

POLLEN-TUBE GROWTH IN CROSSES BETWEEN  
BALANCED CHROMOSOMAL TYPES OF  
*DATURA STRAMONIUM*<sup>1</sup>

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Received January 18, 1929.

TABLE OF CONTENTS

	PAGE
Statement of problem and methods employed.....	538
Pollen-tube growth in diploid plants.....	541
Pollen-tube growth in tetraploid plants.....	544
Tetraploid × diploid crosses.....	545
Diploid × tetraploid crosses.....	546
Triploid × diploid crosses.....	547
Triploid × tetraploid crosses.....	547
The pollen of triploids.....	549
Pollen-tube growth in triploid plants.....	551
Diploid × triploid crosses.....	552
The pollen of haploid plants.....	552
Diploid × haploid crosses.....	553
Tetraploid × haploid crosses.....	553
Triploid × haploid crosses.....	556
Haploid × haploid crosses.....	556
Haploid × diploid crosses.....	557
Haploid × tetraploid crosses.....	557
Distance of penetration of pollen tubes.....	557
DISCUSSION.....	557
Abnormal pollen tubes, and conditions influencing pollen-tube growth.....	557
Pollen germination.....	561
Conditions in haploids in relation to the origin of types.....	561
Developmental selection.....	563
SUMMARY.....	566
LITERATURE CITED.....	567

In an investigation on tetraploid plants of *Datura Stramonium* (BLAKESLEE, BELLING AND FARNHAM 1923) it was shown that the cross with diploids ( $2n \times 4n$ ) is not successful. Over 212 attempts between various strains of  $2n$  plants pollinated with pollen from  $4n$  plants failed to set seed, as well as many more attempts made later. The reciprocal cross ( $4n \times 2n$ ) produces some seeds, and this combination has given rise to triploid ( $3n$ ) plants, but even this cross is difficult to accomplish and, of the few seeds obtained, only a small proportion have been brought to germination.

<sup>1</sup> One of a series of cooperative investigations made possible by grants from the AMERICAN ASSOCIATION FOR THE ADVANCEMENT OF SCIENCE (1922-23) and from the JOSEPH HENRY FUND, NATIONAL ACADEMY OF SCIENCES (1924-26).

The present investigation on pollen-tube growth was undertaken in an effort to find an explanation for the difficulties encountered in making this cross. We are also including tests of pollen-tube growth in combinations of crosses between the balanced chromosomal types,  $4n$ ,  $3n$ ,  $2n$ , and  $n$  plants. These were tested with their own pollen and that of other members of this balanced series, all of them belonging to Line 1A, except a few of the tests made in the years previous to 1927, which belonged either to Line 1 or Line 1A.

The plants of Line 1 were seed-grown from a strain of purple-flowered armed, many noded plants originally obtained from Washington, D. C. This line had been rendered homozygous through many generations of inbreeding. The plants of Line 1A which were used in these tests represent a strain developed from a single haploid plant of Line 1.

Two unimportant combinations employing the pollen of  $3n$  plants ( $4n \times 3n$  and  $n \times 3n$ ) were omitted. The former was made in previous years and shows the same general conditions as  $2n \times 3n$  or  $3n \times 3n$ . The cross  $n \times 3n$  was not tested. The general procedure and technique employed was similar to that of our other investigations on pollen-tube growth (BUCHHOLZ and BLAKESLEE 1928b, c) in which cut flowers were pollinated and kept for a given period of time (usually 12 hours) under conditions of constant temperature.

From microscopic slides prepared by dissection of the styles killed after known time intervals the following diagrams and statistical tables were prepared. Photographic reproductions of several such preparations are shown in figures 25-29. In the diagrams, the pollen tubes are represented as growing from the stigma toward the right. Only the forward ends of pollen tubes were counted and are plotted in the appropriate space (3 mm intervals), those whose ends appeared normal (see figure 28) are plotted above the datum line, those whose ends appeared swollen or abnormal due to bursting (see figure 25) are plotted below the datum line. Pollen grains which remained ungerminated on the stigma together with those which burst on the stigma during germination, two classes not always separated with certainty in making the counts, are plotted together, and, for the sake of simplicity, in the form of a single vertical bar at the left, half above and half below the datum line. These were actually observed scattered over the curved surface of the stigma through several space intervals. The counting was greatly facilitated by the use of two hand tally counters and a microscope equipped with a graduated mechanical stage with vernier, and an ocular with cross hairs. The field

width between cross hairs was .33 mm and nine of these transects were combined in the diagrams to represent 3 mm intervals.

In figures 25 to 28 are shown  $2n$  styles with pollen tubes from  $2n$  and  $4n$  parents. Figure 25, with pollen from  $4n$ , shows the region just below the expanded stigma (6–13 mm from top of stigma) and figure 27 shows the similar region with pollen from  $2n$ . Figure 26 with pollen from  $4n$ , and figure 28 with pollen from  $2n$ , show the regions near the point reached by the longest pollen tube. Figures 25 and 26 are photographs from the same preparation as the diagram of figure 8 ( $2n \times 4n$ ) and figures 27 and 28 are from a test similar to that shown in figure 2 ( $2n \times 2n$ ).

The diagrams, figures 1 to 24, illustrate in detail the distribution of the pollen tubes in representative slides from the various tests. These and all other similar counts not represented by diagrams are given in table 2. In the tables the germinated portion of the gametophytes are grouped into two kinds, normal-appearing A and B, and abnormal-appearing C and D. Group A represents all pollen tubes which have grown to a point half as long as the longest normal pollen tube or longer, Group B are laggards or those which have grown less than half of this distance. Likewise, Group C represents burst or abnormal pollen tubes which have grown half, or more than half, of the distance of the longest normal pollen tube, and D represents the abnormal group which have grown less than half of this distance. Thus, if our diagrams, figures 1, 2, 3, and so forth were each divided by a vertical line at the mid-point, there would be four quadrants, two above and two below the datum line, lettered and related thus:

BA
DC

In table 2 both the actual numbers, and also the percentages, are given for individual tests. The percentages given in the first few columns are based on the total number of pollen grains accounted for in the test, while the percentages in the columns under Groups A, B, C, and D are based on the pollen tubes alone, not including the pollen remaining ungerminated on the stigma. Thus the pollen of a tetraploid plant on its own stigma usually shows about 24 percent ungerminated, and the percentage distribution of the A, B, C and D groups is based only on the 76 percent which germinated.

For convenience, we have prepared table 1 which shows only the general averages in percentage of each group of tests in table 2. Since table 1 does not include an equal number of tests of each type of cross, we are giving the total number of pollen grains accounted for (total gametophyte population) and also the number of styles whose counts were included in

each type of cross, in order that these may serve roughly as a measure of the statistical value of the data on which the percentages are based. The diagrams are given as representative examples showing the more exact distribution of the pollen tubes.

Not all of the pollinations were obtained in 1927. Some were made in previous years (but all since 1921) and under slightly different conditions, as indicated in the diagrams.

#### POLLEN-TUBE GROWTH IN DIPLOID PLANTS

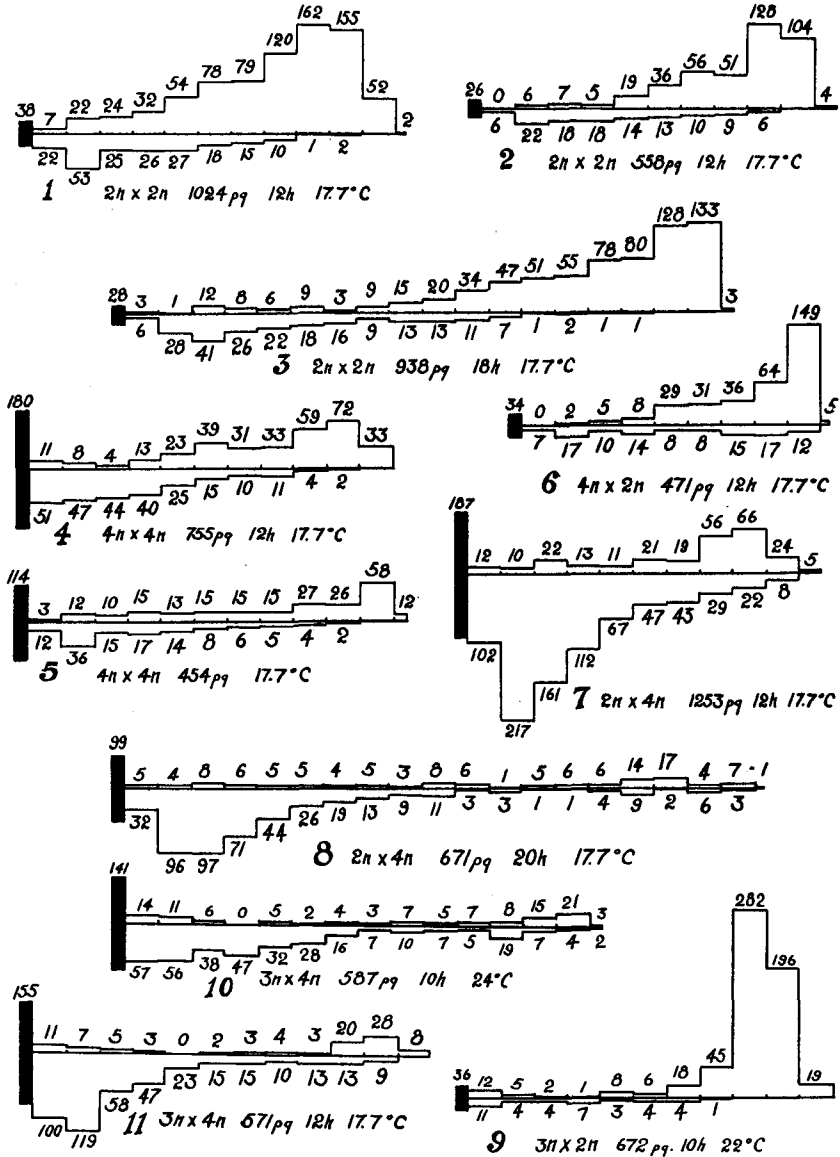
Figures 1 and 2 represent the curves of distribution of the ends of the pollen tubes in the styles of normal diploid plants after 12 hours at 17.7° C. Figure 3 belongs to the same set of tests, but was left growing for 18 hours. Self pollinations were the same as sib-crosses. The proportion of ungerminated pollen grains remaining on the stigma was small, being only about 4-5 percent. Their number is given and represented in the diagrams by the heavy vertical bar at the left.

These diploids give us distribution curves which serve as our standards of comparison. It will be seen in the diagrams and in figure 28 that the mode of frequency of the ends of normal-appearing pollen tubes was near the advancing front. The pollen tubes lagging behind the mode may represent to some extent individuals which were slow in germination due to imperfect contact with the stigma or retarded individuals due to crowding, but the abnormal pollen tubes, those below the datum line, and some of the slow-growing normal tubes may have been due to differences in nuclear or cytoplasmic constitution.

The mode of distribution of the abnormal pollen tubes was in the region near the stigma as indicated very accurately by the diagrams. It is also shown in the tables by the proportions in Groups C and D. The abnormal pollen tubes were present in small proportion in the pistils of all normal diploid plants, whether from self pollinations or sib crosses (see figure 27). They are found in the purest inbred strains. Their proportion is not always quite the same, and their abundance seems to bear some relation to the number of ungerminated pollen grains, and also to the number of retarded or slow-growing pollen tubes in the normal group represented above the datum line (Group B of tables). Thus, if there are few burst tubes, there is very often a greater proportion of retarded pollen tubes or ungerminated pollen grains.

The burst or abnormal pollen tubes (Groups C and D) may have been due in part to chromosomal irregularities from non-disjunction in microsporogenesis. They resemble the types of abnormal tubes which we have

found present in great abundance among the  $(2n+1)$  chromosomal types which we have been investigating by the same methods. However, BELLING (BELLING and BLAKESLEE 1924b) observed only 0.4 percent non-disjunction in  $2n$  plants, so that in the main they may be due to other causes.



FIGURES 1-11.

more burst tubes than we might expect in the region of the mode of frequency of normal tubes.

#### POLLEN-TUBE GROWTH IN TETRAPLOID PLANTS

Figures 4 and 5 represent typical pollen-tube distribution curves in seed-grown Line 1A tetraploid plants ( $4n \times 4n$ ), after 12 hours, at  $17.7^\circ \text{C}$ . Both the proportion of burst pollen tubes and the proportion of ungerminated pollen grains were much higher than in diploid plants. The ungerminated pollen was about 24 percent and this count included pollen grains burst on the stigma during germination.

As in diploids, the normal-appearing portion of the pollen-tube population (those above the datum line) are grouped into a distinct mode near the advancing front. The tables as well as the diagrams show that in comparison with  $2n$  plants, there are more slow-growing normal pollen tubes in proportion to the number found grouped in the mode (figures 1 and 2). Figure 4 shows proportionately fewer lagging normal pollen tubes (13 percent in group B) than figure 5 (17 percent in Group B), but the effect of the greater crowding in figure 4 may have been to cause a greater proportion of bursting among these slower-growing pollen tubes. This is confirmed by the relative proportions found in the D groups.

The burst and swollen tubes were crowded mostly into a second mode located in the region 3–15 mm from the top of the stigma. They are much more conspicuous objects than normal tubes, and frequently a number of pollen tubes terminate in the same mass of extruded protoplasm, indicating that interference may have taken place.

The great abundance of burst pollen tubes may be explained in part by the proportionately high non-disjunction in the microsporogenesis of tetraploids, which amounts to about 30 percent (BELLING and BLAKESLEE 1924a).

We may estimate the excess of abnormal pollen tubes and ungerminated pollen grains of tetraploids over those of diploids from the data in table 1. This excess represents a difference of about 32 percent. Thus the present tests of pollen-tube growth show that 100 pollen grains from tetraploids yield about 32 more individuals which show abnormal performance or fail to germinate entirely, than this number of pollen grains from diploids. This is in close agreement with BELLING'S estimate of the total non-disjunction.

It should be pointed out in this connection that chromosomal irregularities such as a high non-disjunction is also indicated in the megasporogenesis of tetraploid plants. This condition is reflected in the number of

very small aborted ovules found on the enlarged region of the placenta among the seeds which may be observed to the best advantage in immature seed capsules. The percentage of unenlarged or aborted ovules in tetraploids is always high and varies between 35 and 50 percent, while in diploids this value is usually between 6 and 9 percent.

#### TETRAPLOID $\times$ DIPLOID CROSSES

In the cross  $4n \times 2n$ , pollen-tube growth is nearly the same as when diploids are self-pollinated. A number of such tests were made in different seasons, and figure 6, a 12-hour test at  $17.7^{\circ}\text{C}$ , will serve to illustrate these. Figure 17 shows another test in which Group A is greater, even though the population is larger. Though neither of these represent conditions of great crowding (the stigma and style of tetraploid plants are quite large), the spread of the A group is slightly greater in figure 17 than in figure 6. The more pronounced peak in the curve of figure 6 may be due to less crowding, but both tests seem to show favorable conditions of pollen-tube growth.

There is, obviously, no possibility that unfavorable pollen-tube growth might be responsible for the low fecundity of the cross  $4n \times 2n$ . The plants coming from this cross are chiefly  $3n$  and  $2n$ , the latter resulting presumably from parthenogenesis of  $2n$  female gametes. The number of seeds obtained from this cross is very low in proportion to the number of pollen tubes which appear to be available for fertilization. Thus in 1920-21 (BELLING and BLAKESLEE 1922, table 2) 41 capsules were obtained from 62 attempts,  $4n \times 2n$ . These 41 capsules contained 167 seeds, an average of 2.6 seeds per capsule.

We may safely assume that at least 500 pollen tubes may enter the ovary in the pollinations  $4n \times 2n$ . In estimating the seed to pollen-tube ratio, therefore, we can calculate 1 seed per 192 pollen tubes reaching the ovary. Furthermore, only 16 seedlings were obtained from 167 seeds, which gives a ratio of one seedling per 1900 pollen tubes reaching the ovary. The number of seedlings which are actually triploid plants, and therefore the result of fertilization, is still lower (one  $3n$  plant per 13000 pollen tubes reaching the ovary). This ratio would be widened still further if records of all attempts at pollinations were included, rather than data from seed-bearing capsules only.

There are always many aborted ovules and small seeds obtained in these crosses,  $4n \times 2n$ , even though the seed capsules contain only a few viable seeds. Some of these ovules have enlarged considerably and may be classified from external appearance as small seeds. There are very many

others which enlarged only slightly. Still others too microscopic to be included in seed counts fail to enlarge at all, and correspond in size to the abortive ovules observable scattered over the placental surface in immature capsules of tetraploid plants.

From this, it is obvious that the relative infertility of the cross  $4n \times 2n$  is due to causes other than pollen-tube growth, such as failure of proper union of the sperms with both egg and polar nucleus, failure of the zygotes to develop after fertilization, or nutritional difficulties resulting in death of the embryo during seed development. Combined with this early abortion we have poor seed germination.

#### DIPLOID $\times$ TETRAPLOID CROSSES

In the cross  $2n \times 4n$ , a distinctly different picture of pollen-tube growth is obtained (see figures 25 and 26). We have shown in a previous publication (BUCHHOLZ and BLAKESLEE 1927b) that the distribution curves for pollen tubes of this cross have a backward skew. If a separation is made of the abnormal and normal-appearing pollen tubes, we obtain distribution curves similar to the one shown in figure 7, which shows the results of a 12-hour test at 17.7°C. The pollen of the  $4n$  plant actually germinates better on the diploid stigma than it does on the  $4n$  stigma; on  $2n$ , 85 percent germinate; on  $4n$ , 76 percent germinate. All of our tests of this cross made at various times during the past few years show conditions very similar to these.

The proportion of abnormal pollen tubes is very large. Figure 7 shows a condition in which only 21 percent of the total pollen-tube population remained normal-appearing after 12 hours. This is one of the most favorable of our tests. Among the germinated portion of the gametophyte population, the A group varied from 10–18 percent as shown in table 2 with an average of only 14 percent.

Figure 8 shows one of these styles with a population of 671. This test was made at the same time as the one used for figure 7 but the pollen tubes were kept growing for 20 hours. Here the normal-appearing pollen tubes comprise only 18 percent, while the abnormal or burst portion represents 67 percent of the total population. The A group is only 12 percent (table 2) and at this stage bursting is still taking place. Thus, there is little probability that more than a few scattered individuals of the remaining pollen tubes would have reached the ovary. These tests were conducted under conditions favorable to pollen-tube growth (BUCHHOLZ and BLAKESLEE 1927b, c). In the field or greenhouse at variable high temperatures, the conditions are less favorable.



Even if an occasional pollen tube should reach the ovules, the chances of obtaining seeds are, as we have shown in the reciprocal cross, only about 1 in 192, and the chances for the survival of seedlings are not over one in 1900. The sterility of the cross  $2n \times 4n$  is, therefore, due primarily to difficulties in pollen-tube growth.

#### TRIPLOID $\times$ DIPLOID CROSSES

The cross  $3n \times 2n$  has been successfully made many times. It is from this cross that the primary ( $2n+1$ ) chromosomal types have been obtained repeatedly in our cultures. In a recent publication (BLAKESLEE 1927) 936 such offspring are tabulated, showing aside from 270 normal plants, 498 ( $2n+1$ ) primaries, and 310 double ( $2n+1+1$ ) forms with 13 unidentified plants.

Pollen-tube growth is very favorable in this cross. Figure 9 represents such a case and this is from a 10-hour test made in a previous year, kept at 22°C. The triploid plants used in these tests for styles as well as pollen parents were scions from a grafted Line 1A plant and were therefore derived from the same plant used in other breeding experiments. Since we knew that this cross gives favorable pollen-tube growth under various conditions, we did not repeat these tests in 1927.

The distribution of the pollen tubes of figure 9 with the small proportion of burst pollen tubes found, indicates conditions of very favorable pollen-tube growth. Tables 1 and 2 show that the A group comprises 88 percent of the pollen-tube population which is a much higher figure than that in any other combination which we have tested. There are fewer laggard pollen tubes than in  $2n \times 2n$  crosses, but this may be partly explained by the fact that the styles of triploids are considerably larger than those of diploid plants so that crowding is lessened.

#### TRIPLOID $\times$ TETRAPLOID CROSSES

Figures 10 and 11 show the distribution of the pollen tubes in a test of the cross  $3n \times 4n$ . Three such tests were made in 1927, 12 hours at 17.7°C., and two were made in previous years, 10 hours at 24°C. All five tests are included in the tabulation, and are consistent in showing unfavorable pollen-tube growth. An average of 27.6 percent of the pollen fails to germinate, in comparison with 24 percent in the cross  $4n \times 4n$ , and 14.6 percent in the cross  $2n \times 4n$ . If we consider the germinated portion of the gametophyte population, we will note that Group D includes 62 percent, Group C 14 percent, Group B 9 percent and Group A 15 percent (averages, table 1). Except for the differences in the ungerminated portion

TABLE 1  
Summary for each combination of tests.

TYPE OF CROSS	NUMBER OF TESTS	GRAND TOTAL (Game-tophytes recorded)	UNGERMINATED BURST POLLEN ON STIGMA		POLLEN TUBES SWOLLEN OR BURST WITHIN STYLE		POLLEN TUBES WITHIN STYLE APPEARING NORMAL		TOTAL NUMBER OF TUBES	POLLEN TUBES SWOLLEN OR BURST IN REGION CORRESPONDING TO GROUP B		POLLEN TUBES BURST AFTER GROWING LONGER THAN GROUP B		RETARDED NORMAL POLLEN TUBES		FORWARD GROUP OF NORMAL POLLEN TUBES		GROUP A, PERCENT BASED ON GRAND TOTAL
			Number	Percent	Number	Percent	Number	Percent		Number	Percent	Number	Percent	Number	Percent	Number	Percent	
1n×1n	2	396	123	31.0	100	25.3	173	43.7	273	61	2.41	39	14.3	79	28.9	94	34.4	23.7
1n×2n	1	264	15	5.7	22	8.3	227	86.0	249	16	6.4	6	2.4	24	9.6	203	81.5	76.8
1n×4n	1	557	81	14.5	378	67.9	98	17.6	476	266	55.9	112	23.5	52	10.9	46	9.7	8.3
2n×1n	3	2039	608	29.8	138	6.8	1293	63.4	1431	84	5.9	54	3.8	243	17.0	1050	73.3	51.5
2n×2n	3	3514	145	4.1	639	18.2	2730	77.7	3369	501	14.9	138	4.1	421	12.2	2309	68.8	65.7
2n×2n	2	1079	984	91.2	55	5.1	40	3.7	95	47	49.5	8	8.4	26	27.4	14	14.7	1.2
2n×3n	2	3380	498	14.7	2238	66.2	644	19.0	2882	1963	68.2	275	9.5	196	6.8	448	15.5	13.3
2n×4n	4	542	212	39.1	42	7.7	288	53.2	330	25	7.6	17	5.2	49	14.8	239	72.4	44.1
3n×1n	1	672	36	5.4	42	6.2	594	88.4	636	30	4.7	12	1.9	32	5.0	562	88.4	83.6
3n×2n	1	1120	1009	90.1	67	6.0	44	3.9	111	66	59.4	1	0.9	25	22.5	19	17.1	1.7
3n×3n	1	2404	660	27.6	1343	55.8	397	16.5	1740	1127	64.7	216	12.4	148	8.5	249	14.3	10.4
3n×4n	2	725	252	34.8	64	8.8	409	56.4	473	38	8.0	26	5.5	28	5.9	381	80.6	52.5
4n×1n	2	1137	63	5.5	215	18.9	859	75.6	1074	118	11.0	97	9.0	82	7.6	777	72.4	67.4
4n×2n	2	1209	294	24.2	368	30.4	547	45.3	915	316	34.5	52	5.7	134	14.6	413	45.2	34.2

of the pollen, these tests are very similar to the  $2n \times 4n$  cross and indicate very unfavorable conditions of pollen-tube growth. It is possible that occasionally a few pollen tubes might reach the ovary, and if difficulties in fertilization or survival of zygotes are not as great as in the cross  $4n \times 2n$  we might expect an occasional seed. Offspring from this cross, however, have not yet been obtained.

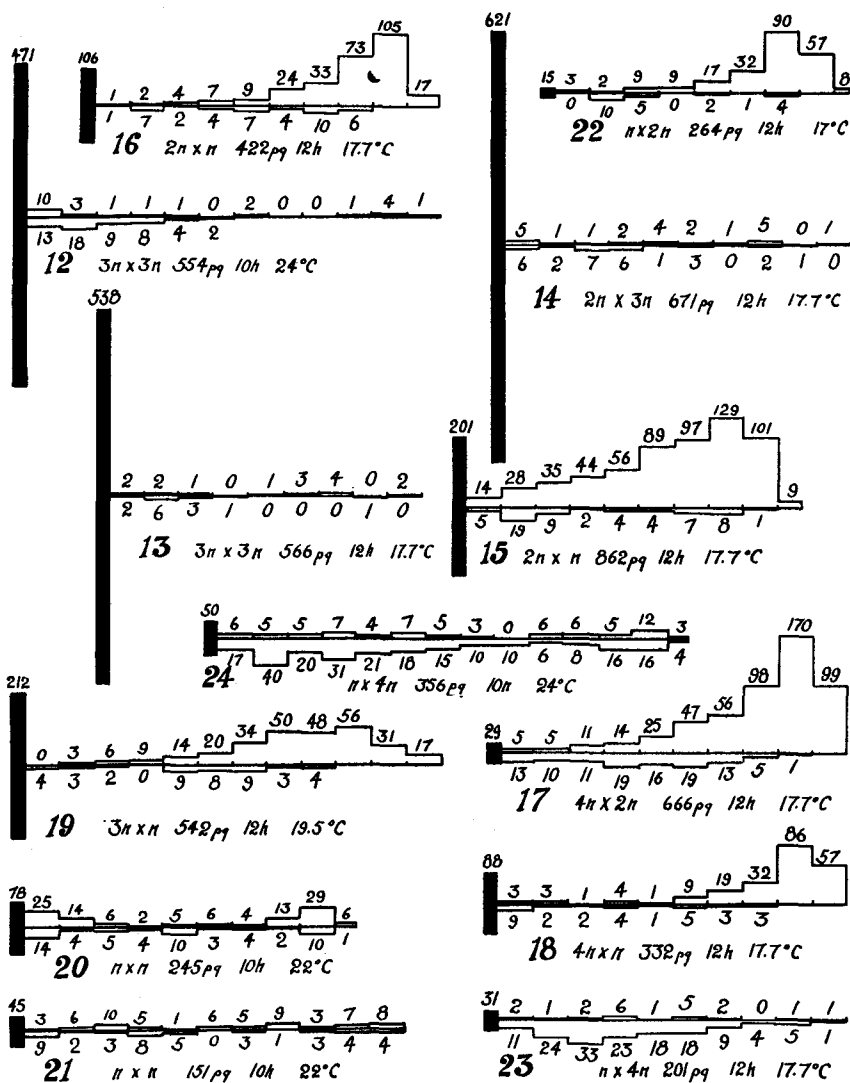
#### THE POLLEN OF TRIPLOIDS

The pollen of triploids contains a great range of types with variable chromosome numbers. There are a few grains with 12 chromosomes, which are the same as those produced by diploid plants, and a few with 24 chromosomes, similar to those from tetraploids, with a great preponderance of pollen grains having chromosome numbers between these two extremes.

After the formation of microspores, there is an important period of growth in the male gametophyte, before the pollen is actually matured as such. This is true of the pollen in all normal diploid plants as well as in triploids and tetraploids. The microspores of diploid plants have a volume of about 2900 cubic microns immediately after reduction and at their maturity the volume of the pollen grains is about 70,000 cubic microns, which represents a 24-fold increase in volume. In triploids during this stage of growth of the microspores, a considerable proportion, 43.6 per cent (BLAKESLEE and CARTLEDGE 1926 and 1927) of the pollen becomes abortive. This abortive pollen probably represents a fraction containing the most unfavorable chromosomal combinations, which are unable to develop further as gametophytes and do not reach the stage of matured pollen grains.

Mature pollen grains in *Datura* have two nuclei, the tube nucleus and the generative nucleus; the former, a larger spheroidal body only slightly denser than the cytoplasm; the latter a much smaller, very dense rod-shaped structure, as observed in stained pollen grains from normal plants cleared in chloral hydrate. Before a pollen grain is matured from a microspore, it must not only enlarge greatly and become filled with reserve food substances, but its nucleus must undergo at least one division. Evidently not all of the pollen grains of a triploid plant are able to pass successfully through these stages of development and many microspores abort in various stages. An examination of this pollen shows evidence of a great range in the sizes of the shrunken aborted grains, as well as of the grains which survive to become rounded out and stain deeply. There is, therefore, a distinct phase of gametophytic selection here, and the inference is

obvious, that this elimination is to some extent differential, that the gametophytes with deficiencies and chromosomal combinations highly unfavorable to the continued growth of the gametophyte, are the ones



FIGURES 12-24.

eliminated early in various stages as aborted pollen, while the combinations more nearly normal for the gametophytes are the ones which increase their volume greatly and develop to maturity. Their mature size is prob-

ably in part proportional to their chromatin content, at least for the grains with 12, 24, and 36 chromosomes, in which the chromosome conditions are  $n$ ,  $2n$  or  $3n$ . In the great majority which have 12 plus several extra chromosomes, the sizes are probably also more or less conditioned by the combinations of extra chromosomes present.

#### POLLEN-TUBE GROWTH IN TRIPLOID PLANTS

Figure 12 shows the condition in a pollination  $3n \times 3n$ , in which there were 471 pollen grains ungerminated on the stigma, plus 83 which had germinated and produced pollen tubes. There were also many of the aborted grains (not plotted) which remained observable on the stigma. The 83 which had germinated represent only 15 percent of the pollen grains matured; 54 of them had burst within the region of the style near the stigma. Only 6 tubes, or possibly 8, may be considered by their performance as having 12 chromosomes, or a proportion of the total gametophytes between 1 and 1.5 percent. Figure 12 shows the results of a 10-hour test made prior to 1927 exposed at  $24^{\circ}$  C. Figure 13 is a 12-hour test made in 1927 at  $17.7^{\circ}$  C. and shows a much lower percentage of pollen germination on the stigma. Only 5 percent of the pollen grains germinated, against 15 percent in figure 12. The difference between these two tests is less pronounced when we consider only the normal-appearing pollen tubes (see also table 2) which are 3 percent in figure 13 against 5 percent in figure 12. If Group A and Group B are compared, we find that figure 12 has more in the B group while figure 13 has more in the A group. Basing the percentages in the A group on the total number of pollen grains accounted for, the percentages are very nearly equal; 1.6 percent for figure 12 and 1.7 percent for figure 13. The difference between the two tests is therefore one which may be attributed to the number of pollen grains with extra chromosomes which happened to germinate under the different conditions of the two tests, or to an experimental error through loss of ungerminated pollen during manipulation.

There is obviously very little crowding among the gametophytes which germinate and begin to grow within the style. Probably the pollen grains with a single extra chromosome may germinate, if the combinations are able to grow at all, but only the pollen tubes with 12 chromosomes will be likely to reach the ovary in most cases. The pollen of triploids cannot be crowded sufficiently on a stigma to provide more than a limited number of pollen tubes with 12 chromosomes and this, therefore, results in the fertilization of only a very few of the ovules in an ovary. Though fewer seeds are obtained from the selfing of triploid plants,  $3n$  parents produce

seeds in considerable numbers when pollinated with the pollen of diploid plants. In another publication (BLAKESLEE 1927) an analysis of the progeny of triploid plants has been given.

#### DIPLOID $\times$ TRIPLOID CROSSES

The pollen of triploids, when germinated on the stigmas of diploid plants, gives very similar results to those just described. Figure 14 represents such a cross. The proportion of the pollen which may represent gametophytes with 12 chromosomes may be estimated from the data in table 2, by taking the number of pollen tubes in the A group and calculating their percentage of the total number of pollen grains accounted for under total gametophytes. In (27-211-1), this is 1.2 percent and in figure 14, this is 1.3 percent.

The expected random assortment of chromosomes in triploids has been confirmed by BELLING (BELLING and BLAKESLEE 1922). Such assortment should produce only 0.024 percent pollen grains with 12 chromosomes, the tubes from which should be found in our group A (compare tables). In this group of normal-appearing tubes, however, we have found about 1.5 percent of unaborted pollen, or about 0.85 percent of the total pollen population applied to the stigma. This is about 35 times the number expected. This figure would be decreased if any considerable number of pollen grains which had burst or remained ungerminated on the stigma were lost during manipulation.

From this fact we may conclude that in triploids some, as yet unknown, factors are responsible for the number of chromosomes in the male gametophytes offered to the processes of developmental selection. A somewhat similar situation is found in the female gametophytes of triploids (BELLING and BLAKESLEE 1922).

#### THE POLLEN OF HAPLOID PLANTS

Haploids produce two classes of pollen, pollen by "pseudo-reduction," in which the 12 chromosomes separate so that there are 6-6, 5-7, 4-8, and so forth, or always less than one complete set of 12 chromosomes, passing into the resulting microspores (BELLING and BLAKESLEE 1926) and pollen by non-reduction, in which the number of chromosomes is 12. The pseudo-reduction microspores very generally abort, and there is quite a range in the sizes of the aborted grains, indicating that some of them may have enlarged before they aborted. BLAKESLEE and CARTLEDGE (1926, 1927) have estimated these aborted grains at 88 percent of the pollen count at the time when the pollen is mature, and have found that the conditions

under which the plants are growing result in slight differences in the proportion of "bad pollen". Grafted scions of the haploid plant 20745(4) were used in these tests on pollen-tube growth.

It is the remaining 12 percent of the pollen attributed primarily to non-reduction with which we are concerned in these tests of haploid pollen. It has been presumed that this pollen has 12 chromosomes. However, the different tests employing the pollen from haploids show that some differences exist, for we have a high proportion of this pollen (23 percent–42 percent) unable to germinate on the stigmas of  $2n$  plants, or the stigma of any member of the balanced series.

#### DIPLOID $\times$ HAPLOID CROSSES

Since the functional pollen of haploids is scanty it was necessary to combine the pollen of several anthers in making these tests, in order that the number of fully matured grains applied to the stigma might be comparable to our  $2n \times 2n$  tests. Our counts of the proportion of aborted pollen, made on the stigmas of several of the tests (data not included in the diagrams or tables) gave only 61–81 percent (average 76 percent) aborted pollen, but it is probable that some of the aborted grains were lost during the manipulation of mixing the pollen and pollinating the stigmas, and we are not certain that all abortive pollen grains present on the stigmas of our preparations are observable.

Figures 15 and 16 show the pollen-tube distribution curves of crosses  $2n \times n$ . Aside from the ungerminated pollen, these distribution curves are similar to those of  $2n \times 2n$  tests and it is obvious that a very high proportion of these pollen tubes behave as if they had 12 chromosomes. These tests resemble diploid selfs and sib crosses even in respect to the details of the crowding effect. Figure 15, which has twice as many pollen tubes as figure 16, has a much greater proportion of retarded tubes (a larger B group, table 2) while another case (27–181–1, table 2) of intermediatesized population, shows the intermediate condition of distribution.

Some abnormal pollen tubes are found, resembling the abnormalities of the  $2n \times 2n$  tests but in much smaller proportion, less than half as many, which may be significant. It is possible that a chromosomal complex, which in a cytoplasm derived from a diploid would permit germination, prevents germination when the cytoplasm is derived from a haploid.

#### TETRAPLOID $\times$ HAPLOID CROSSES

Figure 18 shows a diagram of the pollen-tube distribution curve in a cross  $4n \times n$ . In this case, 26 percent of the matured pollen grains failed

TABLE 2  
*Pollen-tube distribution in individual styles.*

TYPE OF CROSS AND IDENTIFICATION NUMBER	GRAND TOTAL (Chromophyt-testcoordinated)	UNGER-MINATED POLLEN REMAINING ON STIGMA	POLLEN GRAINS BURST ON STIGMA	PERCENT UNGER-MINATED AND BURST POLLEN	POLLEN TUBES APPEARING SWOLLEN OR BURST WITHIN STYLE		POLLEN TUBES APPEARING NORMAL		TOTAL NUMBER OF POLLEN TUBES	POLLEN TUBES SWOLLEN OR BURST IN REGION CORRESPONDING TO GROUP B	POLLEN TUBES BURST AFTER GROWING LONGER THAN GROUP B	RETADED NORMAL POLLEN TUBES	FORWARD GROUP OF NORMAL POLLEN TUBES				
					Number Percent		Number Percent										
					Number	Percent	Number	Percent									
$n \times n$ (figure 20)	245	25	53	31.9	57	23.3	110	44.9	167	33	19.8	24	14.4	48	28.7	62	37.1
$n \times n$ (figure 21)	151	26	19	29.8	43	28.5	63	41.7	106	28	26.4	15	14.2	31	29.2	32	30.2
$n \times 2n$ (figure 22)	264	7	8	5.7	22	8.3	227	86.0	249	16	6.4	6	2.4	24	9.6	203	81.5
$n \times 4n$ (figure 23)	201	8	23	15.4	146	72.7	24	11.9	170	106	62.4	40	23.5	15	8.8	9	5.3
$n \times 4n$ (figure 24)	356	20	30	14.0	232	65.2	74	20.8	306	160	52.3	72	23.5	37	12.1	37	12.1
$2n \times n$ (figure 15)	862	50	151	23.3	59	6.8	602	69.9	661	38	5.7	21	3.2	158	23.9	444	67.2
$2n \times n$ (27-181-1)	755	130	171	39.9	38	4.9	416	55.2	454	26	5.7	12	2.6	65	14.3	351	77.4
$2n \times n$ (figure 16)	422	21	85	25.1	41	9.7	275	65.2	316	20	6.3	21	6.6	20	6.3	255	80.7
$2n \times 2n$ (figure 1)	1024	6	32	3.7	199	19.4	787	76.8	986	165	16.7	34	3.5	173	17.5	614	62.3
$2n \times 2n$ (figure 2)	558	6	20	4.7	116	20.7	416	74.6	532	73	13.7	43	8.1	34	6.4	382	71.8
$2n \times 2n$ (figure 3)	938	7	21	3.0	215	22.9	695	74.1	910	179	19.7	36	4.0	68	7.5	627	68.8
$2n \times 2n$ (26-3)	600	5	19	4.0	54	9.0	522	87.0	576	47	8.1	7	1.2	96	16.7	426	74.0
$2n \times 2n$ (26-4)	394	11	18	7.4	55	13.9	310	78.7	365	37	10.1	18	4.9	50	13.7	260	71.3
$2n \times 3n$ (figure 14)	671	497	124	92.5	28	4.2	22	3.3	50	22	44.0	6	12.0	13	26.0	9	18.0
$2n \times 3n$ (27-211-1)	408	279	84	89.0	27	6.6	18	4.4	45	25	55.6	2	4.4	13	28.9	5	11.1
$2n \times 4n$ (figure 7)	1253	38	149	14.9	807	64.4	259	20.7	1066	664	62.3	143	13.4	71	6.7	188	17.6
$2n \times 4n$ (27-154-2)	610	29	52	13.3	449	73.6	80	13.1	529	404	76.3	45	8.5	36	6.8	44	8.3
$2n \times 4n$ (27-160-2)	846	30	101	15.5	530	62.7	185	21.8	715	479	67.0	51	71.1	38	5.3	147	20.6
$2n \times 4n$ (figure 8)	671	19	80	14.8	452	67.3	120	17.9	572	416	72.7	36	6.3	51	8.9	69	12.1



TABLE 2 (Continued).

TYPE OF CROSS AND IDENTIFICATION NUMBER	GRAND TOTAL (Gametophytes recorded)	UNGERMINATED POLLEN REMAINING ON STIGMA	POLLEN ORAINS BURST ON STIGMA	PERCENT UNGERMINATED AND BURST POLLEN	POLLEN TUBES APPEARING SWOLLEN OR BURST WITHIN STYLE		POLLEN TUBES APPEARING NORMAL		TOTAL NUMBER OF POLLEN TUBES	POLLEN TUBES SWOLLEN OR BURST IN REGION CORRESPONDING TO GROUP B	POLLEN TUBES BURST AFTER GROWING LONGER THAN GROUP B	RETARDED NORMAL POLLEN TUBES	FORWARD GROUP OF NORMAL POLLEN TUBES		
					Number	Percent	Number	Percent						Group C	Group B
$3n \times n$ (figure 19)	542	74	138	39.1	42	7.7	288	53.2	330	25	7.6	49	14.8	239	72.4
$3n \times 2n$ (figure 9)	672	5	31	5.4	42	6.2	594	88.4	636	30	4.7	32	5.0	562	88.4
$3n \times 3n$ (figure 13)	566	485	53	95.0	13	2.3	15	2.7	28	12	42.8	5	17.9	10	35.7
$3n \times 3n$ (figure 12)	554	315	156	85.1	54	9.7	29	5.2	83	54	65.0	20	24.1	9	10.9
$3n \times 4n$ (figure 11)	671	72	83	23.1	422	62.9	94	14.0	516	360	69.8	27	5.2	67	13.0
$3n \times 4n$ (27-196-2)	362	45	73	32.6	171	47.2	73	20.2	244	126	15.7	24	9.9	49	20.0
$3n \times 4n$ (27-196-3)	297	52	72	41.7	136	45.8	37	12.5	173	122	70.5	14	9.2	21	12.1
$3n \times 4n$ (figure 10)	587	53	88	24.0	335	57.0	111	18.9	446	276	61.9	43	9.6	68	15.3
$3n \times 4n$ (23-14)	487	58	64	25.9	279	57.3	82	16.8	361	243	67.3	38	10.5	44	12.2
$4n \times n$ (27-198-2)	393	77	87	41.7	35	8.9	194	49.4	229	20	8.7	16	7.0	178	77.7
$4n \times n$ (figure 18)	332	20	68	26.5	29	8.7	215	64.8	244	18	7.4	11	4.5	203	83.2
$4n \times 2n$ (figure 17)	666	7	22	4.3	107	16.1	530	79.6	637	67	10.5	55	8.6	475	74.6
$4n \times 2n$ (figure 6)	471	9	25	7.2	108	22.9	329	69.9	437	51	11.6	27	6.2	302	69.2
$4n \times 4n$ (figure 4)	755	48	132	23.8	249	33.0	326	43.2	575	218	37.9	31	5.4	249	43.3
$4n \times 4n$ (figure 5)	454	43	71	25.1	119	26.2	221	48.7	340	98	28.9	21	6.2	164	48.2

to germinate on the stigma. The germinated 74 percent were distributed within the style so that Group A contained 83 percent of them. By comparing this with Group A in the tables, it will be noted that this value is high, and indicates conditions of favorable pollen-tube growth. (Another case (27-198-2), not represented by a diagram shows 42 percent of the pollen ungerminated, but the distribution of the germinated portion is very similar to that in figure 18). Neither of these tests represents conditions of crowding, for the  $4n$  pistil is large and there are few retarded pollen tubes. It is apparent that the cross  $4n \times n$  is a possible combination from the standpoint of pollen-tube growth and compares very favorably with the cross  $4n \times 2n$  (figure 17). A small proportion of abnormal pollen tubes produced by the pollen from a haploid is the rule in  $4n \times n$  crosses.

#### TRIPLOID $\times$ HAPLOID CROSSES

Figure 19 shows the distribution of the pollen tubes in a  $3n \times n$  cross in a 12 hour test made in a previous year at 19.5°C. This test was not repeated in 1927. It is similar in essentials to the crosses  $4n \times n$  and  $2n \times n$ , and represents a combination with conditions of favorable pollen-tube growth. The ungerminated portion of the pollen remaining on the stigma is 39 percent and the grouping and distribution of the normal pollen tubes is rather spread and not entirely typical of other favorable combinations.

#### HAPLOID $\times$ HAPLOID CROSSES

Figures 20 and 21 show diagrams of the condition and distribution of the pollen tubes in  $n \times n$  tests. Both of these were made previous to 1927. As in all other cases in which haploid pollen was tested a high proportion (30-32 percent) remained ungerminated on the stigma. Compared with the performance of this pollen when tested on styles of  $2n$ ,  $3n$  and  $4n$  plants, the conditions of pollen-tube growth in  $n \times n$  tests are less favorable, but they are not entirely unfavorable and much more satisfactory than the  $2n \times 4n$  and  $3n \times 4n$  crosses, as will be seen by a study of the proportions in the A and B groups in the tables.

The stigmas and styles of haploid plants are small and the number of pollen grains which could be applied to their stigmas by the same methods used in our other crosses was for this reason smaller. On the other hand, the styles are about  $2/3$ - $3/5$  as long as those of  $2n$  plants, and these short styles present a condition which tends to favor the survival of a sufficient number of normal pollen tubes in selfed haploid flowers to accomplish fertilization.

HAPLOID  $\times$  DIPLOID CROSSES

The cross  $n \times 2n$  was only attempted a few times and not repeated in 1927. Figure 22 shows the pollen tubes in this cross from a 12-hour test of a previous year at  $17^{\circ}\text{C}$ . It seems that here we have high pollen germination and favorable pollen-tube growth, conditions closely resembling  $2n \times 2n$  crosses. It is difficult to explain why this cross should be more favorable than the combination  $n \times n$ , aside from the non-germinating part of haploid pollen. The data here, however, are based on only a single test and a small population. On the whole, the data seem to indicate that the apparently good pollen grains of *Datura* which have been derived from a diploid are better than the similar grains derived from a haploid.

HAPLOID  $\times$  TETRAPLOID CROSSES

Figures 23 and 24 show diagrams of  $n \times 4n$  tests. Here we have a high degree of incompatibility, for nearly all of the pollen tubes burst within the style, a condition essentially similar to the  $2n \times 4n$  crosses. Also, the proportion of the pollen which fails to germinate is around 12–17 percent as it is in  $2n \times 4n$  crosses. The sterility of this combination is likewise due to difficulties in pollen-tube growth.

## DISTANCE OF PENETRATION OF POLLEN TUBES

It will be noted that the distance reached by the longest pollen tube in a given time is very nearly the same, regardless of the style used and the pollen. The differences shown in some of the tests are due to the differences in time and temperature. When the temperature and time intervals are the same, the distance reached by the longest pollen tubes is nearly the same. Slight differences are always found in tests with styles and pollen of the same origin (BUCHHOLZ and BLAKESLEE 1927b) and compared with these variables any differences found in the series of studies would not appear to be significant.

## DISCUSSION

*Abnormal pollen tubes and conditions influencing pollen-tube growth*

There are at least two conditions giving rise to an abnormal swelling and bursting of pollen tubes which must be recognized in these tests of pollen-tube growth between balanced chromosomal types. One is the growth of otherwise normal gametophytes in an unsuitable style. This condition is illustrated by the crosses  $2n \times 4n$ ,  $3n \times 4n$  and  $n \times 4n$ , and appears very similar to the condition found when certain interspecific crosses are attempted in which the primary cause of sterility is definitely

## EXPLANATION OF PLATE 1

Photomicrographs of preparations of the conducting tissue containing pollen tubes, dissected from the styles. The preparations were stained in an aqueous mixture of Fuchsin S, 10 parts, and Light Green S. F., 3 parts, cleared in lactic acid, and spread out by pressure on the cover glass.

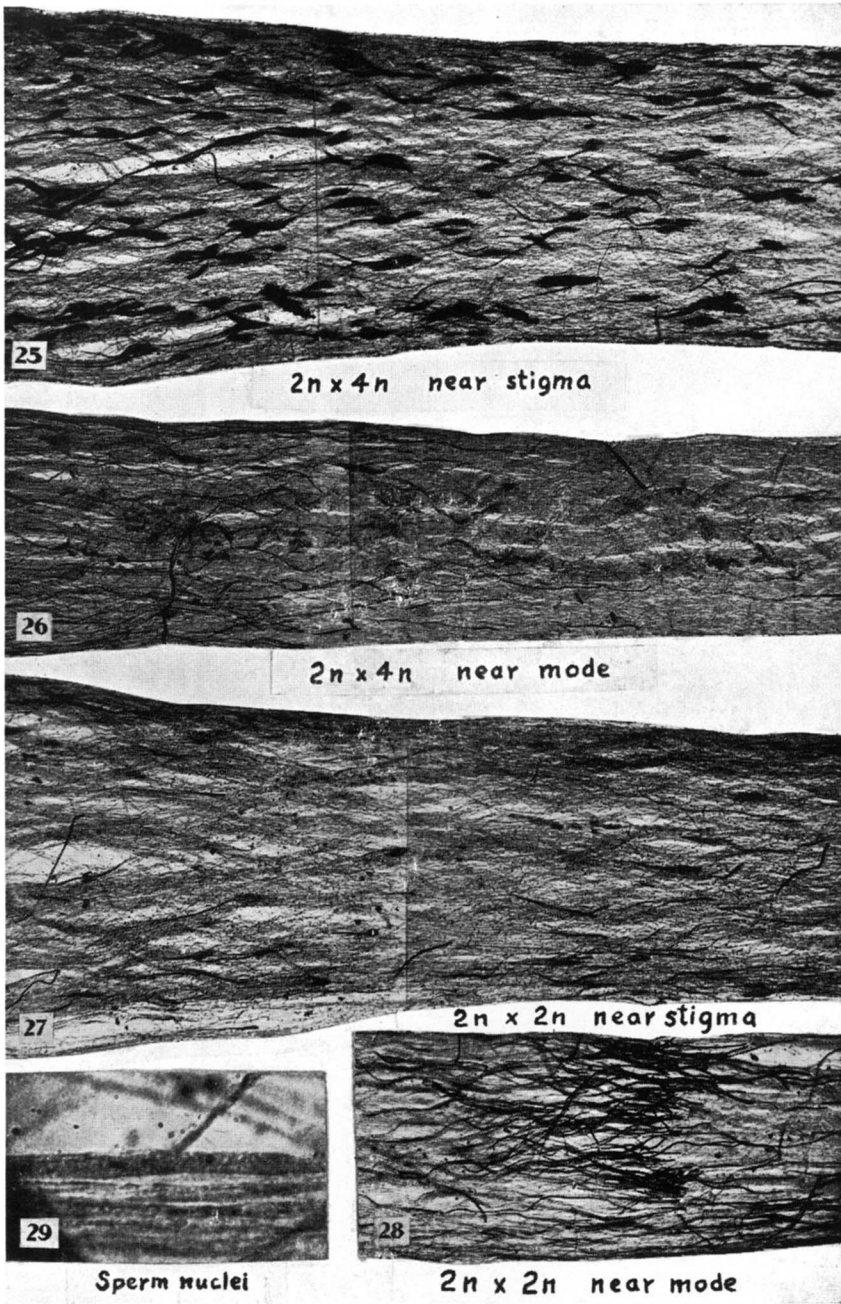
FIGURE 25.— $2n \times 4n$  pollination. Upper portion of style near stigma showing many swollen or burst pollen tubes.  $\times 16$ . See figure 7.

FIGURE 26.— $2n \times 4n$  pollination. Same style as figure 25 in region of longest pollen tubes.  $\times 16$ . See figure 7.

FIGURE 27.— $2n \times 2n$  pollination. Upper portion of style near stigma showing only a few swollen or burst pollen tubes.  $\times 16$ . See figures 1-3.

FIGURE 28.— $2n \times 2n$  pollination. Style similar to figure 27 in the region of mode of distribution of pollen-tube ends.  $\times 16$ . See figures 1-3.

FIGURE 29.—Two sperm nuclei showing in a pollen tube from a preparation stained in Delafield's Haematoxylin.  $\times 300$ .



recognizable as due to difficulties in pollen-tube growth (BUCHHOLZ and BLAKESLEE 1927b). In these crosses, the pollen germinates on the stigma, but the growth of the pollen tubes is followed by swelling and bursting so that sooner or later all or nearly all of the male gametophytes are eliminated. Since the result is much the same whether  $1n$ ,  $2n$ , or  $3n$  styles are used, the cause of the bursting in such cases must be due to something in the styles unsuitable to the growth of pollen tubes having  $2n$  chromosomes.

The other condition which causes bursting of pollen tubes seems to depend upon the nuclear constitution of the gametophytes. In self-pollinations or sib crosses between tetraploids, the high non-disjunction which is known to occur in microsporogenesis is reflected in a correspondingly high proportion of burst pollen tubes, and in a high proportion of ungerminated pollen. Similarly, the small proportion of abnormal pollen tubes found in diploid tests ( $2n \times 2n$ ) is probably due in part to differences arising through non-disjunction in the formation of microspores. We know from other pollen-tube tests of the  $(2n+1)$  chromosomal mutants that similar abnormal tubes are the result of the extra chromosome, and that this chromosomal abnormality may express itself in various other ways; for some by giving rise to ungerminated pollen grains, and for other chromosomes, to pollen tubes which may remain normal-appearing but grow at a retarded rate. The bursting pollen tubes which result from these two conditions, namely, unsuitable styles, and nuclear constitution of the gametophytes, are not distinguishable from their appearance, and it is from our knowledge of the origin and constitution of the pistil and pollen employed that we are able to distinguish between these two conditions which may cause bursting.

Still other conditions may cause a bursting of a part of the pollen tubes in a population, for instance, the presence of certain genes and this condition has been demonstrated for the gene *tricarpele* (BUCHHOLZ and BLAKESLEE 1927b.) Presumably our plants are homozygous and we are not concerned with gene differences in these tests unless they should represent lethals which arose anew in considerable numbers.

The most favorable conditions of pollen-tube growth are found in  $2n \times 2n$ ,  $4n \times 2n$ ,  $3n \times 2n$  and  $n \times 2n$  combinations. In all of these combinations, when pollen of diploids is employed, more than 93 percent of the pollen germinates on the stigma, and the bursting of pollen tubes within the styles occurs only in low proportions. A large number of the pollen tubes are grouped into a pronounced mode near the advancing front, and any outstanding differences in the shape of the polygon of distribution appear to be due to differences in the amount of crowding.

Another series of very favorable conditions of pollen-tube growth is found in the crosses  $2n \times n$ ,  $4n \times n$  and  $3n \times n$  when pollen of haploid plants is employed. While 23–42 percent of the matured pollen grains from haploids fail to germinate, the portion which does germinate is otherwise similar to the pollen of  $2n$  plants, and shows very favorable conditions of pollen-tube growth. The combination  $n \times n$  seems to show somewhat unfavorable conditions of pollen-tube growth though some seeds are produced on haploids.

Less favorable conditions of pollen-tube growth are found in tetraploids, in both selfs and sib crosses  $4n \times 4n$ . The pollen of these is encumbered with many individual abnormalities, but the 45 percent or less of the pollen tubes remaining after the abnormalities are sifted out are fully potent and are able to accomplish fertilization. The  $4n$  plants have always been found fully capable of maintaining themselves as distinct tetraploid lines. However, modified tetraploid types are known to occur in cultures, plants of  $(4n \pm 1)$ ,  $(4n + 1 - 1)$  and other abnormal constitutions (BLAKESLEE, BELLING and FARNHAM 1923), and if recognized, the use of these should be avoided in making tests of this kind. Some of these may be suspected on account of abnormalities in external appearance and habit, by their failure to set fruit or by having only small seed capsules. They may be positively identified only by cytological methods, and this was done for a few greenhouse plants, which gave results similar to the garden plants used.

Among the garden plants in which special cytological examinations of the plants tested were not made, no abnormal somatic conditions were observable, and none of the pollen-tube preparations obtained from the use of tetraploid plants over a period of several years showed more favorable conditions than the tests obtained in 1927 and used in this study.

The cross  $4n \times 2n$ , in which pollen-tube growth is favorable, is highly sterile because of difficulties in fertilization, or difficulties in the development of  $3n$  zygotes, while in the cross  $2n \times 4n$  the difficulties of pollen-tube growth are added to these other barriers to crossing. Thus  $4n$  plants are almost completely isolated biologically from  $2n$  plants.

In all combinations in which triploid pollen was tested, this pollen was found to be highly impotent, all but a small fraction of it being incapable of germination. The chief difficulties in all pollinations with this pollen are attributable to the abnormal chromosomal conditions, in that most of the pollen grains have several extra chromosomes. The proportion of normal-appearing pollen tubes, presumably with 12 chromosomes is between 1.2 percent and 1.7 percent whether grown on  $3n$  or  $2n$  styles.

*Pollen germination*

In all of the combinations in which the pollen of  $2n$  plants was employed the germination was well above 93 percent. We take this fact to indicate that the experimental conditions of pollen germination on cut flowers were very favorable; they were much more favorable in fact than similar tests on plants growing in the garden.

When the pollen of  $4n$  plants was employed, its germination was only about 75 percent on  $4n$  and  $3n$  stigmas, and about 85 percent on  $2n$  and  $n$  stigmas. We are, at present, unable to account for the difference. The additional pollen tubes produced by the higher germination in these crosses probably only increases the proportion of abnormal or burst pollen tubes in the population.

The pollen from triploids is always highly impotent on all stigmas and this is because it gives a very low germination. This pollen was also tested on  $4n$  stigmas with no better results than on  $3n$  or  $2n$  stigmas, and there is no reason to suspect that it might be better on the stigmas of haploids, a test which we have not yet made. The difference between figure 12 and figure 13 may be due at least in part to an experimental error. In the tests of former years represented by figure 12 no special precautions were taken to prevent the loss of ungerminated pollen from the stigma during staining and mounting. Figures 13, 14 and 27-211-1, in fact, all tests made in 1927 were handled in a manner which would prevent or minimize the error from the loss of ungerminated pollen grains remaining on the stigma.

*Conditions in haploids*

When the pollen from haploid plants was employed, the tests showed from 23-42 percent (general average 32 percent) of the matured pollen ungerminated. This is true without exception of all other tests of this pollen made in previous years. A few of these former tests were included, but most of the preparations had faded so badly that the pollen tubes were too obscure to be counted accurately and these slides were therefore not counted and tabulated. With regard to the relative proportion of ungerminated pollen on these old slides, they show from inspection that there is always a similar large proportion of matured pollen grains which fails to germinate.

The cause of non-germination of a large proportion of the pollen of  $n$  plants is not fully understood. The bursting pollen tubes are also present in small proportions in tests employing pollen of  $n$  plants and may be attributed to similar causes. These might represent new genes, causing



abnormalities in the pollen, and therefore wholly or partially lethal. The gene tricarpel (BUCHHOLZ and BLAKESLEE 1927a) had its origin in a haploid plant, and though it is transmitted through the pollen, its transmission is accomplished with a high pollen-tube mortality.

Three hypotheses suggest themselves as offering possible explanations. On the hypothesis that these abnormal pollen grains and pollen tubes represent gene mutations, they would be classifiable as lethals, genes which are automatically sifted out in the processes of gametophytic selection. MULLER and ALTENBURG (1919) have shown that lethals occur in *Drosophila* much more abundantly than genes which survive to become visible. Our *Datura* records to date for genes derived from a haploid plant (BLAKESLEE, MORRISON and AVERY 1927) have given between 2 and 3 percent genes which survive developmental selection and give visible effects in the sporophyte generation. The proportion of visible genes to lethals or those not transmitted with recognizable regularity would therefore have to be in the proportion of 1:11, 1:12 or more, if this hypothesis accounts for all of the 32 percent non-germinating pollen grains.

It might be urged against this hypothesis that relatively few visible gene mutations are known in *Datura* from any source (BLAKESLEE, MORRISON and AVERY 1927), and this hypothesis would assume a surprisingly high basic mutation rate. The basic mutation rate, however, has been found to be very much lower in diploids. The various processes of developmental selection (BUCHHOLZ 1922, BUCHHOLZ and BLAKESLEE 1922, 1927a) which are gradually becoming more fully understood and appreciated, offer a possible explanation for their continued elimination of new genes and thus may account for the general scarcity of easily recognized gene mutants in *Datura*. It is possible that in addition to a higher basic mutation rate in haploid plants we have a less severe elimination of new genes in an  $n$  plant under the special conditions at the time when it is producing its  $2n$  offspring.

Another possible explanation for these non-germinating pollen grains is that they represent gametophytes with chromosomal deficiencies, some of the microspores with less than 12 chromosomes arising from pseudo-reduction.

If even a small proportion of the total product of pseudo-reduction possessed only 11 chromosomes and yet was capable of reaching maturity, this class might be of considerable significance as a component of the matured grains in view of the high amount of pollen abortion. However, we have assumed that chromosomal deficiencies result in abortive pollen,

since all  $(2n-1)$  deficiencies (chiefly sectorial branches) thus far recognized have produced at least 50 percent abortive pollen (BLAKESLEE and CARTLEDGE 1926, 1927). Thus, in an anther of a flower from a  $(2n-1)$  branch, in which the output of pollen with 12 chromosomes is 50 percent, gametophytic selection in the pre-pollination stage apparently eliminates half of the pollen output within the anther or all of the pollen grains with 11 chromosomes. A third possible explanation is that these ungerminated pollen grains, together with the pollen tubes which burst in the style, represent pollen grains with 13 or 11 chromosomes, brought about by non-disjunction in sporogenous tissue. BELLING and BLAKESLEE 1926, report two such cases. Non-disjunction in the division following non-reduction would also give rise to pollen grains with 13 and 11 chromosomes. That such non-disjunctions may occur in haploids is suggested by the relatively high percentages of non-disjunctional types produced by a haploid (BLAKESLEE, MORRISON and AVERY 1927), although their occurrence might be due in part to non-disjunction in the early zygote, which therefore could not be of significance in connection with the pollen. Any non-disjunction associated with non-reduction might be of considerable significance in haploids due to the large proportion of grains which abort, as has been suggested in the preceding paragraph.

On the basis of known facts, it is possible that all three hypotheses are to some degree correct. There is good reason to believe that both the abnormal pollen tubes and the non-germinating pollen grains produced by a haploid plant are in some way connected with a high number of spontaneous chromosomal and gene mutations found in haploid progeny.

#### *Developmental selection*

The process of developmental selection, which has been alluded to in previous paragraphs, has been discussed in previous publications (BUCHHOLZ 1922, BUCHHOLZ and BLAKESLEE 1922, 1927a). These experiments on *Datura* serve as further illustrations of some of the specific eliminating effects of this mechanism of evolution.

We are concerned with many of the forms of developmental selection in these experiments. In angiosperms, the most important of these are interovular and gametophytic selection. Interovular selection brings about eliminations between ovules within the same ovary; (a) after the fertilization stage. This form of interovular selection is illustrated by the high mortality among the enlarged ovules or developing seeds of *Datura* resulting from the cross  $4n \times 2n$ , or the high selective mortality from the

cross  $3n \times 2n$ . In the cross  $4n \times 2n$ , there are obtained only a few viable seeds per capsule plus very many abortive ovules which had begun to enlarge but which failed to mature. The term interovular selection implies that the ovules containing individuals lost during seed development may have been crowded out during embryonic stages in competition with other ovules containing more vigorously growing individuals. Of course, many zygotes are lethal due to their genetic constitution and might not be able to survive under the most favorable conditions. Enlarged ovules which are eliminated may include those in which fertilization was not complete, resulting in an embryo without endosperm or endosperm without a fertilized egg, as well as those containing perfect embryos. An ovule containing an embryo derived by parthenogenesis would tend to be eliminated if this weaker individual is growing in competition with  $2n$  zygotes in the ovary of a  $2n$  plant. On the other hand, the parthenogenetic individuals which are produced in the seed capsule from a  $4n \times 2n$  cross, where they would be  $2n$  (or  $2n \pm 1$ ), would probably be among the survivors, as our genetic data indicate.

Interovular selection may also take place: (b) before the fertilization stage, bringing about a selective elimination between ovules within the same ovary, probably due to the type of female gametophytes which they contain. We have observed many ovules which had pollen tubes extending into the micropyles but which had remained unenlarged at a stage when neighboring ovules had increased their volume many times and contained embryos. The detailed study of these early aborting ovule types presents some difficulties, and we have not pursued our investigations of these small aborted ovules beyond the point of definitely ascertaining the fact that these stages of elimination exist.

Gametophytic selection is of two forms or phases: (a) between female gametophytes; and (b) between male gametophytes. That which may occur between female gametophytes in *Datura* within the same ovule (that is, between the four megaspores or their products) has not been investigated. Our experimental studies have been confined very largely to a study of (b) the gametophytic selection in male gametophytes. We have come to distinguish between two stages of the latter: gametophytic selection between male gametophytes within the anthers before the maturity of the pollen; and gametophytic selection between male gametophytes after the pollen reaches the stigma. This latter stage is most successfully subjected to experimental study and our investigations have been directed chiefly toward a better knowledge of this form of developmental selection.

The gametophytic selection which takes place within the anthers during the development of the pollen grains from microspores is a form or phase of developmental selection. As pointed out in the previous pages, a 24-fold enlargement, accompanied by a nuclear division, takes place during these stages, and in some types many microspores are eliminated as aborted pollen. The great range in the sizes of the aborted pollen grains of triploids and haploids indicates that eliminations occur in all stages from the smallest microspores on to the stages approaching pollen maturity. The facts seem to indicate that, on the whole, this pre-pollination stage of gametophytic selection eliminates most largely the deficiency gametophytes with less than 12 chromosomes, while the gametophytes with extra chromosomes are most largely eliminated by gametophytic selection after pollination.

The phase of gametophytic selection which takes place among male gametophytes after pollination is one of the most important stages in which eliminations occur. This is well illustrated by the tests involving the use of triploid pollen in which the small proportion of pollen tubes with 12 chromosomes are effectively sifted from a large mass of pollen grains with excessive chromosomes. Only a few types of  $(2n+1)$  plants are known to exist in which an extra chromosome may be transmitted through the pollen, and in these instances, the pollen tubes grow at a slower rate. It is in pollinations using the pollen of these  $(2n+1)$  types that we have had some success in our efforts to control the selection of male gametophytes, through manipulations which tend to crowd or not crowd the pollen tubes, by cutting off previously pollinated styles at the proper time, and other similar methods.

Likewise in tetraploids ( $4n \times 4n$ ) the non-germination of 25 percent of the pollen and the early elimination of another 30 percent of the pollen tubes are important means which limit the transmission of newly acquired chromosomal abnormalities, while there is abundant evidence that interovular selection also eliminates a high proportion of abnormal female gametophytes and zygotes.

The effect of the various processes of developmental selection in tetraploids and diploids is, therefore, to render them more or less stable. Tetraploids have been maintained for many years in cultivation as distinct races from generation to generation. Triploid and haploid types are not capable of maintaining themselves; the same processes operate to render these types unstable. In these types, developmental selection tends to eliminate or hinder the reproduction of their kind. Triploids produce relatively few seeds, and may give rise to diploids,

tetraploids, and various  $(2n+1)$ ,  $(2n+1+1)$ , types rather than  $3n$  plants, while haploids give rise to diploids, and to  $(2n+1)$  chromosomal types (BLAKESLEE, MORRISON and AVERY 1927), rather than to haploid types. In addition a haploid induces a relatively high proportion of visible gene mutants. There seems to be good evidence that a much higher proportion of gene and chromosomal abnormalities (perhaps 10-12 times as many) are produced in the pollen of a haploid than can become recognizable in the adult plant.

#### SUMMARY

1. Pollen-tube growth was studied by means of tests made under controlled conditions, using a technique described elsewhere. Pollen-tube distribution curves and statistical tables were prepared showing the condition and distribution of the ends of pollen tubes in the style after comparable time intervals.

2. Abnormalities in pollen-tube growth were found in all plants investigated. These consisted of swollen ends and burst pollen tubes within the style. These occurred only in smaller numbers in selfs and crosses among  $2n$  plants and in the styles of any type pollinated with pollen having 12 chromosomes. When these occurred in excessive proportions, this is regarded as an indication of unfavorable conditions of pollen-tube growth.

3. A high proportion of ungerminated pollen remaining on the stigma is an indication of unsuitable genetic constitution of the pollen.

4. The nature of the style and stigma is an important contributory cause of the abnormalities in pollen-tube growth of certain combinations. Styles of  $n$ ,  $2n$ , and  $3n$  plants were found unsuited to the growth of pollen tubes coming from the pollen of  $4n$  plants. Styles of all types,  $n$ ,  $2n$ ,  $3n$ , and  $4n$  were found to be favorable for the growth of pollen from  $2n$  plants.

5. The pollen of tetraploid plants gave only about 75 percent germination on tetraploid stigmas, and about 85 percent germination on  $3n$ ,  $2n$  and  $n$  stigmas, where the pollen-tube growth within the style is unfavorable.

6. Abnormal pollen tubes due mainly to bursting were found in considerable numbers in  $4n \times 4n$  tests, in which pollen-tube growth was most favorable. This corresponds quite closely to the high non-disjunction in microsporogenesis of tetraploids, which results in many pollen grains with 23 and 25 chromosomes.

7. The pollen of triploid types gives very little germination, either

on their own type of stigma or on that of  $2n$  and  $4n$  types. Only about 10 percent of this pollen is capable of germination, and about 1.5 percent of the pollen grains produce tubes which may be presumed to have 12 chromosomes. This percentage is about 30 times that expected on the basis of random assortment found at reduction and suggests unknown factors affecting chromosome numbers.

8. From 60-70 percent of the small proportion of the pollen grains which mature in a haploid may germinate on the stigma of  $4n$ ,  $3n$ ,  $2n$  and  $n$  types. The pollen tubes within the style have a smaller proportion of burst tubes and present growth conditions otherwise similar to  $2n \times 2n$  tests, except for the tests  $n \times n$ .

9. Three hypotheses are proposed in explanation of the nature of the ungerminated portion of the pollen from haploids. Of these, the suggestion that some may contain new genes which are lethal in the male gametophytic stage, and the suggestion that some may be pollen grains with 13 chromosomes, is in line with genetical evidence; another suggestion that some may be pollen grains with 11 chromosomes arising from pseudo-reduction is improbable but not entirely disproved.

10. The nature and role of developmental selection in relation to the types of offspring produced by the  $n$ ,  $2n$ ,  $3n$ , and  $4n$  types is discussed. While the actual origin of the chromosomal types depends upon cytological processes, developmental selection in its different forms serves as a sieve which determines the type of offspring which may survive to the next generation and modifies the proportions in which these types may appear.

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