Memoirs of the College of Science, Kyoto Imperial University, Series B,
Vol. IV, No. 3, Article 17, 1929.

Chromosome Arrangement.

IX. The Pollen Mother Cells in *Cycas revoluta*, THUNB.

By

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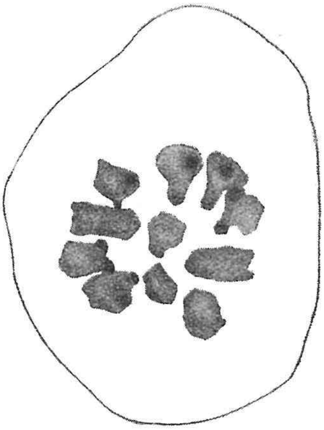
With 16 Text-figures.

(Received January, 26, 1929)

In pollen mother cells of *Cycas revoluta* the chromosomes (gemini) are of various sizes and shapes and form nearly a graded series in regard to size. To see in what manner chromosomes are arranged in the equatorial plate in those cases such as in Cycas having chromosomes of different sizes, some microscopical observations were made with microtome sections of pollen mother cells of *Cycas revoluta*. Owing to difficulty in fixing the material we were not able to observe a sufficient number of cases to enable us to draw a statistical conclusion, but the results so far obtained may furnish us with some ideas about the question.

I. THE NUMBER OF CHROMOSOMES AND THEIR SIZES AND SHAPES.

While engaged, in 1927, at the request of Prof. KUWADA in an investigation with fresh pollen mother cells of *Cycas revoluta* stained with acetocarmine, not for the purpose of determining the number of the chromosomes, we happened to find 11 double chromosomes or gemini in the heterotype division. This number is not in accordance with the result of ISHIKAWA and also seems to be very improbable in gymnosperms (see FUJII, 1910). According to ISHIKAWA the number of chromosomes of *Cycas revoluta* is 24 in the proembryonal phase, a number which has been commonly found in most of the gymnospermous plants hitherto investigated. This number gives the conclusion that the pollen mother cells of *Cycas revoluta* should have 12 gemini in the heterotype metaphase, and we now actually found in them a number which was less than this expected number by one. Thus some closer descriptions of the number as well as the shapes of the chromosomes is necessary, before we enter on our main subject.



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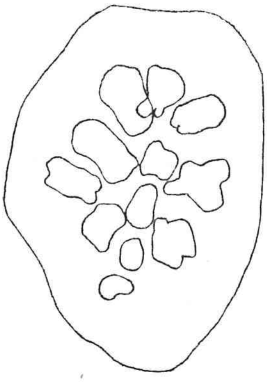

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Fig. 1. Nuclear plate in the heterotype division showing 11 chromosomal elements. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

Fig. 2. The same showing 12 chromosomal elements. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

a) *Pollen mother cells.* Material was collected in the prefecture of Wakayama near Kyoto in the middle of July, 1927 and 1928. As fixing fluids, various mixtures were employed; not only each mixture alone, but also combinations of some of the mixtures. Not all these methods of fixation brought forth satisfactory results in all pollen sacs, but in materials fixed with FLEMMING's strong solution and the Bonn modification of FLEMMING's solution a good result was obtained in some of the pollen sacs. In materials fixed with a half-diluted solution of BENDA containing no trace of acetic acid the results were also found to be pretty good. All section were cut 12 microns thick, and HEIDENHAIN's haematoxylin was exclusively used for staining.

C. J. CHAMBERLAIN[1] has found 12 chromosomes in the pollen mother

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[1] Cited from COULTER and CHAMBERLAIN (1925).

cells of *Dioon edule*, and the same number in the mitosis giving rise to the ventral canal and egg nuclei, and in cells of the endosperm of the same plant. In *Stangeria paradoxa* (CHAMBERLAIN) and *Macrozamia Fraseri* (LIGHT) the same haploid number of chromosomes has also been reported[1]. Miss F. G. SMITH[2] has found in pollen mother cells of *Ceratozamia* the same number of chromosomes in 46 cases, 11 chromosomes in three cases, and 13 chromosomes in one case, out of 50 cases observed. In *Zamia* she has found 12 chromosomes in all the 25 cases examined. REYNOLDS also reports that he has counted 12 chromosomes in cells of the endosperm of *Microcycas* with the exception of two cases where he has found 14 chromosomes.

In the heterotype division of the pollen mother cells of *Cycas revoluta*, 11 chromosomes (gemini) where clearly counted in the majority of cases (Fig. 1). In only 8 cases out of 140 cases observed, the number was 12 (Fig. 2). The counting in polar view was made in 126 cells. In the cases where 12 chromosomes are counted, there is found generally a pair of chromosomes of nearly the same size and shape lying near each other (Figs. 2 and 4). Only in one case out of the eight cases observed, one observed in the stage of prophase being excepted, this paired arrangement was obscure. In the homotype division the chromosomes are much longer than those in the heterotype division, so that exact counting was rather difficult with a few exceptions where 11 chromosomes were counted.

In the majority of cases, whatever the total number might be, 11 or 12, there were found in the heterotype nuclear plate two large chromosomes or gemini of a rod shape, lying horizontally. These two large chromosomes are distinguishable from the other chromosomes not only by their larger size and the horizontal recumbent position, but also by their being behind the other chromosomes in the process of disjunction. The other chromosomes are massive in shape and are of different sizes (Fig. 1). In this paper the two large chromosomes

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[1] Cited from SCHÜRHOFF (1926).

[2] Cited from COULTER and CHAMBERLAIN (1925).

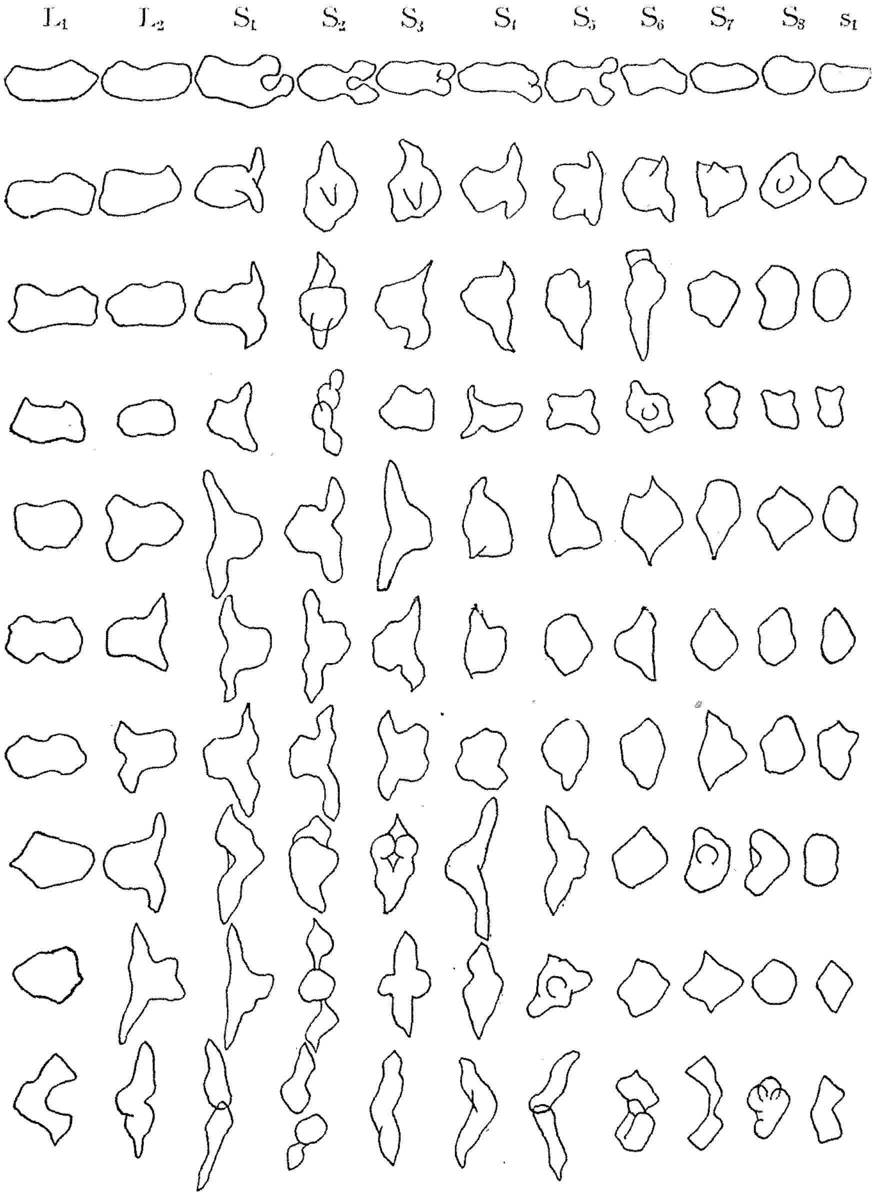


Fig. 3. 11 chromosomal elements in early or late metaphase or meta-anaphase in heterotype division arranged in a line according to their size and shape. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

Fig. 4. 12 chromosomal elements in early or late metaphase in heterotype division arranged in a line according to their size and shape. A pair of the elements in the last column is reproduced here in its original relative position. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

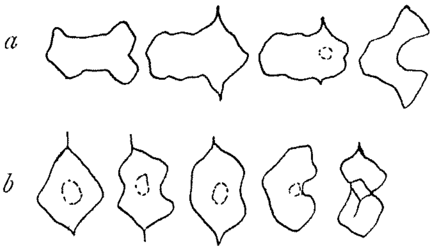


Fig. 4 *a–b*. Gemini in early and late metaphase indicating their points of spindle fiber attachment. *a*. L_1 or L_2. *b*. Smaller gemini. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

are designated L_1 and L_2, and the smallest one s_1, the remaining 8 chromosomes of intermediate sizes being named S_1, S_2...S_8 respectively. The chromosomes L_1 and L_2 are of almost equal size and shape, and are hardly distinguishable from each other. They are about twice as large as s_1, the smallest one (comp. Figs. 3 and 4).

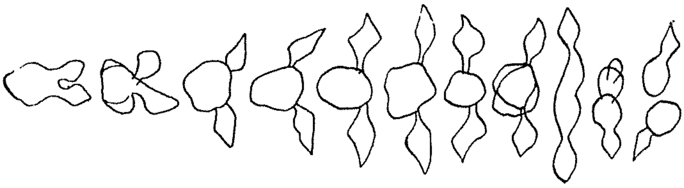
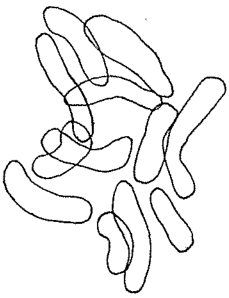


Fig. 6. S_2-*geminus in different stages of disjunction from early metaphase to meta-anaphase.* Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

The investigation has not proceeded so far as to determine the point of the spindle fiber attachment of each chromosome, but in some of the chromosomes it was not very hard to determine. In L_1 and L_2 the attachment is found near the end of the chromosomes, but strictly speaking, it may be subterminal at least in one of the chromosomes L_1 or L_2 (Fig. 5, *a*). In the other five massive chromosomes ($S_1 - S_5$) which are larger than the remaining four ($S_6 - S_8$ and s_1), it is terminal (Fig. 3). In the latter named four chromosomes it has not been determined whether the attachment is terminal or non-terminal, but at least in one of them it is median or submedian (Fig. 5, *b*).



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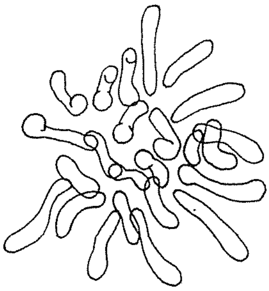

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Fig. 7. A twelve-chromosome group from an endosperm cell. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

Fig. 8. A twenty-four chromosome group from a nucellus cell. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

Besides L_1, L_2 and s_1 there is in the heterotype metaphase a characteristic chromosome or geminus (S_2), which has a subterminal constriction in each component of it. When the components are drawn each to the opposite poles, this constricted part becomes very conspicuous making the whole appearance of the geminus very unique. (Fig. 6).

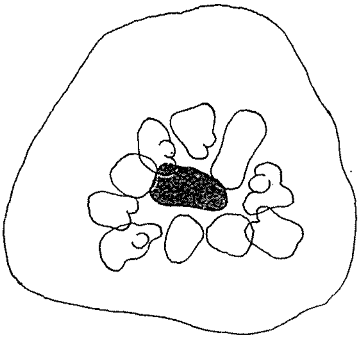
In some of the chromosomes of medium size (S_1-S_6), the portions attached to the spindle fiber are deeply stained, as compared with the main body of the chromosomes which is stained only to a light colour (Fig. 1). These portions may be comparable with the "polar granules" described in animal chromosomes.

b). *Endosperm.* Some observations of the number of chromosomes were made in cells of the endosperm. Material was collected in the middle of August in the prefecture of Wakayama, and was fixed by the Bonn modification of FLEMMING's solution. The results were pretty satisfactory.

So far as counting was made there were found always 12 chromosomes (Fig. 7), a result which was to be expected from ISHIKAWA's result. They are rod-shaped and of varying lengths. The longest one is almost twice as long as the shortest one.

c). *Nucellus.* Observations were made in the preparations in which the chromosomes of the endosperm were counted. In this tissue the chromosomes are thinner than those in the endosperm tissue. Owing to this situation of the chromosomes and also to the 2n condition of the number of chromosomes of the tissue exact countings were possible only in a few cases. In these cases the number was 24 (Fig. 8), just the same number as given by ISHIKAWA in the proembryonal phase.

Though our investigation has not yet proceeded far, enough to enable us to discuss the origin of the variation in the chromosome number in different phases of the life cycle in *Cycas revoluta*, the results we have so far obtained seem to suggest that the chromosome number 11 is derived from 12 which is the prevailing number in gymnosperms, and we seem to find a certain suggestion as to the sex determination of this dioecious plant in the fact that this derived number is found in the microsporogenesis.



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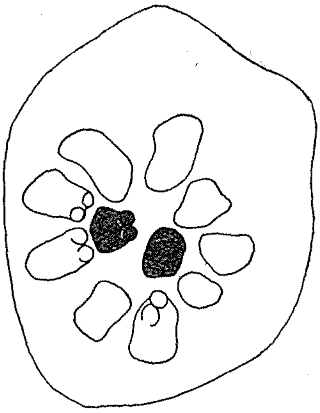

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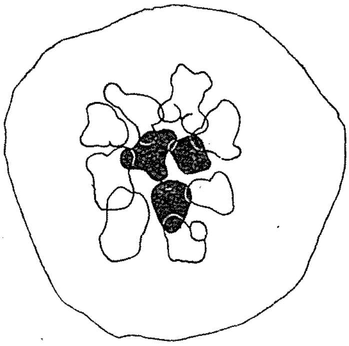

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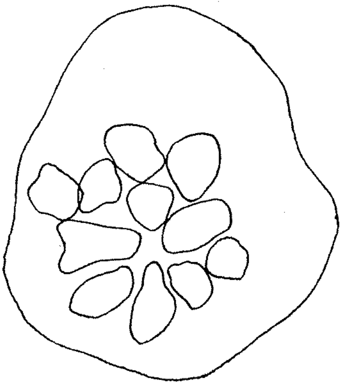

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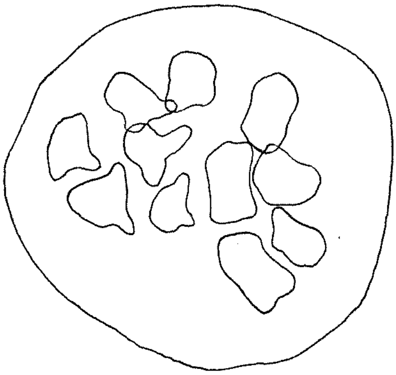

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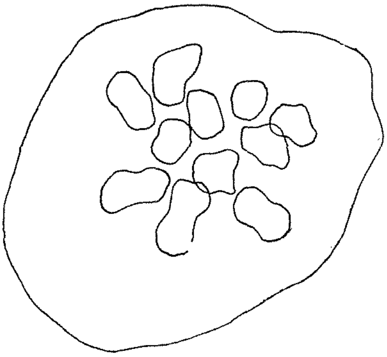

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Fig. 9—14. Explanation in the text. In Fig. 9—11, the inner chromosomes are shown in solid black. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

II. ARRANGEMENT OF CHROMOSOMES.

In this investigation, only the predominating case having 11 chromosomal elements is concerned.

The form of chromosome arrangement can be classified into the following three distinct and three intermediate types :—

Distinct types :

Type I. Ten chromosomes form practically a ring having the remaining one in its center (Fig. 9).

Type II. Two chromosomes occupy the central or inner positions being surrounded by the other 9 (Fig. 10).

Type III. Three chromosomes are found in the inner region, the chromosomes forming the ring being 8 in number (Fig. 11).

Intermediate types :

Type IV. A case where discrimination as to whether the number of the inner chromosomes is 1 or 2 is difficult (Fig. 12).

Type V. A similar case where it is dfficult to discriminate whether the number is 2 or 3 (Fig. 13).

Type VI. Another case where there is the same difficulty in discriminating whether the number is 3 or 4 (Fig. 14).

The number of observed cases of these different types is given in Table I.

TABLE I.

Type		Number of inner chromosomes	Number of observed cases	Percentage
Distinct ;	I	1	3	2.50
	II	2	53	44.16
	III	3	39	32.50
Intermediate ;	IV	1 or 2	5	4.16
	V	2 or 3	17	14.16
	VI	3 or 4	3	2.50
Total			120	99.98

From Table I it is seen that cases belonging to Type II are the most numerous, being 44.16% of all the cases observed, and cases belonging to Type III comes next in frequency of occurrence, the frequency value (32.5%) being also distinctly higher than that of cases belonging to the other types of arrangement. If we assume that the intermediate types represent those cases where some of the chromosomes of the distinct types are slightly displaced by the action of the fixing fluid, or those where the arrangement is in transition to Type II or III, these frequency values for Types II and III will rise higher. According to MAYER, in the case of floating magnets, the stable form is like Type III, and therefore, if the chromosomes are comparable with floating magnets there must exist a certain complexity in the case of *Cycas revoluta*.

In Table II only in respect to the chromosomes L_1, L_2 and s_1 the numbers of possible combinations of any two inner chromosomes obtainable on the assumption that every chromosome has an equal chance of occupying an inner position, and the observed numbers of the corresponding cases (Fig. 15), together with their numerical values expressed in percentage, are given, and in Table III those for the case where there are three inner chromosomes (Fig. 16). In these tables the chromosomes previously designated S_1, S_2,............S_8 are all represented by S.

TABLE II.

Two inner chromosomes.

Combination	Number of combinations	Expected percentage	Observed number	Observed percentage
s_1L_1 s_1L_2 (Fig. 15*a*)	$\left.\begin{array}{l}({}_1C_1)({}_1C_1)=1\\({}_1C_1)({}_1C_1)=1\end{array}\right\}2$	3.63	4	7.54
s_1S (Fig. 15*c*)	$({}_1C_1)({}_8C_1)=8$	14.54	12	22.64
L_1L_2	${}_2C_2=1$	1.81	0	0
SL_1 SL_2 (Fig. 15*b*)	$\left.\begin{array}{l}({}_8C_1)({}_1C_1)=8\\({}_8C_1)({}_1C_1)=8\end{array}\right\}16$	29.09	11	20.75
SS (Fig. 15*d*)	${}_8C_2=28$	50.90	26	49.05
Total	${}_{11}C_2=55$	99.97	53	99.98

TABLE III.

Three inner chromosomes.

Combination	Number of combinations	Expected percentage	Observed number	Observed percentage
$s_1L_1L_2$	$({}_1C_1)({}_2C_2) = 1$	0.60	0	0
$\left\{\begin{matrix} s_1SL_1 \\ s_1SL_2 \end{matrix}\right.$ (Fig. 16*a*)	$\left.\begin{matrix} ({}_1C_1)({}_8C_1)({}_1C_1) = 8 \\ ({}_1C_1)({}_8C_1)({}_1G_1) = 8 \end{matrix}\right\} 16$	9.69	7	17.94
s_1SS (Fig. 16*c*)	$({}_1C_1)({}_8C_2) = 28$	16.96	16	41.02
L_1L_2S	$({}_2C_2)({}_8C_1) = 8$	4.84	0	0
$\left\{\begin{matrix} SSL_1 \\ SSL_2 \end{matrix}\right.$ (Fig. 16*b*)	$\left.\begin{matrix} ({}_8C_2)({}_1C_1) = 28 \\ ({}_8C_2)({}_1C_1) = 28 \end{matrix}\right\} 56$	33.93	10	25.64
SSS (Fig. 16*d*)	${}_8C_3 = 56$	33.93	6	15.38
Total	${}_{11}C_3 = 165$	99.95	39	99.98

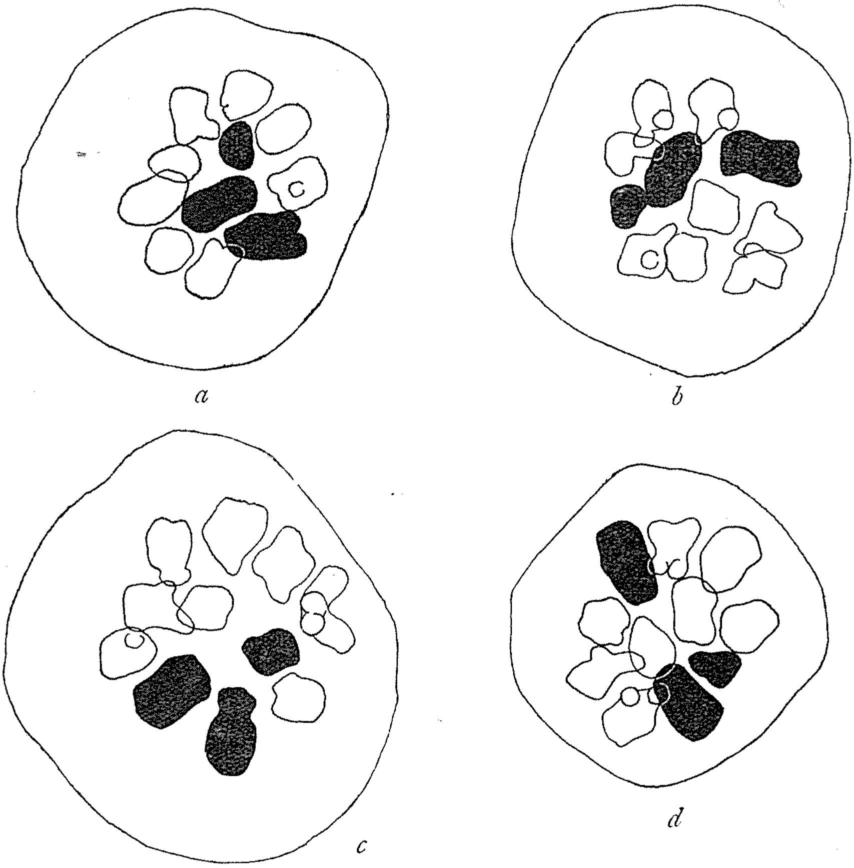

Fig. 15 *a–d*. Case having two inner chromosomes. In all the figures the two large chromosomes L_1 and L_2, and the smallest chromosome s_1 are shown in solid black. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

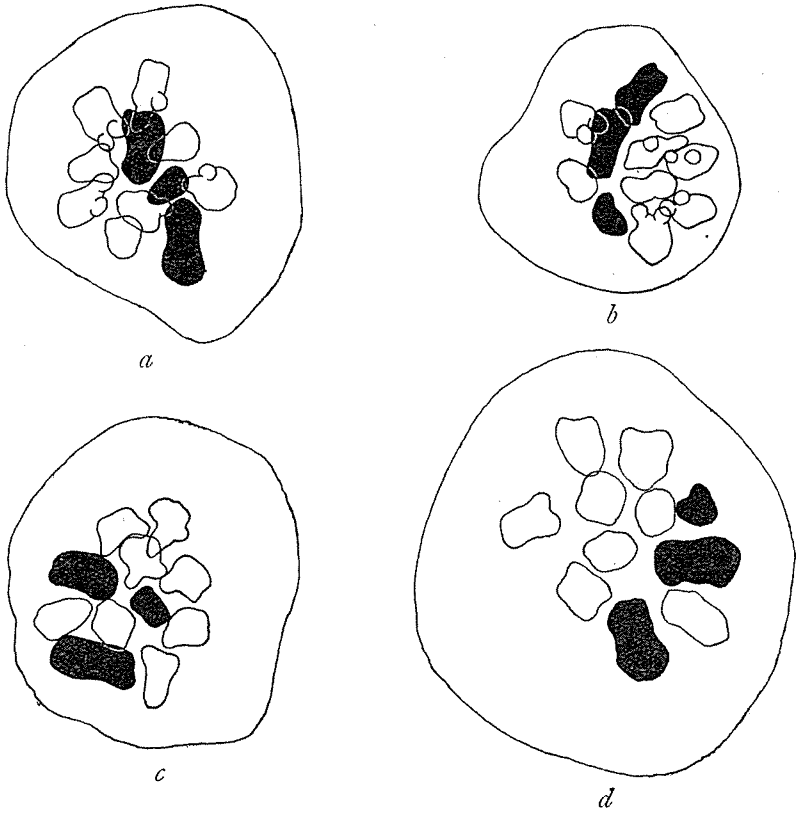

Fig. 16 *a–d*. Cases having three inner chromosomes. The chromosome L_1, L_2 and s_1 are shown in solid black as in Fig. 15. Camera drawing: apochr. imm. 2 mm. × comp. oc. 12.

From these tables we can readly obtain the observed and expected percentages of the numbers of cases where either the chromosome L_1 or L_2, or both occupy the inner positions, and those of cases where the small chromosome s_1 is inside: They are given in Table IV.

TABLE IV

Combination	From Table II		From Table III	
	Observed %	Expected %	Observed %	Expected %
Either L_1 or L_2	28.29	32.72	43.58	43.62
Both L_1 and L_2	0	1.81	0	5.44
s_1	30.18	18.17	58.96	27.25

From Table IV, we see that while the chromosome s_1 has a greater tendency to occupy the inner positions than was to be expected, on the basis of the assumption we made, the chromosomes L_1 and L_2 occupy the inner positions as frequently as we can expect on the same basis. It may be also a noteworthy fact that no case having both L_1 and L_2 at the same time in the inner region of the arrangement form was found. either in the cases having two inner chromosomes or in those having three.

The result obtained for s_1 may not be convincing, because there is a certain risk that some of the other small S-chromosomes such as S_3 or S_7 may be mistaken for s_1. To avoid this risk, we examined the frequency with which these three chromosomes occupy the inner positions in any of the possible combinations, and compared the results obtained with the expectable frequency. In Table V these frequencies for the case where two chromosomes occupy the inner positions are shown, and in Table VI those for the case where three chromosomes take the inner positions. In these two tables, all the chromosomes other than S_7, S_3, and s_1 are represented by the same letter X.

TABLE V.

Combination	Number of Combinations	Expected frequency	Observed frequency
s_1S_7 S_7S_8 s_1S_8	${}_3C_2 = 3$	2.89	15
s_1X S_7X S_8X	$({}_3C_1)({}_8C_1) = 24$	23.12	28
XX	${}_8C_2 = 28$	26.98	10
Total	${}_{11}C_2 = 55$	52.99	53

TABLE VI.

Combination	Number of combinations	Expected frequency	Observed frequency
$s_1S_7S_8$	${}_3C_3 = 1$	0.23	5
s_1S_7X S_7S_8X s_1S_8X	$({}_3C_2)({}_8C_1) = 24$	5.67	13
s_1XX S_7XX S_8XX	$({}_3C_1)({}_8C_2) = 84$	19.85	16
XXX	${}_8C_3 = 56$	13.23	5
Total	${}_{11}C_3 = 165$	38.98	39

Tables V and VI show that when the combinations contain only one X or no X, the small chromosomes S_7, S_8 and s_1 occupy the inner positions more frequently than we expect from the view point of probability, and that the chromosomes from S_1 to S_6 have a much lesser tendency to occupy the inner positions on an average than expected, because we know that the chromosomes L_1 and L_2 both of which are designated X in these tables similarly to the S-chromosomes take the inner positions as frequently as is expected.

If we, then, examine the expected and observed frequencies in respect to L's (L_1 and L_2), S's (S_1, S_2,......and S_6), and s's (S_7, S_8 and s_1) we obtain Tables VII and VIII for the cases where there are two and three inner chromosomes respectively.

TABLE VII.

Combination	Number of combinations	Expected frequency	Observed frequency
s s	${}_3C_2 = 3$	2.89	13
s L	$({}_3C_1)({}_2C_1) = 6$	5.78	10
s S	$({}_3C_1)({}_6C_1) = 18$	17.34	21
L L	${}_2C_2 = 1$	0.96	0
S S	${}_6C_2 = 15$	14.45	4
L S	$({}_2C_1)({}_6C_1) = 12$	11.56	5
Total	${}_{11}C_2 = 55$	52.98	53

TABLE VIII.

Combination	Number of combinations	Expected frequency	Observed frequency
s s s	${}_3C_3 = 1$	0.23	4
s s L	$({}_3C_2)({}_2C_1) = 6$	1.41	2
s s S	$({}_3C_2)({}_6C_1) = 18$	4.25	11
s S S	$({}_3C_1)({}_6C_2) = 45$	10.63	8
L L S	$({}_2C_2)({}_6C_1) = 6$	1.41	0
L L s	$({}_2C_2)({}_3C_1) = 3$	0.70	0
L S S	$({}_2C_1)({}_6C_2) = 30$	7.09	3
S S S	${}_6C_3 = 20$	4.72	0
L S s	$({}_2C_1)({}_6C_1)({}_3C_1) = 36$	8.50	11
Total	${}_{11}C_3 = 165$	38.94	39

The results given in Tables VII and VIII show a certain resemblance to the results of KUWADA's experiments with floating magnets ("Chromosome Arrangement." I.). In these experiments it has been shown that in the case where there are two inner chromosomes, the combination L_1 and L_2 is of very rare occurrence and probably forms a very unstable configuration, and in the results of our microscopical investigation this combination has not been found at all (Table VII). The experiments show also that in the cases where there are three inner chromosomes, the combinations sSS, LLS, LLs, LSS, SSS, and LSs do not occur, being transformed to the forms having two inner chromosomes. The results of our microscopical observations show that in the cases of the combinations LLS, LLs and SSS (Table VIII) this is really true, but some cases having the combinations sSS, LSS and LSs have been observed. These are, however, except the case of LSs, less than the expected numbers. That these latter cases are actually found microscopically seems to be due to the fact that while in KUWADA's experiments it is assumed that there are two L's, four S's and five s's, in the case of the microscopical investigation, owing to difficulty in discriminating the small S chromosomes except S_7 and S_8 from the larger S's, six chromosomes have been taken as S instead of four, and three as s instead of five. To put it in other words, there are two s's in KUWADA's sense among our six S chromosomes, and therefore there seems to be a certain possibility that such combinations as sSS or LSS may be realized in a certain measure.

SUMMARY.

1. In the heterotype division of pollen mother cells the number of gemini was 11 in 132 cases out of 140 cases observed, and 12 in 8 cases. In the case of 12 chromosomal elements a pair of elements lying near each other was generally found. The chromosomes are of different sizes, forming a graded series in regard to size. There is found in every case, whatever the total number may be, 12, or 11, a pair of large chromosomes (L_1 and L_2) which are distinguished from the other elments not only by their large size but also by other characteristics in their behaviour during division. A small geminus is also found, but it was difficult to identify it in some cases, because it is not distinctly smaller than the other small elements.

In the homotype division, counting of the chromosome number was possible only in a few cases, where it was shown to be 11 in all the countings.

In the endosperm the number of chromosomes is 12 so far as the present investigation is concerned, and in the nucellus tissue it was 24, though counting was possible only in a few cases.

2. The chromosome arrangement was investigated in pollen mother cells having 11 chromosomal elements. The form of arrangement (having 3 inner chromosomes), which resembles the stable configuration of MAYER's floating magnets, does not present the first maximum of frequency, but the second. The first maximum is represented by the form having two inner chromosomes. The two large elements (L_1 and L_2) occupy the inner positions of the arrangement as frequently as we can expect from the point of view of probability. The small element

has been shown on the other hand to have a greater tendency to occupy an inner position than we can expect from the same point of view. The result of observations also shows that in both cases of arrangement where there are two and three inner chromosomes respectively, the combinations in which some of the small chromosomes are contained occur more frequently than expected, while the combinations containing' no small chromosome are of less frequent occurrence than expected.

I desire to acknowledge my indebtedness to Prof. KUWADA, under whose suggestion and direction this investigation was made, and also to express my sincere thanks to Mr. KINASHI of the Botanical Institute for the kind help he has accorded me in the collection of material, and also to Prof. KUWADA for his kindness in giving me permission to use some of his material.

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