

Canna crosses II.

The chromosome numbers of *Canna glauca*, *C. glauca* × *indica* F₁, *C. aureo-vittata* and *C. aureo-vittata* gigas

by J. A. Honing.

In the first paper on *Canna* crosses ¹⁾ I could confirm for my strain of *C. indica* the number of chromosomes found by BELLING ²⁾ as haploid 9. Now it was necessary to determine the chromosome numbers of the other *Canna* species used in my crossings, as the irregularities e. g. in the ratios of the F₂ offspring after crossing *C. glauca* with *indica* were perhaps to be ascribed to irregular nuclear divisions on account of differences in the number of chromosomes of the parents. This was of course a possibility (already indicated by LOTSY ³⁾) specially to be controlled after BELLING's detection of five triploid strains of *C. indica*. The following is the result of investigations of pollenmothercell divisions. *)

The *Canna* flower has only one half stamen fertile (one theca), the other half being petaloid. The quantity of pollen seems to be dependent in a high degree on the outer circumstances, as sometimes in young not yet opened flowers the theca is empty, whilst that of the next flower in the same spike is very well supplied. It also occurs that one loculamentum has no pollen and the other of the same theca is provided with it, or that in a small part of a loculamentum the pollen mothercells are well developed and the rest has perished. Thus the formation of pollen may be very irregular, but this irregularity concerns the not yet divided pollenmothercells and is not the more or less constant effect of disturbances during the reduction division. In the meiosis of *C. indica*, *C. glauca* and their F₁ however till now I have never found irregularities to such an extent that abnormal and not viable pollen should be the result.

¹⁾ Mededeelingen Landbouwhoogeschool Wageningen, Vol. 26, paper 2, 1923.

²⁾ BELLING, J. The behavior of homologous chromosomes in a triploid *Canna*. Proc. National Acad. of Sciences Vol. 7, p. 197—201, 1921.

³⁾ LOTSY, J. P. Genetica V., p. 381, 1923.

*) The preparations were fixed with alcohol-acetic acid or with Bouin solution and stained with HEIDENHAIN's iron-alum-haematoxylin, the figures sketched with the aid of Abbe's camera lucida. Objectives Zeiss apochromatic immersion 90 × and 120 ×, homogeneous immersion 50 × and fluorit 100 × with different compensating oculars.

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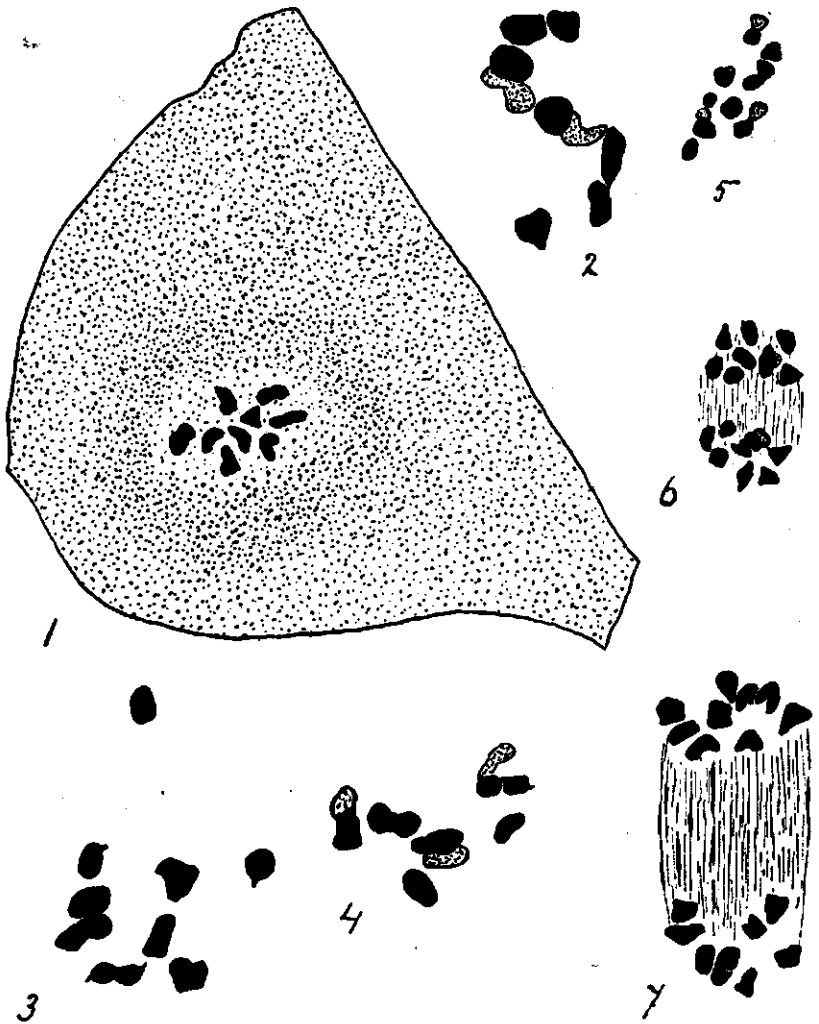


Fig. 1. *C. indica*, polar view of homotypic anaphase plate 2400 ×
 " 2. *C. glauca*, chain of bivalents in heterotypic metaphase 2880 ×
 " 3. " , heterotypic metaphase 2880 ×
 " 4. " , " " 2880 ×
 " 5. " , beginning of heterotypic anaphase 2880 ×
 " 6. " , heterotypic anaphase 2160 ×
 " 7. " , " " 2400 ×

Canna glauca.

The chromosome number of *C. glauca* is the same as that of *C. indica*, namely haploid 9, as was already mentioned by TISCHLER¹⁾ in his list of chromosome numbers under my name and with the year 1923, quite correctly and in good faith, though it was not yet published. In over fifty cases the number 9 in metaphase or anaphase was certain and the figures 2—7 do not need further discussion, at least so far as the number of chromosomes is concerned. As to the appearance in chain form something has to be said later on in connection with the different views of BOEDIJN and CLELAND.

C. glauca × *indica* F₁.

In early diakinesis or end strepsinema (fig. 8) it is evident that at least the majority of chromosomes is paired; on the other hand, in late diakinesis it is quite certain that gemini and univalent chromosomes both are present, and of the latter probably the larger ones will belong to the *glauca* set, the smaller ones to the *indica* set (figs. 9—11).

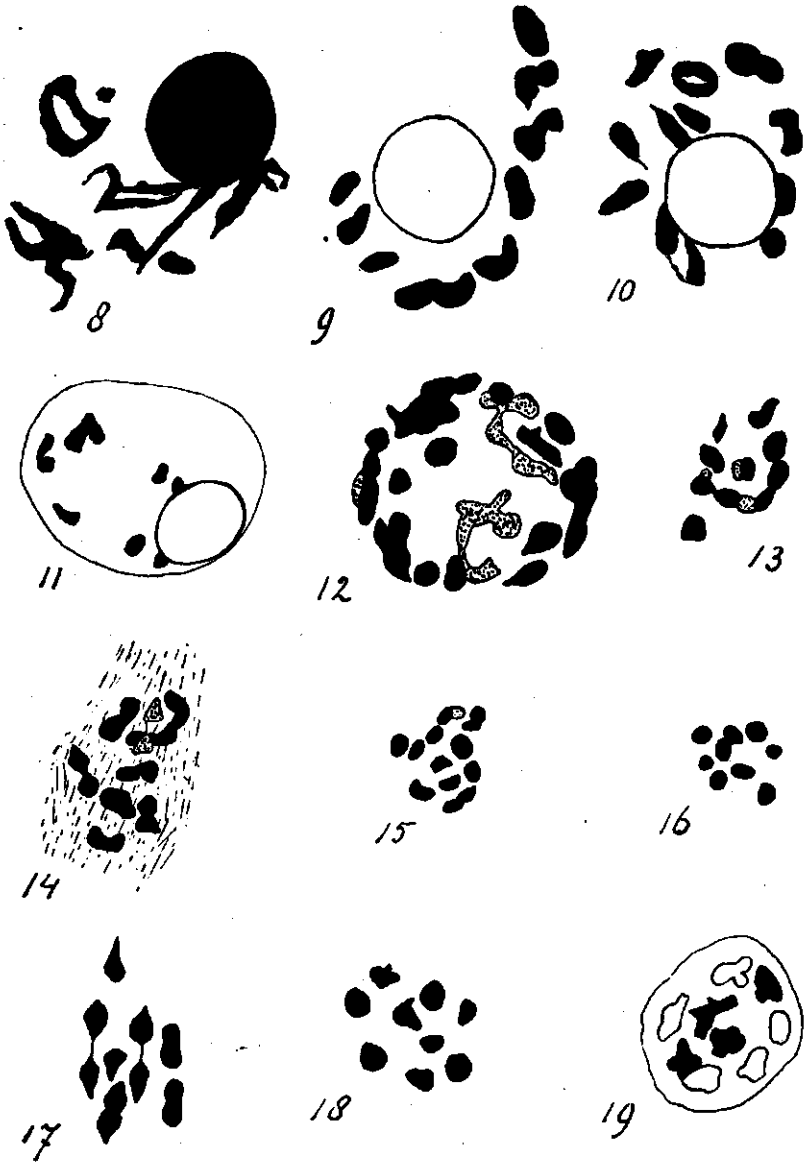
Chains were found in the stage of the chromosomes being scattered through the spindle, viz. two bivalent ones in fig. 14 and 5 univalents with probably two bivalents in fig. 13. In fig. 14 the doubleness of nearly all gemini is visible.

The latter phenomenon may occur even in the metaphase which is not always so plainly composed of 9 gemini as in fig. 16. Sometimes the equatorial plate consists of gemini — either recognizable by their outline as double-chromosomes or not — and univalents together. I believe this occurrence of univalents in the metaphase not to be a case of precipitancy of some gemini to enter into the anaphase, but on the contrary the continuation of aversion or inability of pairing, already shown in the diakinesis. In fig. 15 I see a ring of 4 gemini and two univalents with two univalents in the centre and outside the ring 2 gemini (of which the one to the right is clearly double) with 2 univalents.

The splitting of gemini in the beginning of anaphase does not occur as a rule for all pairs at the same time and the equatorial plate may be even then rather irregular for the not yet divided ones (fig. 17).

Although the haploid number of chromosomes could be determined in over a hundred cases, there were in the preparations only nine anaphase plates complete in one section. This number is of course far too small to allow of the conclusion that the

¹⁾ TISCHLER, G. Pflanzliche Chromosomen-Zahlen. *Tabulae Biologicae* Bd. IV. 1927.



C. glauca × *indica* F₁

- Fig. 8—10. portion of a diakinesis, chromosomes partly unpaired
2160 ×
- " 11. portion of a diakinesis, chromosomes partly unpaired
1200 ×
- " 12. diakinesis in degeneration 2880 ×
- " 13. side view of spindle, chain of 5 uni- and 2 bivalents
2160 ×

anaphase will always be regularly $9 + 9$, but the contrary I have not seen.

Canna aureo-vittata.

The chromosome number of *C. aureo-vittata* is also the same as that of *C. indica* and this similarity was already in anticipation even more likely than that of *C. glauca* and *indica*. For the resemblance of *aureo-vittata* with *indica* goes very far. When a systematist would assert *aureo-vittata* to be a variety or subspecies of *indica*, I should believe it immediately. The figs. 20—23 give metaphase plates, the one of fig. 23 being rather abnormal with three swollen and badly stained gemini. Typical is the fine thread between two bivalent chromosomes in fig. 20, which may be left from the adhering of two bivalents in chain form in former stages.

Canna aureo-vittata gigas.

1923 I had made some crosses *C. aureo-vittata* deep yellow \times pale yellow (both originally received from the botanical garden in MONTEVIDEO) with a view to analyse the yellow flower factors and to compare them with those in *C. indica* (hypostatic) and in *C. glauca*. 1926 I noticed in two F_2 numbers, the Nrs. 812 and 814 with 51 resp. 11 blooming plants, in each one specimen with noticeably slow growth, very broad leaves, larger staminodes and beginning its flowering rather late in the season. Both were quite sterile, neither after free pollination nor after pollination by hand a single seed could be obtained. Judging from the robust appearance they were supposed to be hero or *gigas* types, i. e. triploid or tetraploid.

The next year among an F_1 of 1328 individuals there occurred again 5 broad leaved, larger flowered and late blooming plants, children of three different mothers. Retracing the line of ancestors it was easily found that all the seven giants were descendants of one and the same F_1 plant (see table). When we count those numbers in which the new type appears separately, there are seven

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- Fig. 14. side view of spindle, nine pairs of which two adhering 2160 \times
 " 15. heterotypic metaphase, 6 gemini and 6 univalents 1600 \times
 " 16. heterotypic metaphase, 9 gemini 2160 \times
 " 17. portion of heterotypic anaphase, side view, differences in time of separation 2880 \times
 " 18. heterotypic anaphase plate, polar view 2880 \times
C. glauca \times *indica* F_2 n $^\circ$. 25
 " 19. diakinesis with 9 double chromosomes 2880 \times

CANNA AUREO-VITTATA DEEP YELLOW Nr. 508 — 2 × PALE YELLOW Nr. 507 — 1.

Year.	Gen.	Number of plants.
1924	F ₁	F ₁ : 33
1925	F ₂	F ₂ : 846
1926	F ₃	F ₃ : 944
1927	F ₄	F ₄ : 1328 3151


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    graph TD
      F1[Nr. 600 (33)*] --> F2_670[Nr. 670 (105)]
      F1 --> F2_671[Nr. 671 (139)]
      F1 --> F2_672[Nr. 672 (168)]
      F1 --> F2_673[Nr. 673 (239)]
      F1 --> F2_674[Nr. 674 (40)]
      F1 --> F2_675[Nr. 675 (155)]
      
      F2_670 --> F3_805[Nr. 805 (90)]
      F2_670 --> F3_806[Nr. 806 (208)]
      F2_670 --> F3_807[Nr. 807 (252)]
      
      F2_671 --> F3_808[Nr. 808 (221)]
      F2_671 --> F3_809[Nr. 809 (62)]
      
      F2_672 --> F3_810[Nr. 810 (6)]
      F2_672 --> F3_811[Nr. 811 (3)]
      F2_672 --> F3_812[Nr. 812 (51)]
      
      F2_673 --> F3_813[Nr. 813 (40)]
      F2_673 --> F3_814[Nr. 814 (11)]
      
      F2_674 --> F3_874[Nr. 874 (128)]
      F2_674 --> F3_876[Nr. 876 (177)]
      
      F2_675 --> F3_873[Nr. 873 (290)]
      F2_675 --> F3_877[Nr. 877 (71)]
      
      F3_805 --> F4_863[Nr. 863 (2)]
      F3_805 --> F4_864[Nr. 864 (15)]
      
      F3_806 --> F4_865[Nr. 865 (12)]
      F3_806 --> F4_866[Nr. 866 (115)]
      
      F3_807 --> F4_867[Nr. 867 (328)]
      F3_807 --> F4_868[Nr. 868 (151)]
      
      F3_808 --> F4_871[Nr. 871 (290)]
      F3_808 --> F4_872[Nr. 872 (39)]
      
      F3_809 --> F4_875[Nr. 875 (3)]
      F3_809 --> F4_878[Nr. 878 (71)]
      
      F3_810 --> F4_879[1 gigas]
      F3_810 --> F4_880[3 gigas]
      
      F3_811 --> F4_881[1 gigas]
      F3_811 --> F4_882[1 gigas]
      
      F3_812 --> F4_883[1 gigas]
      F3_812 --> F4_884[1 gigas]
      
      F3_813 --> F4_885[1 gigas]
      F3_813 --> F4_886[1 gigas]
      
      F3_814 --> F4_887[1 gigas]
      F3_814 --> F4_888[1 gigas]
      
      F3_874 --> F4_889[1 gigas]
      F3_874 --> F4_890[1 gigas]
      
      F3_876 --> F4_891[1 gigas]
      F3_876 --> F4_892[1 gigas]
      
      F3_873 --> F4_893[1 gigas]
      F3_873 --> F4_894[1 gigas]
      
      F3_877 --> F4_895[1 gigas]
      F3_877 --> F4_896[1 gigas]
      
      F4_863 --> F4_Total[3151]
      F4_864 --> F4_Total
      F4_865 --> F4_Total
      F4_866 --> F4_Total
      F4_867 --> F4_Total
      F4_868 --> F4_Total
      F4_869 --> F4_Total
      F4_870 --> F4_Total
      F4_871 --> F4_Total
      F4_872 --> F4_Total
      F4_873 --> F4_Total
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      F4_885 --> F4_Total
      F4_886 --> F4_Total
      F4_887 --> F4_Total
      F4_888 --> F4_Total
      F4_889 --> F4_Total
      F4_890 --> F4_Total
      F4_891 --> F4_Total
      F4_892 --> F4_Total
      F4_893 --> F4_Total
      F4_894 --> F4_Total
      F4_895 --> F4_Total
      F4_896 --> F4_Total
  
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Among a total number of 3151 plants there are 7 gigas or 0,222 %, all descendants of Nr. 675, i. e. of one of the six F₁ individuals used as motherplants for following generations.

*) The number of flowering plants of every sowing is given between ().

giants out of 600 or 1,167 % as against 2551 hybrids of four generations without gigas-types.

In a parallel cross with 742 hybrids of four generations there never occurred a gigas nor in the 506 pure *aureo-vittata*, i. e. 310 constant deep yellow ones of four generations from 12 mother-plants, and 196 pale yellow ones also of four generations and from 9 mothers.

As the quantity of pollen was very small I only tried to get seeds by selfing and by crossing with normal *aureo-vittata* as pollenparent. The results were once more very meagre: 5 seeds (4 seedlings) after selfing, 2 seeds (1 seedling) after crossing with normal *aureo-vittata* and 12 seeds (3 seedlings) after free pollination of two motherplants.

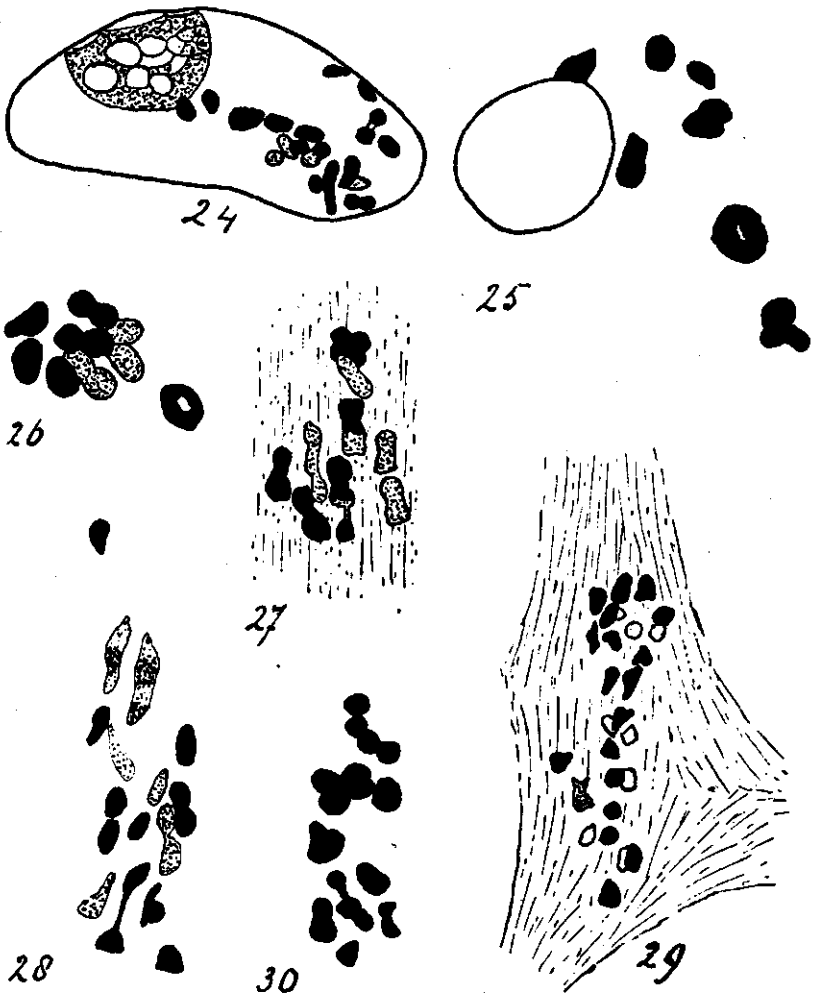


C. aureo-vittata

- Fig. 20. heterotypic metaphase, the fine thread between two bivalents probably resulting from chain formation 2880 ×
 " 21—22. heterotypic metaphase 2880 ×
 " 23. " " somewhat irregular and with 3 gemini swollen and faintly stained. 2880 ×

Part of the young flowers have been used to control the meiosis of pollenmothercells in order to get certainty about the nature of the new type and to search for an explanation of the high degree of sterility in this originally so fertile species.

First of all it must be said that the new type is indeed a real gigas form with double the quantity of chromatin. This expression is more exact than doubled number of chromosomes would be, because the doubling in number may fail to occur in some pairs of chromosomes, sometimes even in all nine pairs together. In the latter case there are in the heterotypic metaphase nine very big — tetravalent — chromosomes. (figs. 31 and 32). On the other hand the normal doubling also exists, as in two corresponding heterotypic anaphase plates together 35 (in all probability out of 36) chromosomes could be counted (fig. 43) or as is certain from the side view of heterotypic anaphase in fig. 41 or from the homotypic



C. aureo-vittata gigas

- Fig. 24. diakinesis with paired and unpaired chromosomes 1600 X
 " 25. portion of diakinesis 2880 X
 " 26. contraction after diakinesis 2880 X
 " 27—30. chromosomes scattered through the spindle
 " 27. 9 tetravalents, partly degenerating 2880 X
 " 28. 1 tetra- and 17 bivalents, partly in degeneration 2880 X
 " 29. multipolar spindle with bi- and univalents 1920 X
 " 30. tetra-, bi- and univalents 2880 X

metaphase plate in fig. 44. Between these extremes there are pollenmothercells with sets of uni-, bi- and tetravalent chromosomes mixed, a conclusion drawn as well from the number as from the size and the form of the chromosomes.

The individual behaviour of the chromosomes becomes visible in diakinesis. In fig. 24, a not totally complete diakinesis, 20 chromosomes could be counted, so at least 2, but probably some more pairs have not united to bivalents. Also fig. 25 proves the differences in inclination for pairing. While scattered through the spindle the chromosomes may be arranged as nine tetravalents (fig. 27), but also as uni-, bi- and tetravalents (fig. 30) or as bi- and univalents (fig. 29). The same holds good for the heterotypic metaphase: 9 tetravalents in the figs. 31 and 32, bivalents probably only in fig. 34, bi- and univalents in fig. 35, tetra-, bi- and univalents in the figs. 36, 38 and 39. Sometimes one set of 9 seems to be regularly arranged in a somewhat flattened part of a metaphase plate, whilst the other is more clumped together (fig. 34), or the other is partly split in univalents (fig. 35). Breaking up of chromosomes in metaphase also occurs (fig. 40).

Sometimes the spindle is curved, flat-convex or even concave-convex.

In heterotypic anaphase and telophase lagging chromosomes are to be found (figs. 41 and 45). This elimination of chromosomes may be one of the causes of sterility, for instance when a tetravalent is lagging or two homologous bivalents, but it does not seem to be necessary that the loss of univalent chromosomes, of which the homologous ones are present in the for the rest diploid nucleus, should result in non viable daughter cells. The fact is that after formation of the cell wall a large part of the dyads look far from normal, also when no trace of diminution of chromosomes can be discovered. The chromatin stains badly, whilst the cytoplasm holds the haematoxylin too well in differentiation.

Very often there is no meiosis at all and direct nuclear division takes place (figs. 46—48). This proof of degeneration is still more accentuated by the occurrence of micronuclei (fig. 48). Also after the heterotypic division the homotypic one may fail to come and instead of it a fragmentation is to be seen (fig. 49). That this fragmentation is not a regular amitotic division is probable in the cases of dyads of which one daughter cell has died before fixation, which is rendered likely by the deep staining and by the higher turgescence of the sister cell (fig. 50). On the other hand sometimes the formation of more than four pollen cells is to be found.

In short with so many irregularities and so much degeneration the high degree of sterility is comprehensible. The well-known



31



32



33



34



35



36



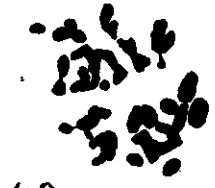
37



38



39



40



41



42



43



44

lesser fertility of *gigas* types as for instance in *Oenothera* or WINKLER's ¹⁾ *Solanum nigrum* giants is here far more strongly pronounced and TISCHLER's ²⁾ remark „das sich die Chromosomenzahl „ungestraft“ nicht ins Übermasz anhäufen lässt“, made about aposporic mosses, may be applied to *Canna aureo-vittata*.

As to the point of difference between BOEDIJN ³⁾ and CLELAND ⁴⁾, which is discussed by OEHLKERS ⁵⁾ and to which SINOTÔ ⁶⁾ and Miss LELIVELD ⁷⁾ added their comments, I can only say that I

1) WINKLER, H. Über die experimentelle Erzeugung von Pflanzen mit abweichenden Chromosomenzahlen. Ztschr. f. Bot. Bd. 8, S. 417—531, 1916.

2) TISCHLER, G. Allgemeine Pflanzenkaryologie S. 601, 1922.

3) BOEDIJN, K. Der Zusammenhang zwischen den Chromosomen and Mutationen bei *Oenothera Lamarckiana*. Rec. d. trav. botan. néerlandais XXII, S. 173—261, 1925.

4) CLELAND, R. E. Meiosis in pollen mother cells of *Oenothera Franciscana sulfurea*. The bot. Gaz. LXXVII, p. 149—170, 1924.

5) OEHLKERS, F. Sammelreferat über neuere experimentelle *Oenothera*-arbeiten II. Ztschr. f. ind. A. u. Vererb. XLI, S. 359—375, 1926.

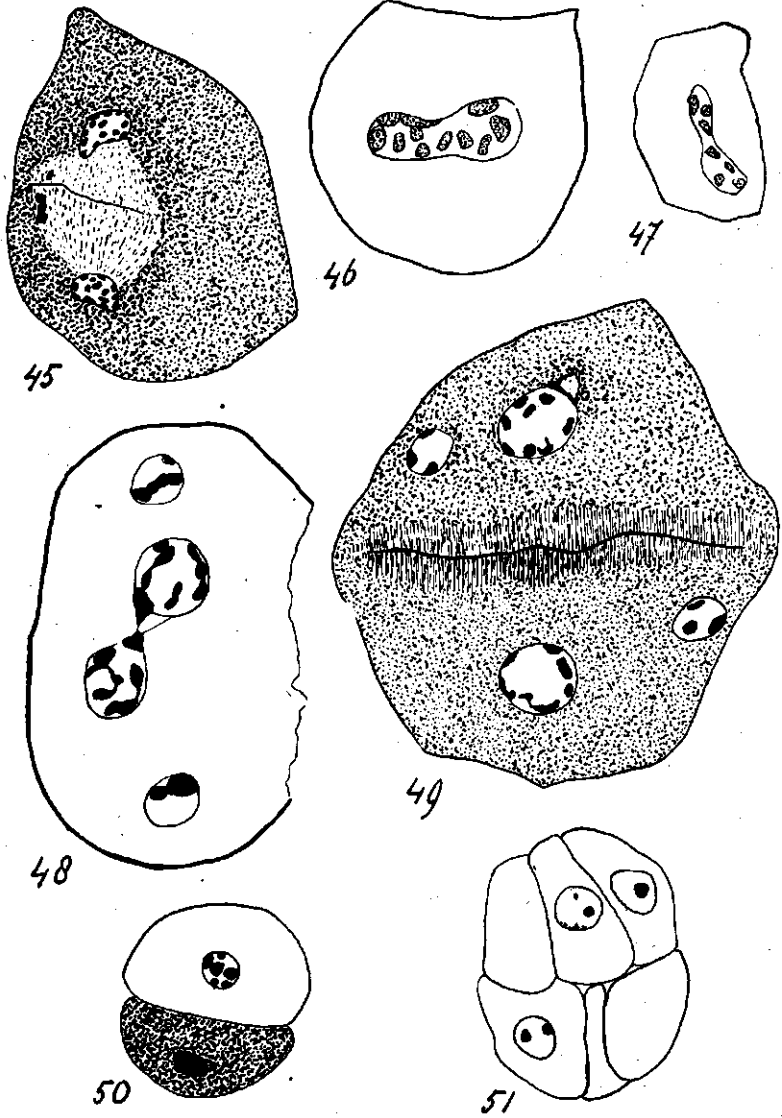
6) SINOTÔ, Y. Microsporogenesis in *Oenothera sinuata* L. The Botan. Mag. Tokyo XLI, p. 225—234, 1927.

7) LELIVELD, J. A. Some remarks on the cytology of *Oenothera*. Rec. d. trav. botan. néerlandais XXVa, p. 237—243, 1928.

C. aureo-vittata gigas

Fig. 31—40. heterotypic metaphase

- „ 31—32. 9 tetravalents 3200 ×
- „ 33. incomplete pairing of chromosomes 3200 ×
- „ 34. one set of 9 bivalents separated from the other partly more contracted set 2880 ×
- „ 35. one set of 9 bivalents and the other probably consisting of 6 bi- and 6 univalents 2880 ×
- „ 36. 6 tetra-, 3 bi-, 2 univalents and 1 ? 2160 ×
- „ 37. incomplete pairing of chromosomes 3200 ×
- „ 38. 1 tetravalent, 15 bivalents and 2 univalents (not absolutely certain) 2800 ×
- „ 39. tetra-, bi- and univalents 3200 ×
- „ 40. breakdown of chromosomes 2880 ×
- „ 41. heterotypic anaphase in side view, two lagging chromosomes 2880 ×
- „ 42. portion of heterotypic anaphase with three pairs already separated, one not yet, and two chromosomes in degeneration 2880 ×
- „ 43. two corresponding heterotypic anaphase plates with together 35 chromosomes 3200 ×
- „ 44. homotypic anaphase plate 2880 ×



C. aureo-vittata gigas, abnormalities

- Fig. 45. heterotypic telophase with lagging chromosomes 770 ×
 " 46. direct division of nucleus 770 ×
 " 47. " " " " 430 ×
 " 48. " " " " and micronuclei 770 ×
 " 49. first meiotic division followed by a direct division 860 ×
 " 50. dyad of which one cell is overstained (dead before fixation) 430 ×
 " 51. six daughter cells instead of four 370 ×

find chains and free chromosomes in one and the same theca and on one and the same slide. Also in free bivalent chromosomes in heterotypic metaphase the adhering in former stages is evident from the short thick threads (compare fig. 2 with 3 and 4 of *C. glauca*, figs. 13 and 14 of *C. glauca* × *indica* F₁ with 15 and 16). So I can understand the more intermediate position of OEHLKERS.

I must immediately emphasize that in fig. 2 the chain consists of bivalent chromosomes, just as the two joined ones in fig. 14 are bivalents; in fig. 13 however there is a chain of five univalents (connected with two bivalents). The latter case only may be compared with CLELAND's and SINITÔ's figures. When there is a chain of bivalents one is not absolutely forced to enter into the question of parasyn-desis or telosyn-desis; with BOEDIJN we could consider the chain as proceeding from merely accidental adherings, for *Canna* a not very likely conclusion in relation to an arrangement as in fig. 2. But in case of univalents in chain formation it is somewhat more difficult to persist in the opinion that the chain should have been formed accidentally after normal parasyn-desis.

Now my preparations have been made to determine the number of chromosomes and consequently I have not many slides with prophase stages suitable for studying the question para- or telosyn-desis. From some nuclei it is quite certain that parasyn-desis occurs (fig. 8), but, on the other hand, there are some others that could be understood at least as well as telosyn-detic chains or portions of chains than as instances of parasyn-desis. I must leave the possibility of both ways of chromosome arrangement in *Canna* undecided. Further discussion and comparison with the results of other authors as EMERSON, HÅKANSSON, SCHWEMMLE etc, is of no use; new material has to be studied.

SUMMARY.

1. The chromosome number of *Canna glauca* and *C. aureo-vittata* is haploid 9, just as in *C. indica*.
2. The *C. glauca* × *indica* F₁ hybrid shows often univalent chromosomes in diakinesis, scattered through the spindle and in metaphase.
3. *C. aureo-vittata* *gigas* appeared 7 times (1926 twice, 1927 5 times) among 4399 individuals of *aureo-vittata* of 4 generations, all 7 giants being descendants of one single plant in 1924. It is nearly sterile.

4. The *gigas* shows in heterotypic metaphase plates 9 tetravalent chromosomes or 18 bivalents or bi- and univalents or even tetra-, bi- and univalents together.

5. Lagging chromosomes are found in heterotypic anaphase and telophase; very often direct nuclear division takes the place of meiosis. This proof of degeneration is accentuated by the occurrence of micronuclei.

6. In *Canna* sometimes chains of bivalent chromosomes are formed, but also of univalents. I must leave undecided the possibility of both ways of chromosome arrangement, para- and telosyndesis.
