclei have been observed, nor any areas with doubled chromosome number. The irregular distribution of the fertile seeds over the plant is however somewhat against regarding the formation of fertile spores as purely a matter of chance. Some F_1 plants have not as yet produced any seeds, and on the fertile ones several capsules contain no seeds while others have eight, nine, or more. The seeds are not however as a rule aggregated in a mass, suggesting a strip of tetraploid somatic tissue, but are separated by infertile ovules. The hypothesis that in the egg the formation of a restitution nucleus is favoured or hindered by conditions at the time of reduction is most in agreement with the observed distribution of fertile seeds. Restitution nuclei have been found in every F_1 plant of which the pollen has been examined. There are however many more cases in which two or three chromosomes are excluded than those in which all are included in a single plate.

The absence or rarity of tetraploids in the seedlings resulting from natural pollination as well as the frequent production of triploids under these conditions by the F_2 shows that the tetraploid pollen is unable to compete with the diploid pollen, that there is in fact very marked selective fertilisation.

SUMMARY.

A number of hybrids between *Digitalis purpurea* ♀ and *D. ambigua* ♂ were obtained. Two plants among the hybrids showed a low degree of fertility. From the others no seeds were obtained.

The F_2 generation differed from the F_1 principally in size, but there was no segregation of the parental characters. About 75 per cent. were highly fertile.

The chromosome numbers of the parent species and of the F_1 hybrid are 28 (n) and 56 ($2n$) in each case. In the F_2 hybrid (artificial pollination) the numbers are 56–112, while all plants obtained from open pollination of the F_1 are triploid (84–$2n$) and sterile.

The viable spores of the F_1 result from the failure of the reduction division and the formation of restitution nuclei.

Digitalis purpurea × *ambigua* is an additional example of a constant intermediate hybrid resulting from chromosome doubling.

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EXPLANATION OF PLATES XIX AND XX.

Plate XIX.

Fig. 1. *Digitalis purpurea* L.
Fig. 2. *D. ambigua* Murr.
Fig. 3. *D. purpurea* × *ambigua* F_1.
Fig. 4. *D. purpurea* × *ambigua* F_2.

In each case beside the flower is shown the inner surface of the lower petals and the markings on the throat.

Fig. 5. *D. purpurea* (yellow) × *D. ambigua*.
Fig. 6. *D. purpurea* (white) × *D. ambigua*.

For the original drawings of this plate we are indebted to Mr A. J. Wise of the R.H.S. Wisley Gardens.

PLATE XX.

Fig. 1. *D. purpurea* L. Metaphase I; pollen mother cell, 28 bivalent chromosomes. × 3600.

Fig. 2. *D. purpurea* × *ambigua* F_1, somatic metaphase in root-tip; 56 chromosomes. × 3600.

Fig. 3. *D. ambigua* Murr. Metaphase I; pollen mother cell, 28 bivalent chromosomes. × 3600.

Fig. 4. *D. purpurea* × *ambigua* F_1. Metaphase-anaphase in pollen mother cell, showing distribution of chromosomes throughout the spindle. Fifty-four can be distinguished. × 3600.

Fig. 5. F_1. Restitution nucleus in interphase I. All the chromosomes are included in a single dumb-bell-shaped nucleus. × 2200.

Fig. 6. F_1. Restitution nucleus of same stage as Fig. 5. The nucleus is strongly lobed. × 2200.

Figs. 7 and 8. F_1. Adjacent pollen mother cells. Fig. 7 shows the single plate resulting from a single restitution nucleus; Fig. 8 three plates resulting from a distribution of the chromosomes in the first division into two large groups and a small one. × 2200.

Fig. 9. F_1. United spindles in anaphase of second division in pollen mother cell. × 3600.

Fig. 10. Tetrads and dyads in F_1 pollen. × 1400.

Fig. 11. F_2. *D. purpurea* × *ambigua*. Fifty-six bivalents; secondary association is shown by several bivalents. × 3600.

Fig. 12. F_2. Metaphase plates of second division in pollen mother cells. × 3600.

All figures drawn with an Abbé camera lucida at stage level, 1·5 mm. Zeiss apochromatic objective, N.A. 1·3.


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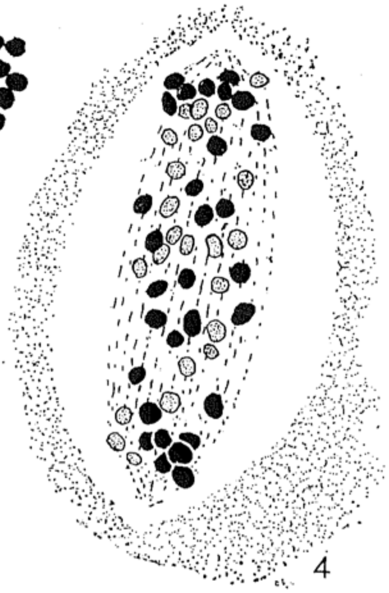
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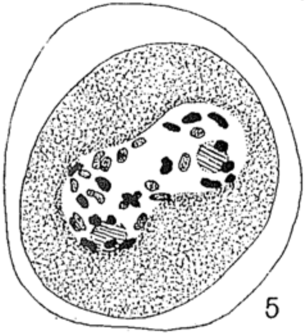
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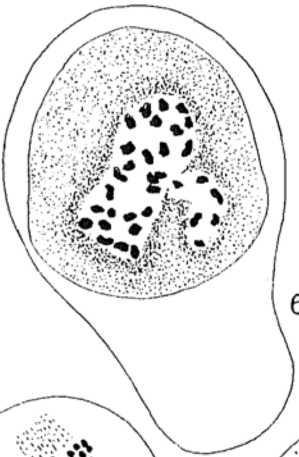
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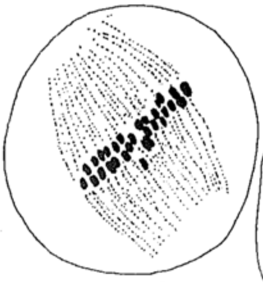
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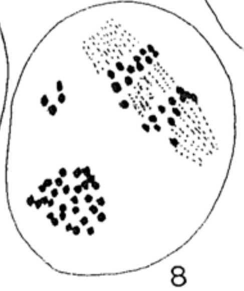
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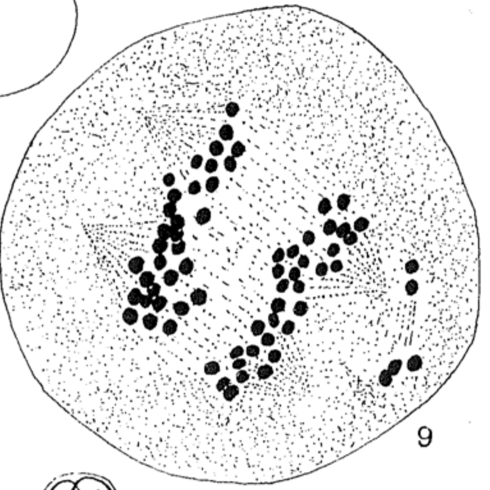
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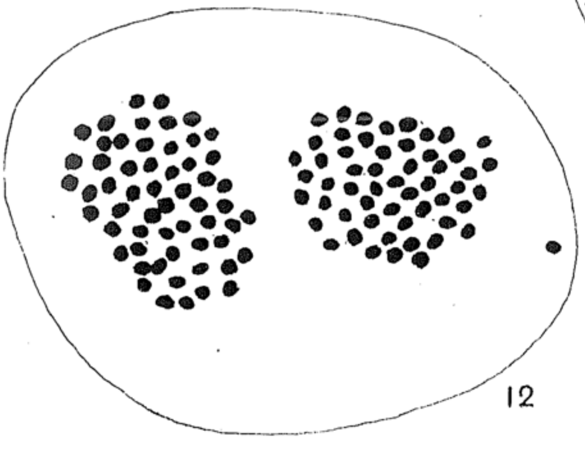
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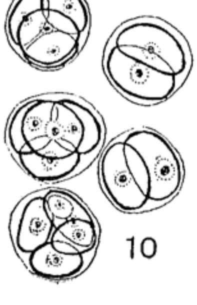
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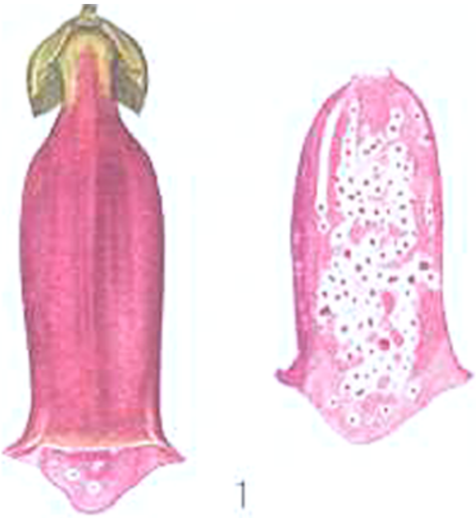
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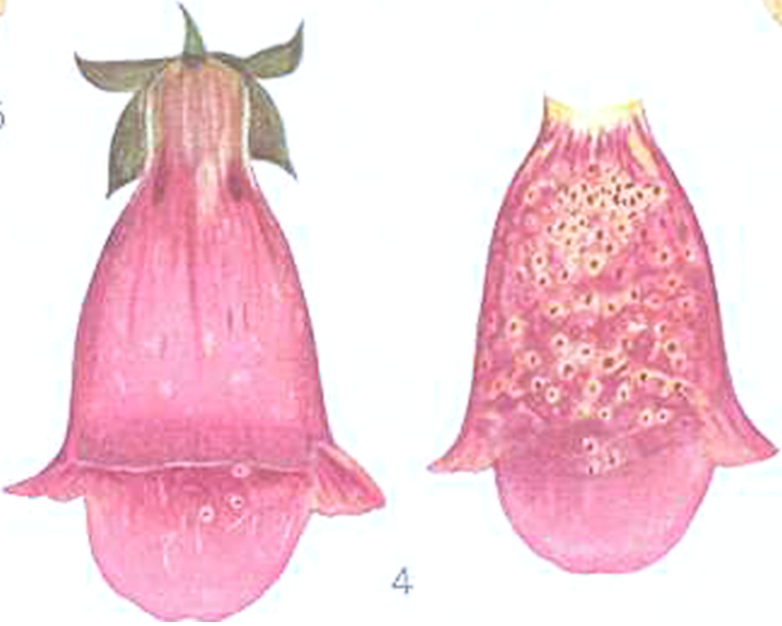
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