Pro ducing at will of fertile diploid and tetraploid gametes in *Duc van Thol, Scarlet (Tulipa suaveolensRoth)*.

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With 22 figures in text and on plates IV and V.

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I. Introduction.

It is a great pleasure to the writer to have this paper published in the Vierteljahrsschrift der Naturforschenden Gesellschaft in Zürich, on the Occasion of the 70th anniversary of his esteemed teacher and friend Professor HANS SCHINZ.

In 1920 and 1921 through particular external causes the experiment succeeded to cause in the *Hyacinth* the development of pollen-grains with a larger number of nuclei than normal, or with nuclei which contained the somatic number of chromosomes instead of the haploid number. Later, in 1923, the attempt to originate triploid off spring from diploid parents by pollination with duplicated pollen-grains was crowned with success. See nos. 1921-1926 a, 1927 c-1927 e and 1928 b.

In 1923 similar experiments were begun at the *Tulips.* The results hereof have partially been published in short notes and papers. See nos. 1926 b, 1927 a, 1927 b, 1928 a und 1928 c.

In this paper the writer intends to exclusively report the results obtained with *Duc van Thol, Scarlet (Tulipa suaveolens)* and chiefly concerning the karyologic research and the fertility of the pollen-grains.

lt has become obvious to him that one can at will produce diploid and tetraploid pollen-grains or not, according to the external circumstances under which the bulbs are grown. The genetic constitution of *Scarlet Duc* is such that the treatment need not be extraordinarily different from these methods of culture. Diploid and tetraploid pollen-grains appeared to originate already with different Dutch ways of growing.

In 1926 and 1927 *Scarlet Duc* was grown and treated in 17 different ways. Thus it was possible to state, generally at least, under which conditions diploid and tetraploid pollen-grains develop and in which circumstances they do not.

The principal cause in the author's opinion is that, owing to the low temperature e. g. 3 • 6°-11 • 6° Centigrade (38°-53° F.) to which the bulbs are exposed in the ground in Holland in October, the meiosis which happens at that time often is stunted in its normal course. Conf. 1928 a.

The experiments *I—XVII* are reported in detail in an other paper whilst at the same time the experiences in this relation at other *Duc van Thol-varieties* are mentioned. Where, in this paper, the necessity arises to mention one of the above experiments the narre *Scarlet Duc* is followed by /, //, &c.

The reports on the experiments with *Tulips* are not done with yet. For this reason the writer has not yet added the references to other literature nor his discussion. "No one has, so far as I know, reported the occurrence of tetraploid gametes, but it is possible to imagine them, theoretically, as a result of non-disjunction of daughter-chromosomes after the homeotypic division, if the preceding reduction were omitted, (as during the formation of diploid gametes)", M. NAVASHIN states, concerning the question how pentaploid forms of *Crepis capillaris* may have originated. (Genetics 10, p. 589, 1925).

The writer thinks that this paper may be regarded as the beginning of answering the question in general.

II. Duc van Thol, Scarlet and its origin.

The *Duc* van *Thol-varieties* are the earliest-flowering *garden-Tulips,* often being in flower before the end of March and in full flower during the first weeks of April. As a rule they rarely exceed six inches in height.

The flower is pointed in the bud and cup-shaped when open. They are principally suitable for pot-cultivation.

Duc van Thol,Scarlet, Fig. 1, is characterized by small, dark-scarlet flowers which are being forced in thousands for Christmas, as it excels by its ability to be forced.

It is bred from seed at Overveen, near Haarlem.

The *Duc van Thol-varieties* are often regarded as descendants of *Tulipa suaveolens* ROTH, a rather rare species, occurring in Southern Russia, as their peculiarities are very much alike.

Reference is given in this relation to the paper, also concerning *Duc van Thol-Tulips* which the author expects to publish next.

III. The karyologic research of the Pollen-grains.

In order to be able to observe distinctly the shapes and sizes of the chromosomes as well as to count their number, the pollen-grains were brought into aceto-carmine which was prepared in three different ways, about the same as indicated by BELLING in The American Naturalist, Vol. 55, p. 573-574, 1921, and in Biol. Bull. of the Marine Biol. Laborat., Vol. 50, Nr. 2, p. 160-162, 1926.

To that effect the following stages were Chosen:

1st the time when the tobe-cell in the microspore begins to divide to form the generative cell;

2nd the time when the generative cell is dividing again to form the two male gametes.

Hereby the number of chromosomes could be established, whilst at the same time the answer would be found to the question whether the large pollen-grains which were supposed to contain diploid or tetraploid nuclei differed in certain respects, as to the development from monoploid pollen-grains.

Had this question to be answered positively, then the supposition had a better foundation that the possibility exists to intentionally cause the forming of triploid and pentaploid *Tulips*.

A. On March the Ist, *Starlet Duc* X had grown so far that the vegetative nuclei, in many pollen-grains, were dividing. The bud

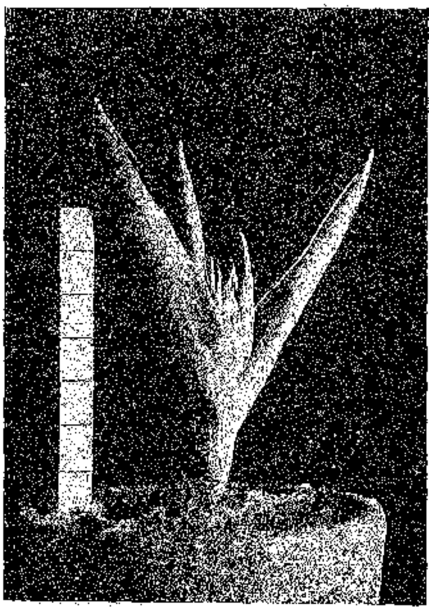


Fig. 2

is still green then, the stalk not stretched, Fig. 2. The pollen was teased out with steel blades and needles in drops of aceto-carmine; to which no ferric hydrate was added. The solution of glacial acetic acid was not stronger than 25 %. Crystals of chloral hydrate were added. Object glassec: 75 X 25 mm; cover-glasses: 50 X 22 mm. On the 8th and 9th of March the slides were examined. The method followed appeared to work very satisfactorily. In this young flowering stage the chromatin of the nucleus is now of a bright carmine colour and is very clearly contrasted with the much paler colour of the cytoplasm which moreover is slightly troubled. When the nucleus is dividing,

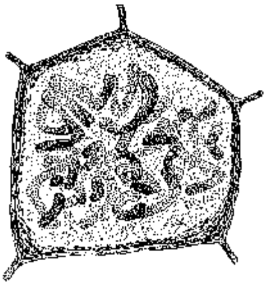


Fig. 8

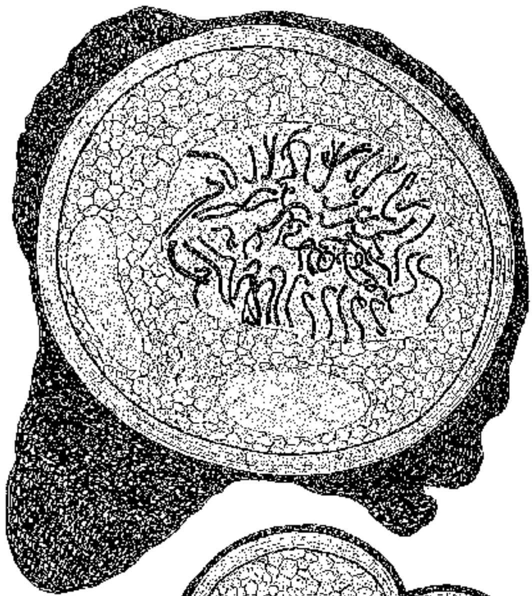


Fig. 12

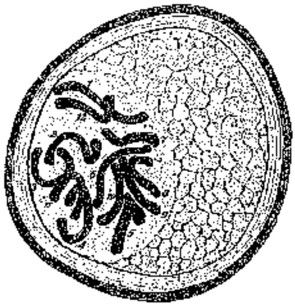


Fig. 10

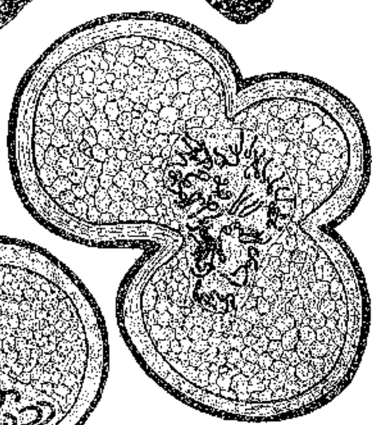


Fig. 13

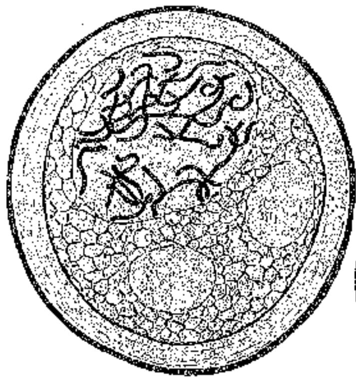


Fig. 11

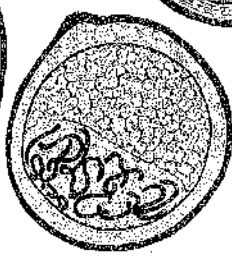


Fig. 9

the chromosomes are of a deep bluish-red or slightly brown-red. In the anthers some microspores occurred in which the generative nucleus was already formed. The partition-wall of the tube-cell was often not yet bent globular, but level. This partition-wall was of a fine bright yellow. The same colour characterized the intine. The exine was of a troubled yellow.

Many sterile quartets (tetrads) occurred, and at the same time often many duets (dyads) of the same size. As the nuclei of the duets had a volume of approximately twice the volume of the nuelei of the quartets, the supposition arose that the meiosis had not happened in the normal way in the first case, whilst it had in the second case. But moreover numerous microspores occurred of three sizes which gave the impression of being fertile. It now appeared that the vegetative nucleus

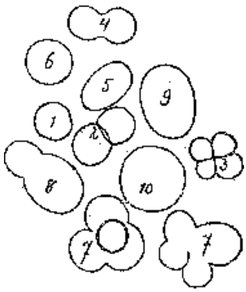


Fig. 14

of the smallest of these pollen-grains contained 12 chromosomes, 6 long ones and 6 short ones, Figs. 9 and 10.

The vegetative nucleus of the pollen-grains of medium size contained 24, 12 long ones, 12 short ones, Fig. 11. That of the largest ones was characterized by 48 chromosomes, 24 long ones and 24 short ones, Figs. 12 and 13.

It is clear that *Scarlet Duc* is a diploid variety, so it is characterized by 24 chromosomes, 12 long and 12 short ones, in the somatic nuclei, Fig. 8.

At the beginning of the research it appeared invariably that the large (diploid and tetraploid) pollen-grains very often were ellipsoidal, whilst the pollen-grains of normal size, the monoploid ones, were characterized by a globular shape. See Figs. 6, 7 and 14. Only recently, after examining a great many more varieties of *Tulips,* it has become clear to which circumstance the globular shape is related.

After stating that the larger pollen-grains of *Scarlet Duc* were either diploid or tetraploid, it was demonstrated clearly that the diploid Pollen-grains occur in the forms as presented by the numbers 2, 4, 5 and 6, Fig. 14. In the pollen-grains indicated by 2 a diploid nucleus is present. Though both meiotic divisions were not completed the intine and exine had still taken the shape of a duet; the partition-wall however failed. The exine had remained thin and transparent.

Still it occurs repeatedly that the partition is present so that the haplosis (heterotypic division) may have happened normally. Such duets are often sterile.

At the pollen-grain no. 4 the exine is built normally all over. The stringing still points to the tendency to form duets in normal cases. This is indicated at no. 5 by the ellipsoidal shape. At no. 6 the pollen-grains have become completely globular like the monoploid (no. 1).

In many figures the various shapes of diploid pollen-grains are to be found. See Figs. 6, 7, 14, &c. The pollen-grains which appeared to be tetraploid not seldom had a shape as indicated by no. 7. In the same anthers such shapes were always accompanied by quartets as indicated by no. 3.

While the research was being done, such quartets were nearly always sterile. The sizes were rather deviating which must point to a non-simultaneous abortion. The originating of the 4 sphere-segments (no. 7) demonstrates the tendency to form a normal quartet, which tendency the cell-wall still possesses.

As with the originating of the, diploid pollen-grains, the first meiotic division (haplosis, heterotypic division) is stunted.

But fiere, in contrast with that which happened with the pollen-grains which remained diploid, the nucleus was stimulated to effect a splitting of all 24 chromosomes, so as to originate a nucleus with 48 chromosomes.

Just as the originating of the 4 sphere-segments is the visible demonstration of the building of a normal quartet, this splitting of chromosomes which, to a certain extent, Shows a relation with the second meiotic division (homeotypic division) in normal cases, is the cause that the duet becomes a quartet of which each sphere-segment contains a nucleus of 12 chromosomes.

In the case related, complete disjunction has not taken place, but only separation of the equationally split chromosomes.

The chromosomes have remained united as one nucleus. According to the theory of chiasmatypy, in the case of the coming into being of a normal quartet, the two homologous chromosomes may be subject to a breaking and a fusion in one or more points at some stage in the prophase of the first meiotic division, so that by mutually exchanging the segments, homologous chromosomes have originated, characterized by new constitutions. Has this stayed away at the chromosomes out of which the diploid and tetraploid pollen-grains are constituted? Do these chromosomes consist of the same segments as the chromosomes of the pollen-mothercells? This has still to be examined.

It need not be stated that also after the originating of the generative nuclei the total number of chromosomes in the 4 monoploid pollen-grains is equal to the number in a tetraploid pollen-grain.

B. On March the 8th, slides were prepared of the pollen of *Scarlet Duc* X in 3 different ways:

ist the same liquid was applied as in A. A crystal of chloral hydrate is added to the drops on the object-glass;

2nd to the aceto-carmine ferric hydrate is added abundantly. After the settling of the superfluous ferric hydrate and the clarification the liquid is applied. As in ist, a very Small quantity of chlöral hydrate is added;

3rd to a mixture of 9 parts alcohol 90 % and 1 part glacial acetic acid a sufficient quantity of solution of ferric hydrate in acetic acid is added about as BELLING indicates. On the ist of March the anthers are brought into this. On March the 8th the pollen in the slides is transferred to the aceto-carmine, as in A.

In none of the 3 cases the chromosomes could be observed as distinctly as in A.

In the first two cases this was caused by the circumstance that in the lapse of time between the Ist and the 8th of March the exine had thickened in such a measure that its peculiar markings made it impossible to distinctly observe the contents of the pollen-grains. In the first case the pollen-grains were of a much darker colour than in the second case. The sizes of the nuclei were clearly visible.

In the third case the pollen-grains were quite black. The largest looked like ink-blots. Through the extraordinary contrast with the surroundings they often were visible separately with the naked eye, especially when the slide was being held up against the light. This always was the case with the tetraploid pollen-grains. Here moreover the differentes in shape see Figs. 12, 13 and 14 — could be observed also without the aid of a microscope.

In all three cases the slides produced an excellent material, suited for photomicrographs to give a general view of the haploid, diploid and tetraploid pollen-grains, Fig. 7.

C. On the 11th of March slides were prepared from the pollen of flowers of ,*Starlet Duc* ./X on the verge of flowering:

1st in aceto-carmine as in A;

2nd in iron aceto-carmine, as in B, 2nd, not adding chloral hydrate however. On the 14th of March the slides were examined. In the latter case the contents of the pollen-grains was strikingly P ale, as in the case of B, 2nd, though chloral hydrate was added thtme.

So this adding of chloral hydrate does not influence this probably. In these slides the outlines of the pollen-grains were so distinct and exact as to give the most excellent results for photomicrographs or drawings.

In the first case, where the pollen had been fixed and stained as in A, but where it had developed slightly more, though not so far yet that the markings of the exine prevented a clear observation of the chromosomes, stages were found at the tetraploid and diploid as well as at the haploid pollen-grains, where the generative nucleus was still dividing.

It occurred a few times that the tube-cell had already formed a short tube.

Moreover slides were prepared of the pollen used for B, 3rd.

3rd it is brought into a liquid, as in B, 1st.

4th it is brought into a liquid, as in B, 2nd.

In these two cases the chromosomes are not to be distinctly observed, in 4th slightly better than in 3rd.

D. On the 17th of March once more slides were prepared of *Starlet Duc* IX from the pollen of the flowers nos. 1, 2, 3, 4, 5 and 10.

1st in the liquid as in A;

2nd in the liquid as in C, 2nd;

3rd in the liquid as in C, 2nd, now newly made, exactly as BELLING'S prescription.

On the 21st of March the slides were examined. Again the chromosomes are to be most distinctly observed in the first case, though the markings of the exine marr the clearness.

In the second case everything is too pale again, as in C, 2nd. In the 3rd case the chromosomes are not well observable either. The pollen-grains are slightly lighter coloured than in the first case.

From the above it appears that for several reasons it is preferable:

Ist to fix the pollen-grains an the moment that either the tube-cell or the generative cell is in its division-stage, so tha t its nucleus is in metaphase;

2nd to fix and to stain the pollen-grains simultaneously in ordinary aceto-carmine of which the solution of glacial acetic acid may be weaker than 45 % as BELLING (1921) indicates, whilst the addition of chloral hydrate may be omitted.

The research was best accomplished by alternately applying the magnifications of 520 X and 1120 X.

IV. A. constant **relation between the number of chromosomes and the surface of the nucleus and that between the number of chromosomes and the surface of the cell.**

One has only to refer to manuals as those of SHARP, TISCHLER and WILSON to get a sufficient insight into the researches done by many investigators concerning further affirmation of the conclusions put down by R. HERTWIG, TH. BOvERI and J. J. GERASSIMOFF, relating to a constant relation between the volume or the surface of the nucleus and the volume of the cytoplasm,

Fig. 1

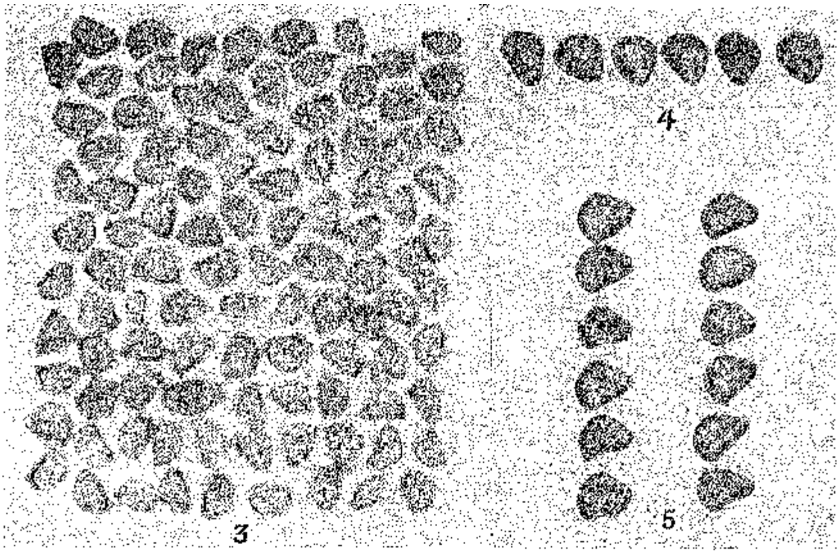


Fig. 3, 4, 5

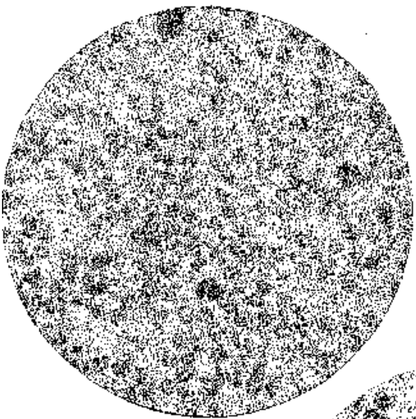


Fig. 6a

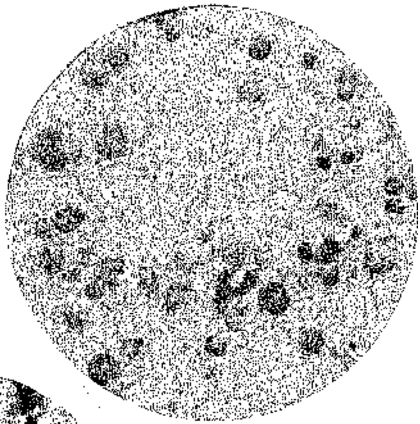


Fig. 6b

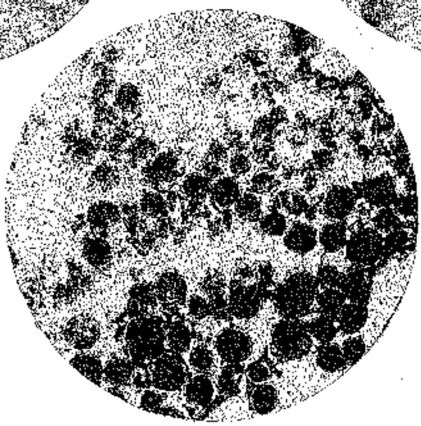


Fig. 7

and further between the volume or the surface of the nucleus and the number of chromosomes.

It need not be stated any more that in the first case it is t h e d i f f er e n c e in s i z e of the nuclei and of the cells which caused the author to accept the supposition that pollen-grains were formed of which the nuclei should contain more than 12 chromosome s.

Repeatedly the volume and surface of the nuclei and of the cells have been calculated.

When comparing pollen-grains — containing the same number of chromosomes in their nuclei — from different flowers which are even in the same flowering stage, then these sizes very often diff er fairly much. The cause of this is that one pollen-grain sometimes has developed more than the other one, or that it has grown under better conditions.

The diameters (2 a) of the globose monoploid, diploid and tetraploid vegetative nuclei of the pollen-grains amounted to the average of 32 • 69, 47 • 11 and 65 • 38 ,u so that the relations were established at 34, 49 and 68, e. about 5, 7 and 10. The moment was thus chosen that no generative cell had originated yet.

Thus the relations of the surfaces (47T a^2) are 25, 49 and and 100, i. e. about 1, 2 and 4, which corresponds with the relation between the numbers of chromosomes (12, 24 and 48).

From this calculation the conclusion clearly follows that the relation between the number of chromosomes and the surface of the nuclei corresponds in a much greater measure than the relation between the number of chromosomes (12 : 24 : 48) and the volumes ($\div$ a^5) of the nuclei (about 5 : 14 : 40).

As diameters of the monoploid, diploid and tetraploid globose pollen-grains were found: 56, 75 and 100 $_A$t. So the relation is about 2, 3 and 4. Here also the relations between the surfaces of the pollen-grains (4, 9 and 16) and those of the numbers of chromosomes (12, 24 and 48) agree much more than the relations of the volumes (8, 27 and 64) and those of the numbers of chromosomes.

J. D. OPPENHEIM states in "Onze Tuinen", April 15th. 1927, p. 230 that he found the diameter of the pollen-grains of *Darwin-*

Tulip Mahagonie to be 57 At. As the chromosomal complement of the diploid *Darwin-Tulips* agrees with that of the *Duc* van *Thol-Tulips* — conf. 1925 a, References — one Sees that these statements correspond with those of the writer.

V. **The germinating of the monoploid, diploid and tetraploid pollen-grains.**

On the 6th of January 1927 the pollen of *Scarlet Duc VIP* was brought an to the stigmas of some flowers of the *Darwin-Tulip William Copland.* The greenhouse-temperatures on the

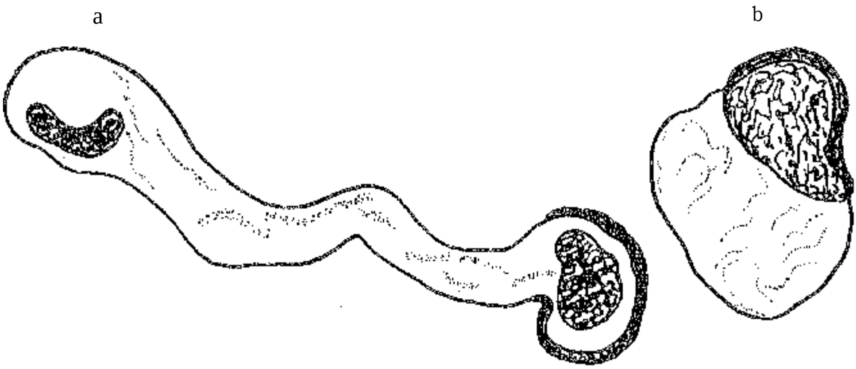


Fig. 15

6th, 7th and 8th of January were 74°, 78° and 75° F., respectively, whilst the degree of moisture was exceedingly high.

After 48 hours (Jan. the 8th) the pistils were cut off; the stigmas were then carefully rinsed in drops of water, deposited an some ten object-classes.

The stigmas of *William Copland* which had been pollinated by the pollen of *Scarlet Duc V* (3rd and 4th of Jan.) were treated in the same way.

In both cases it appeared that the diploid and the tetraploid, as well as the monoploid pollen-grains germinated abundantly, sometimes with extraordinarily long and thick tubes. Of the diploid and tetraploid pollen-grains they often were bag-, bladder-, balloon- or bottle-shaped, Fig. 15.

On the 4th of January too, the pollen of *Scarlet Duc V* was brought on to the stigmas of the *Single Early Flowering Tulips Prince of Austria* and *Cramoisi Brillant* in the same greenhouse. The pistils were cut off on the 8th of Jan., packed in small paper bags and were taken home, same as happened with another 2 pistils of *William Copland.*

On the 10th of January the stigmas were again carefully rinsed, in drops of a solution of methyl green in acetic acid.

On the stigmas of *Prince of Austria* the greater part appeared not to have germinated. Only rather many germinated monoploid pollen-grains were observed. The tubes were often short and 'hick, especially so at the end. The nuclei were often distinctly observed though it was difficult to state their size aecurately.

Fig. 16 a

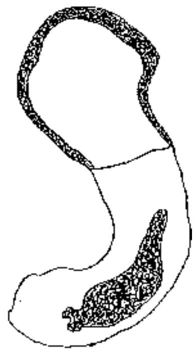


Fig. 16 b

The same result was obtained of *Cramoisi Brillant,* Fig. 16. lt appeared that the pollen-tubes locally have often widened considerably. In rare cases the chromosomes of the generative nuclei which had just originated could be observed, not very distinctly however.

At *William Copland* the pollen had germinated muck better on one stigma than on an other one. The pollen-tubes often showed a very striking thickness.

On. March 11th slides were prepared — in a solution of iron carmine, as stated in Chapter III, und er A and under B, 2nd — of pollen-grains of *Scarlet Duc* which were brought on to the stigmas of *Single Early Tulip La Reine* some days previously (planted outdoors, without protection of a gauze cage). It appeared that few had germinated. Some monoploid pollen-grains had developed a very long tube. They could be rinsed without breaking the tube, Fig. 17.

On April the 23rd, 1927, the pollen of *Scarlet Duc XVII* was brought on to the stigmas of some Kare of flowers of a new, not yet catalogued *Single Early Flowering* seedling which was planted outdoors and transferred to a gauze cage with glass cover before flowering-time.

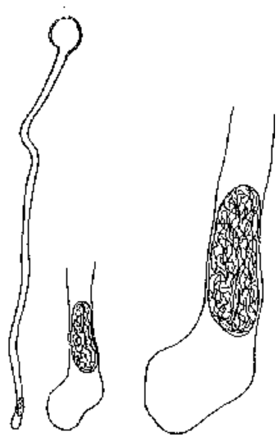


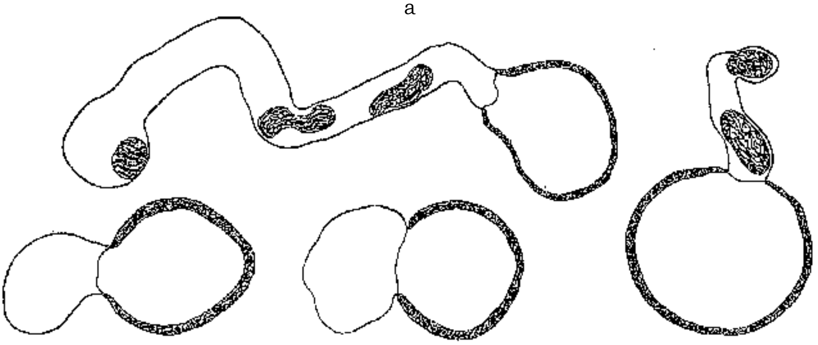
Fig. 17

On the 30th of April, 10 pistils were cut; the stigmas were treated as before, but now in a solution of iron carmine, as stated in chapter III under B, 2nd, with chloral hydrate.

Of this solution a few drops were deposited on each object-glass.

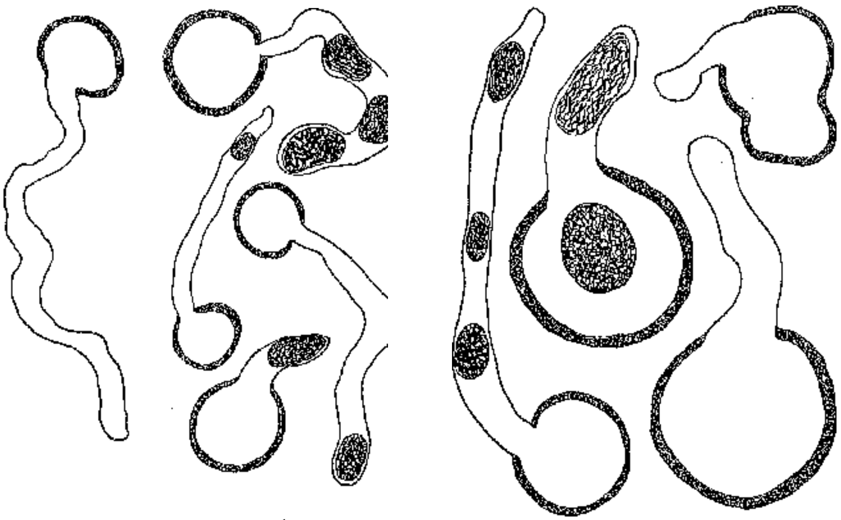
The pollen was examined superficially at first. The number of pollen-grains which had not germinated appeared to be in the majority. Then the slides were prepared. The other 10 pistils remained untouched to find out whether they would grow into capsules. On the 11th of June the slides were definitely examined. Though the majority of the pollen-grains had not germinated, the absolute number of germinated ones was yet large. lt is distinctly shown here that the monoploid pollen-grains germinate quicklier and more easily than the diploid and tetraploid ones. The pollen-tubes of the monoploid ones were nearly

a



b b b

Fig. 18



a b c

Fig. 19

always decidedly longer and of a more normal shape than those of the diploid and tetraploid ones, Fig. 18.

On May the 2nd some 20 flowers of *Single Early Flowering Tulip Hobbema* (planted outdoors, without protection of the gauze tage) were pollinated with *Scarlet Duc XVII.*

On the 7th of May slides were being made in the same way as on April the 30th, and also in the liquid, described as chloral hydrate in B, 2nd. These were minutely examined on June the ist. It looked as if the pollen had germinated much better on some of the stigmas, than on all the others. This probably has to be ascribed to the fact that the first mentioned pistils were in a more favourable receptive state, and not to the facts that the temperature was fairly low and that the pollen had already been kept in the paper bags for some time.

The proportion of germinating of the diploid and tetraploid pollen-grains and of the monoploid ones was the same as stated on June the 11th (Fig. 19).

VI. **The** isolation of **the diploid and tetraploid pollen-grains.**

In many ways the author tried to isolate the diploid and tetraploid pollen-grains from the monoploid ones. This was necessary especially to pollinate separately; for it is not possible to observe them in a drop of water as they have then generally become unfit for the pollination. The method which appeared to be the best one is thus: a dry, clean, not rusty or rough, prepare-needle is inserted — the less the better — into an open anther and then tipped off over a dry object-glass. The clod of pollen is then observed with the microscope (magnification 260 X). It appears to contain some 20 pollen-grains. Such a clod of pollen-grains, left untouched after being tipped of the needle is shown in Fig. 20. It is extraordinary how distinctly the diff erence in size is to be seen, even of these dry pollen-grains. In Fig. 20 the sterile pollen-grains and the fertile monoploid, diploid and tetraploid pollen-grains are indicated respectively by s, h, d and t.

It is recommendable to state first of all where the pollen is situated which has the highest number of diploid and tetraploid pollen-grains, as the numbers of diploid and tetraploid pollen-grains and their shapes in the flowers of plants which have been subject to the same circumstances as well as possible, may still diff er widely, and they will even often diff er in the anthers of one flower and even in different places in the same anther. This will have to be examined first in a drop of water.

Now a second and a third object-glass is used on which some small circles have been drawn with India-ink, in a straight line, in the middle of the glass in the length of it, so as to simplify the observation by means of a Cross-table at the magnification of 260;<.

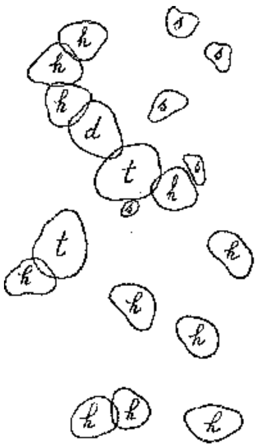


Fig. 20

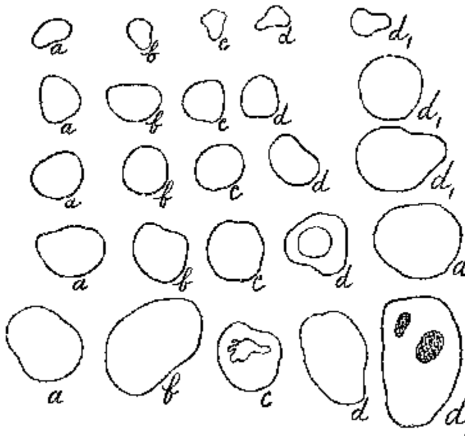


Fig. 21

The small clod of pollen on the first glass is now touched with the point of the previously cleaned needle. Then the needle is again tipped off within the outline of an ink-circle. Thus the pollen-grains are transferred to the second and third object-glass. The pollen-grains within the ink-circles are controlled with the microscope. It then appears that here and there in one of the ink-circles only one pollen-grain was dropped. Should the isolation not have happened as completely yet then the transfer must be carried on.

Experience makes it easy to conclude whether the isolated pollen-grain in tbe circle is a monoploid one or a diploid or tetraploid one. Attention is drawn to Fig. 21 where isolated pollen-grains exclusively are given. In the first row sterile ones, in the others fertile ones, viz monoploid ones in the second row, large monoploid or small diploid ones — this is not to be concluded as easily — in the third row, large diploid or small tetraploid ones in the fourth row and tetraploid in the fifth row. Pollen-grains d are the same as In the latter case the pollen-grains have been swollen in water. In Fig. 22 the tetraploid pollen-grain d_1 is reproduced at a magnification of 560 X. The generative nucleus is in mitosis. The wall of the pollen-grain shows a bell-shape, the beginning of the tube, in the ʸicinity of the vegetative nucleus.

In order to pollinate with diploid or tetraploid pollen-grains only, the object-glass was now carefully touched with the Stigmas

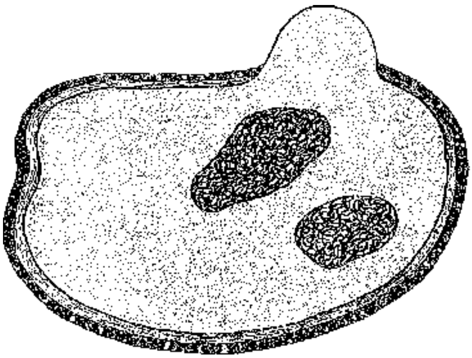


Fig. 22

at the spot where the pollen-grains concerned were situated. See further chapter VII.

VII. The pollination-experiments and their result.

The following cross-experiments were done with the pollen of *Scarlet Duc* which, apart from monoploid pollen-grains, contained diploid and tetraploid pollen-grains as well.

Crosses done	Number of flowers pollinated	Amber of capsules forma	Number of seeds obtained	Nennbar of seeds per eapsule
1 Single Early Tulip Brilliant Star 9 xSearlet Duo XVlcr	9	6	350	58
2 Single Early Tulip Rose Priem 9 xSearlet Dun XVIe	8	5	354	70
3 do. x da.	8	7	609	87
4 do. x do.	8	4	390	97
5 do. x (to.	8	0	0	0
6 Single Early Tolip Rose Prkoee 9 x Searletllue XIVg	8	7	953	107

Cross no. 1 happened indoors, in the greenhouse. The bulbs of *Brilliant Star* were planted in a wooden box.

The other crosses happened outdoors. The bulbs were planted in the full ground.

Each time one row of plants was chosen from the bed, As moulding nearly always appears when winning tulip-seeds — see further — the plants were not placed in a closed cage of glass and gauze; for the same reason the flowers were not enveloped in a parchment paperbag, nor were the unopened anthers taken from the flowers. This latter precaution is superfluous, owing to the self-sterility.

The principal purpose was to get the largest quantity of seeds.

For the erosses 1 and 4 the pollen of *Scarlet Duc XVI* was chosen which contained a very striking quantity of fertile diploid and tetraploid pollen-grains.

Amongst the 350 seeds from cross no. 1 which were rather small, 2 strikingly large seeds were found, Fig. 3.

For the crosses nos. 2--6 the same variety was used as mother-plant.

The various crosses on the field appeared at a regular mutual distance.

Cross no. 5 was a failure altogether.

The capsules from crosses 2 and 3 contained, apart from the good seeds, many germless seeds, even more than good ones. Of cross no. 4 the number of germless seeds was a little smaller. Thus the large number of diploid and tetraploid pollen-grains did not have a stunting influence on the forming of the seeds in this rase. See Fig. 4.

Cross no. 6, the only Gase in which the pollen of *Scarlet Duc XIV* was used and not that of *Scarlet Duc XVI,* produced the best result. See Fig. 5. Few germless seeds occurred. The capsules averagely contained 107 good seeds. It has to be stated here that the spring of 1927 was extraordinarely unsuitable for pollination and fertilisation of flowering bulbs owing to cold and rain.

The good seeds from crosses nos. 2-4 and 6 looked betten than those of cross no. 1 in the capsules of which only 58 probably good seeds were formed. The cause of this mainly is the planting of the bulbs in the full ground outdoors.

The pollination with isolated diploid and tetraploid pollen-grains (see Chapter VI) has yielded only moderate or even fairly bad results this year.

It seems to the writer however that the cause of this is a slight neglect in the method of pollinating.

It has become clear to him in the course of time that the papillas of the Tulip-stigmas are very much liable to wounding when the Pollen is brought on to it with a brush or when an anther is gripped with pincers and stroked along the stigmas.

The result of the wounds is that the cell-sap oozes out of the papillas. This causes mould on the stigmas and seeds. The growers call this "het vuur" and "het vervuren"; vuur (D) Eire; vervuren getting spoiled by "fire". This is caused by *Botrytis parasitica.*

The stigmas are than of a grey colour, caused by the great number of conidia-bearers and conidia.

Later one observes the black sclerotia on the seeds.

The rubbing of the object-glass along the stigma-papillas will have been just as wrong.

It is much better to drop the pollen on the stigmas by tipping it off carefully.

VIII. Perspectives.

It will appear from further papers, as was stated in the Introduction already, that *Scarlet Duc* belongs to the *Tulip-varieties* which possess such a genetic constitution as to enable one to duplicate and to quadruplicate its male gametes.

As this may happen already in Holland, only owing to different ways of culture, the supposition is justified that this is the cause of the originating of the pluriploidy already stated — conf. 1925 a, 1925 b, 1926 a, 1927 c and 1927 d — as was the case at *Hyacinthus orientalis* and *Narcissus Pseudonarcissus.*

Moreover the fact that the development of diploid and tetraploid pollen-grains may be promoted, gives rise to the possibility of produeing at will triploid forms— which was done at the *Hyacinth* already — and even pentaploid forms from diploid parents.

Amongst these Triploids and Pentaploids there may occur (if the *Tulip* reacts as the *Hyacinth* and the *N arcis)* some which are stronger and bigger than the largest diploid forms of the same colour, with a capacity of being easily forced. This may prove to be very important for the growers.

Undoubtedly however, theoretical profits will be gained from the developing of Triploids and Pentaploids next to Diploids, as e. g. in connection with a further penetration into the phenomena of the meiosis and into that of the dominance.

But this may also be important as to the question concerning the meaning of the nucleolus, as the research at the *Hyacinth*

IX. Summary in German (Zusammenfassung).

Duc van Thol, Scharlach, eine Varietät von *Tulipa suaveolens* ROTII, kann unter solchen Umständen gezüchtet werden, dass der Verlauf der Reduktionsteilung (Meiosis) ganz oder teilweise zerstört wird.

Im ersten Fall, wenn die heterotype Teilung (erste meiotische Teilung) und die homöotype Teilung (zweite meiotische Teilung) unterbleiben, entstehen Pollenkörner, deren Kerne die diploide Chromosomenzahl enthalten, nämlich 24, wovon 12 längere und 12 kürzere.

Im zweiten Fall, wenn die heterotype Teilung ausgeschaltet wird, aber die homöotype Teilung zustandekommt, entstehen Kerne, die 48 Chromosomen enthalten, nämlich 24 längere und 24 kürzere.

Oft ist an den Formen der diploiden und tetraploiden Pollenkörner noch klar zu verfolgen, dass erstere zu Dyaden (Duetten), letztere zu Tetraden (Quartetten) sich hätten bilden sollen.

Es ist wertvoll, die Pollenkörner in dem Augenblicke zu fixieren, wo entweder die vegetative Zelle oder die generative Zelle in Teilung begriffen ist.

Die Pollenkörner können ganz gut gleichzeitig in Eisen-Carmin-Essigsäure fixiert und gefärbt werden. Dazu darf die Essigsäurelösung unter 45 % sein. Chloralhydratkristalle hinzuzufügen ist nicht nötig.

Es besteht eine konstante Beziehung nicht nur zwischen der Zahl der Chromosomen und der Oberfläche des Kernes, sondern auch zwischen der Zahl der Chromosomen und der Oberfläche der Zelle (Pollenkorn).

Dadurch, dass die monoploiden, diploiden und tetraploiden Pollenkörner auf die Narben verschiedener Varietäten der *Tulipa Gesneriana* gebracht und nach einiger Zeit abgespült und untersucht wurden, konnte festgestellt werden, dass die diploiden und tetraploiden Pollenkörner wohl zu keimen vermögen, dass aber im allgemeinen die monoploiden Pollenkörner leichter zur Keimung gelangen und deshalb nach einigen Tagen Pollenschläuche zeigen, welche länger und von normalerer Form sind.

Durch Anwendung einer einfachen Methode ist es leicht, die diploiden und tetraploiden Pollenkörner von den monoploiden zu isolieren.

Die Beobachtung von keimfähigen Samen, als Folge von Bestäubungen, welche im Frühjahre 1927 persönlich vorgenommen wurden, lässt die Entwicklung pluriploider Nachkommen erwarten.

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1927e. Nucleolar number and size in diploid, triploid and aneuploid Hyacinths. La Cellule, T. 38, p. 5-65.

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1928b. 1. Transformatie van b]oemorganen bij Tulpen, Hyacinthen en Narcissen.
2. Tri-nucleolaire en tetra-nucleolaire, diploide nakomelingen uit di-nucleolaire, diploide Hollandsche Hyacinthen-variöteiten als ouders. Vijf en twintigste Vlaamsch Natuur- en Geneeskundig Congres, Natuurwetenschappelijk Tijdschrift, *Jg.* 10, No. 3, p. 65.

1928c. Change of the number of Chromosomes and its causes. In the press.

Explanation of Figures.

Figs.1 —5 are photographs, Figs. 6 and 7 are photomicrographs, Figs. 8-22 are drawings. The drawings were in all cases made with the aid of a camera lucida. Drawings 8, 9, 10, 11, 12, 13, 16h, 17, 18a, 18b, 19a, 19b, 19c, 20, 21 and 22 have been reduced to 1/2 in reproduction; drawings 14 and 16a to '/s,

Fig. 1. Starlet Duc IX, flowers 1-5. Of the flower im. 5 the pistil is covered with a piece of silk-paper. The scale an the pasteboard-slip is made out in centimeters, same as with thc other photograph.

Fig. 2. Scarlet Duc X. Moment at which the vegetative nucleus of the tube-cell is dividing.

Fig. 3. Seeds of Single Early Tulip Brilliant Star 9 X Scarlet Duc XVI .

Fig. 4. Seeds of Single Early Tulip Rose Précoce 9 X Scarlet Duc XVI *cf.*

Fig. 5. Seeds of Single Early Tulip Rose Précoee 9 X Scarlet Duc XIV *cr.*
Upper row : small seeds and small germs.
Lower row : large seeds and large germs.

Fig. 6. Scarlet Duc II. Monoploid, diploid and tetraploid pollen-grains in a drop of water, without cover-glass ; a less magnified than b.

Fig. 7. Scarlet Duc X. Monoploid, diploid and tetraploid pollen-grains in iron aceto. carmine.

Fig. 8. Scarlet Duc, equatorial plate in a plerome-cell of a root-tip, from a microtome-slide. Magn. 725 X.

Fig. 9. Scarlet Duc X. Monoploid pollen-grain, vegetative nucleus dividing, metaphase: 12 chromosomes, 6 long and 6 short ones. Magn. 520 X.

Fig.10. Scarlet Duc X. Do., 1-6 : long chromosomes, 7-12 : short ones. Magn. 725 x.

Fig.11. Scarlet Duc X. Diploid pollen-grain, vegetative nucleus dividing, metaphase : 24. chromosomes, 12 long and 12 short ones. Magn. 520 X.

Fig. 12. Scarlet Duc X. Tetraploid pollen-grain, vegetative nucleus dividing, metaphase : 48 chromosomes, 24 long and 24 short ones. Magn. 520 X.

Fig. 13. Scarlet Duc X. As the former, the pollen-grain however not glohular, as in the latter four figures, but composed of four globe-segments three of which are visihle. Magn. 250 X.

Fig. 14. Scarlet Duc IX. Pollen-grains of varions shapes present in one drop of water. Magn. 86 • 6 X.

Fig. 15. Pollen-grains of Scarlet Duc V in a drop of water, without cover-glass ; germinated an the stigmas of Darwin -Tulip William Copland; a: monoploid pollen-grain, b: tetraploid pollen-grain. Magn. 260 X.

Fig. 16. Pollen-grains, probably monoploid, diploid and tetraploid, of Scarlet Duc V in a drop of water; germinated on the stigmas of Single Early Tulip Cramoisi Brillant; a: without cover-glass, magn. 86.6 X ; b : with cover-glass, magn. 260 X.

Fig. 17. Pollen-grain of Scarlet Duc IX in iron aceto-carmine; germinated on the stigmas of Single Early Tulip La Reine. Magn. resp. 58. 5, 260 and 560 X .

Fig. 18. Pollen-grains of Scarlet Duc XVII, in iron aceto-carmine with a crystal of chloral hydrate; germinated on the stigmas of a new Single Early Tulip, not yet named; a: monoploid pollen-grain, b: probably two diploid pollen-grains and one tetraploid. Magn. 250 X.

Fig. 19. Monoploid, diploid and tetraploid pollen-grains of Scarlet Duc XVII, in iron aceto-carrnine with a erystal of chloral hydrate; germinated on the stigmas of Single Early Tulip Hobbema; a: magn. 56 •5 X; b: magn. 130 X, c: magn. 250 X.

Fig. 20. Pollen-grains of Scarlet Duc IX, put on the object-glass in dry state, without cover-glass. Magn. 130 X.

Pollen.grains of Scarlet Duc IX, isolated within a circle, drawn with India-ink on the object-glass and afterwards drawn. Magn. 130 X.

ist row a—d : sterile pollen-grains, dry.

2nd row a—d: fertile pollen-grains as well as the following rows; monoploid pollen-grains, dry.

3rd row a— d: large monoploid or small diploid pollen-grains, dry.

21th row a—d: large diploid or small tetraploid pollen-grains, dry.

5th row a—d: tetraploid pollen-grains, dry; d, d swollen in a drop of water.

Fig. 22. The Pollen-grain drawn last, at a magn. 560 X.