CYTOLOGICAL OBSERVATIONS IN *COFFEA*. IV[1]

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(With Eighteen Text-figures)

CONTENTS

I. Introduction

In one of the previous articles on the cytology of *Coffea* (Krug, 1937) the senior author published a preliminary account on the occurrence of a triploid coffee plant obtained by hybridization between *C. arabica* L. ($2n = 44$) and *C. canephora* Pierre ($2n = 22$). The plant was at that time only in its seedling stage, its triploid nature having been determined at mitosis in root tips. At metaphases some of the longer *canephora* chromosomes could be easily detected. In other articles on the same subject it was suggested that some of the interspecific hybrids grown in Java were highly unproductive due to their triploid nature; a high sterility was expected to occur in these plants.

The present article confirms the hypothesis on the sterility of these triploids, giving a detailed account on the meiotic behaviour of their chromosomes. In the course of our work a few more hybrid plants of the same nature were obtained.

II. Morphological characters of two interspecific triploid hybrids (*Coffea arabica* × *C. canephora*)

The interspecific hybrids as yet studied are intermediate with respect to most of their morphological characters when compared with their parents. The germination of the hybrid seeds is somewhat retarded and the growth at early seedling stages very slow; this may be caused by the

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general lack of sufficient nutritive tissue contained in the seed; in later stages the growth rate is normal. No hybrid vigour is perceptible.

TABLE I

Tree no.	Species and hybrids	Leaf dimensions: Length mm.	Breadth mm.	Index	Angle of veins
45	*C. arabica* var. *bourbon* ($2n=44$)	131·94	53·44	2·47	57°
45 × 37	Triploid hybrid ($2n=33$)	172·00	71·88	2·39	63°
37	*C. canephora* ($2n=22$)	192·50	87·92	2·19	67°
34	*C. arabica* var. *mokka* ($2n=44$)	87·40	29·16	2·99	48°
36 × 34	Triploid hybrid ($2n=33$)	126·34	43·92	2·88	55°
36	*C. canephora* ($2n=22$)	210·62	88·52	2·38	69°

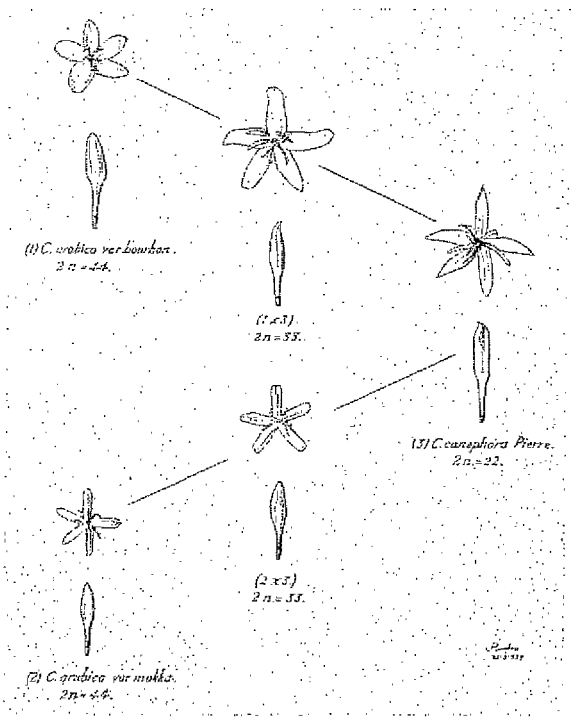

Fig. 1.

Table I contains the leaf dimensions of the parents and hybrids grown under half shade, and Fig. 1 shows differences in flower types ($\times\frac{1}{2}$).

In another instance a cross was made between *C. canephora* and *C. arabica* var. *Murta*, a small-leaved heterozygous variety (Krug, 1938); the morphological characters of the hybrid, not mentioned in the above table, are very similar to those obtained by crossing the var. *bourbon* with *C. canephora*. The results which are being obtained in these interspecific hybridizations are mainly of genetical interest and will be dealt with in future publications on the genetics of *Coffea*.

All these interspecific hybrids have been flowering abundantly, the flowers being normally developed, but practically no fruit set has occurred.

III. CYTOLOGICAL OBSERVATIONS IN TRIPLOID *COFFEA*

(*a*) *Material and methods*

For the study of microsporogenesis of these triploid plants, flower buds were fixed in a solution of three parts of alcohol and one of acetic acid; aceto carmin was used for staining the smears.

All flower buds examined originated from one single hybrid plant (45 × 37–1).

The drawings were made with camera lucida with a magnification of 3350 times; Zeiss 100 × immersion objective and 20 × ocular were used; Figs. 2–18 were reduced $\frac{1}{4}$ for reproduction.

(*b*) *Microsporogenesis*

The initial prophase stages are difficult to observe in detail, the chromatic filaments not showing up very clearly. In this stage one notes that most of them condense close to the relatively large nucleolus; gradually the filament begins to expand throughout the nucleus; however, as long as the nucleolus exists, a good deal of the filaments remain more or less attached to it (Fig. 2). The chromonemata appear merely as dotted lines.

At this initial stage, the nucleus of the pollen mother cell retains its normal round shape, being less intensively stainable than the surrounding cytoplasm.

At the stage when the filaments begin to appear more clearly, the cell volume increases, its cytoplasm becomes less dense and takes up less stain than in earlier stages. Pressing the cover glass on the slide, caryoplasm and cytoplasm become completely undistinguishable from each other. Due to this procedure, the filaments become better separated and a clear doubleness of some of the chromosome strands can sometimes be noted (Fig. 3). The pairing is difficult to be observed due to the con-

densation of the filaments close to the nucleolus in most of the cells. It is however clearly noticeable that the pairing of the filaments becomes gradually more frequent and also that sometimes a third filament tries to attach itself to a pair of chromosomes and apparently mainly at those regions where the two strands are not intimately united.

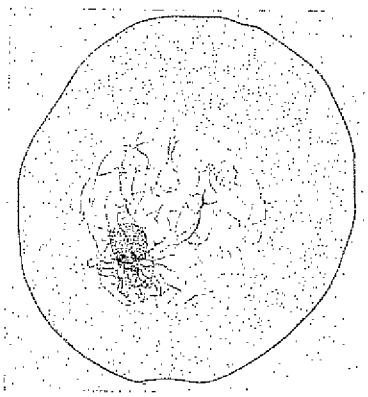

Fig. 2.

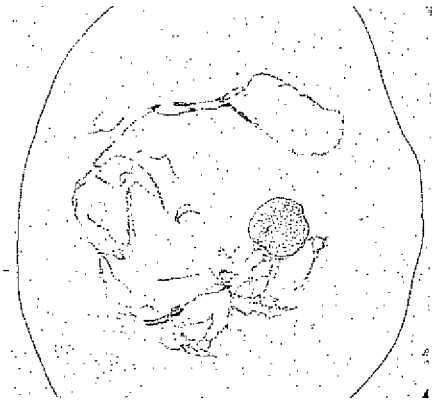

Fig. 3.

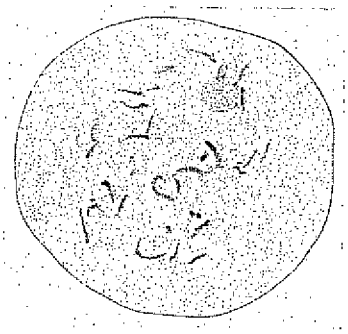

Fig. 4.

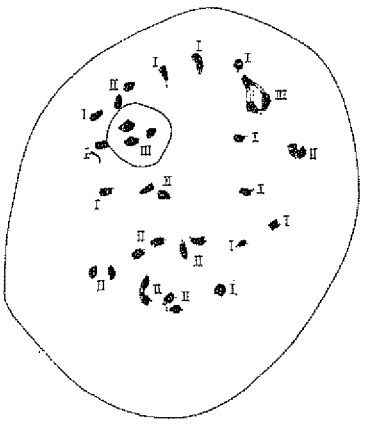

Fig. 5.

The chromatic filaments begin to contract themselves and the chromosomes appear shorter and thicker; they unite, the contracted filaments appearing deeper stained at certain regions, probably due to differences in the degree of contraction along the chromosomes (Fig. 4). As this contraction is terminal for some chromosomes and subterminal or median for others, certain regions of the chromosomes appear at this

stage deeply stained, the remaining part of them nearly disappearing into the cytoplasm (Fig. 5).

At this stage of contraction the nucleolus remains visible, the chromosomes appearing in definite uni-, bi- and trivalent groups, attaining their maximum degree of contraction when passing from diakinesis to metaphase, the univalents taking then a roundish shape.

Table II indicates the frequency of uni-, bi- and trivalent groups in

TABLE II

	Prophases					Metaphases			
Cells	Uni-valents	Bi-valents	Tri-valents	Bi- + tri-valents	Cells	Uni-valents	Bi-valents	Tri-valents	Bi- + tri-valents
A	11	11	0	11	H	6	9	3	12
B	11	8	2	10	I	7	7	4	11
C	11	8	2	10	J	8	8	3	11
D	9	6	4	10	K	6	6	5	11
E	12	6	3	9	L	9	6	4	10
F	16	7	1	8	M	17	5	3	7
G	17	5	2	7	N	29	2	0	2
7	87	51	14	65	O	33	0	0	0
					8	115	43	21	64

Total number of chromosomes 231

% of bivalents	43·3
% of trivalents	18·2
% of bi- + trivalents	61·5

Total number of chromosomes 264

% of bivalents	32·6
% of trivalents	23·9
% of bi- + trivalents	56·5

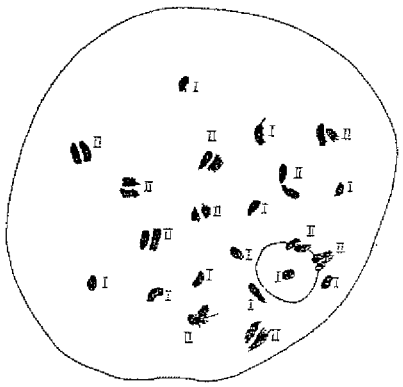

Fig. 6.

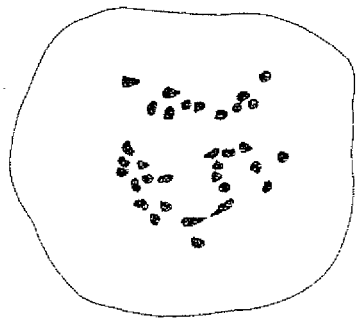

Fig. 7.

diakinesis and metaphase in fifteen examined pollen mother cells. It seems that by passing from one to the other of the above-mentioned stages, the united strands do not separate from each other. The univalents appearing at these stages must therefore be considered as asynaptic chromosomes, not having entered synapsis at earlier stages.

Figs. 5 and 6 show two cases of diakinesis, the first with 11_{I}, 8_{II}, and 2_{III} and the second with 11_{I} and 11_{II}. Fig. 7 represents a metaphase

with 29 univalents and 2 bivalents; it is interesting to note in this figure that the chromosomes are approximately arranged in three groups of eleven. Another metaphase is represented at Fig. 8, where 33 univalents are distributed in two groups, one of 12 and another of 21 chromosomes.

At the beginning of the anaphase, the univalents travel, without dividing themselves, to the poles, their distribution being apparently at random; the movement of the bi- and trivalents is retarded as seen in Fig. 9. The distribution of these elements is extremely irregular,

Fig. 8.

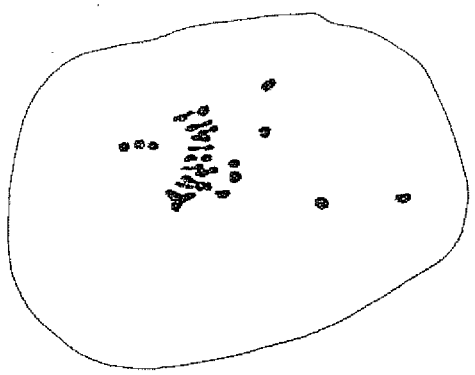

Fig. 9.

sometimes both constituents of the same bivalent group passing to the same pole as can be observed in Fig. 10. Some of these elements retard to such a degree, that they are not included in the resulting daughter nuclei, forming later on micronuclei; the same happens occasionally to some retarded univalent chromosome.

In Table III the distribution of the chromosomes is given as observed in thirty-seven cells at anaphase; it is noted that a certain tendency exists to form daughter nuclei with approximately half of the chromosome set, i.e. with sixteen or seventeen elements; however, due to the irregu-

larities observed during the distribution of the chromosomes, some of them being delayed in their movements to the poles, 54% only of the daughter nuclei contain these numbers.

At the end of the first anaphase sometimes a vacuolization of the chromosomes and the formation of telophasic nuclei containing one or more nucleoli are noted as demonstrated in Figs. 11 and 12. Most commonly, however, the chromosomes do not undergo these changes and start dividing again (second division).

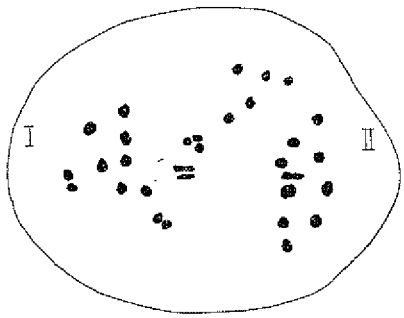

Fig. 10.

TABLE III

Pole 1	"Laggers"	Pole 2	No. of cells
15	2	16	9
16	0	17	6
15	1	17	5
16	1	16	3
14	3	16	2
13	4	16	2
15	0	18	2
14	2	17	1
14	1	18	1
15	3	15	1
13	3	17	1
12	5	16	1
11	5	17	1
13	0	20	1
11	3	19	1
207	33	255	37

When telophasic nuclei are formed at the end of the first division, it is not known if a second division really takes place; it is possible that pollen dyads are formed in these instances instead of tetrads. In about 400 microsporocytes examined however no dyads were found.

At the second division the frequency of lagging chromosomes is smaller than at the first one; in almost 50% (43%) of the divisions observed, the chromosomes divide and their halves are uniformly distributed to the poles. Fig. 13 gives an example of the second division;

at one of the poles 11 chromosomes travel to each side; at the other pole 20 chromosomes go to one side and 18 to the other. There are also 6 remaining chromosomes from the first division; as a total of 30 chromosomes are present at the two poles, the 6 remaining chromosomes have originated from 3 not included in the daughter nuclei after the first

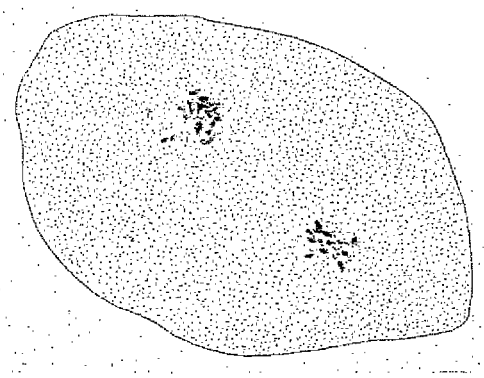

Fig. 11.

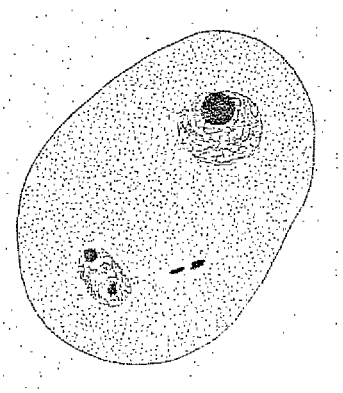

Fig. 12.

division, and which divided farther on. In a similar figure nearly the same distribution of the chromosomes was noted, with the only difference that at one of the poles 19 chromosomes divided themselves normally at second division.

Due to these abnormalities the second division furnishes nuclei averaging less chromosomes than those resulting from the first division; 36·5 % of the microspore nuclei revealed to have 15 or 16 chromosomes.

Only 9·5% of these nuclei had 11 chromosomes and not a single one was found with 22.

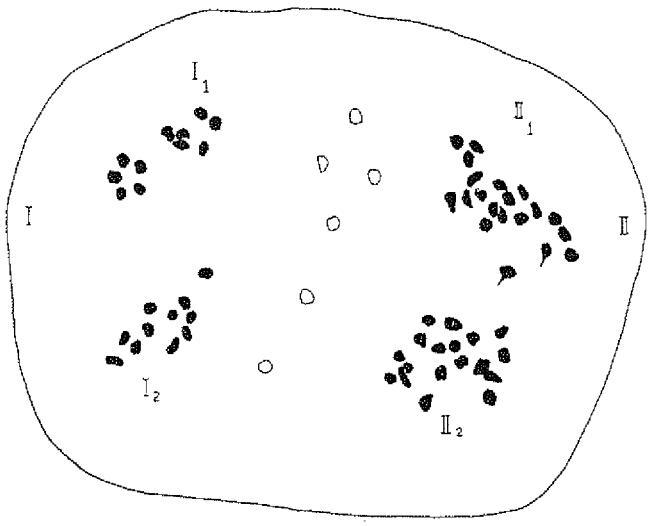

Fig. 13.

Table IV gives the distribution of the chromosomes at second anaphase in 21 cells examined and Table V contains a total summarized frequency distribution of these data.

TABLE IV

2nd anaphase at Pole I			"Laggers" at 1st anaphase	2nd anaphase at Pole II		
Pole I_1	"Laggers"	Pole I_2		Pole I_1	"Laggers"	Pole I_2
18	—	20	4	11	—	9
13	—	13	4	16	—	16
17	—	17	5	11	—	11
17	—	15	1+(1→2)	15	—	15
19	—	19	3→6	11	—	11
20	—	18	3→6	11	—	11
15	—	17	2	15	—	15
16	—	18	3	12	—	14
16	—	16	5	10	2	12
16	—	14	2	15	2	15
16	—	16	4	12	1	13
16	—	16	2	14	2	14
16	1	17	3	12	1	13
16	1	15	0	16	3	15
15	2	15	4	13	—	13
12	—	14	0	19	—	21
13	—	13	2	—	—	—
17	—	17	4	—	—	—
15	—	15	4	—	—	—
14	1	14	3	—	—	—
13	—	13	3	—	—	—

As mentioned above, the lagging chromosomes from the first division sometimes divide at the same time the second anaphase occurs, but are not included in any of the four main nuclei formed; these chromosomes do not dispose themselves in a plate and no spindle is formed (Fig. 13).

Fig. 14 illustrates another aspect of the second division; the arrangement of the chromosomes in this cell may have originated through the following facts: at first division 2 chromosomes got lost in the cytoplasm, 15 going to one pole and 16 to the other; at second division another

TABLE V.

	No. of chromosomes												Total
	9	10	11	12	13	14	15	16	17	18	19	20	21	—
Frequency	1	1	7	5	10	7	14	13	7	3	3	2	1	74

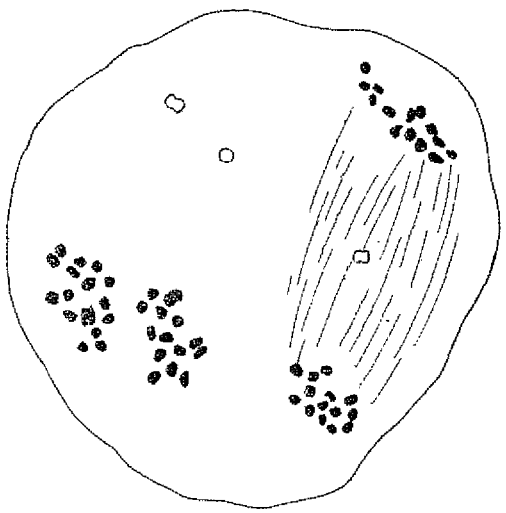

Fig. 14.

chromosome lost itself at one of the poles, 14 chromosomes passing to each side; at the other, 15 chromosomes went to one side and 16 to the other, one of them without dividing.

As a consequence of the abnormal behaviour of some of the chromosomes, microcytes are formed which may contain from 1 to 10 chromosomes due to the fact that they often divide; the viability of these microcytes is however very doubtful.

At the end of the second division, the cytoplasm divides and "tetrads" are formed; as expected, these are nearly always abnormal, containing more than 4 microspores (Figs. 15–18).

Counting the microspores in 400 microsporocytes the results shown in Table VI were obtained.

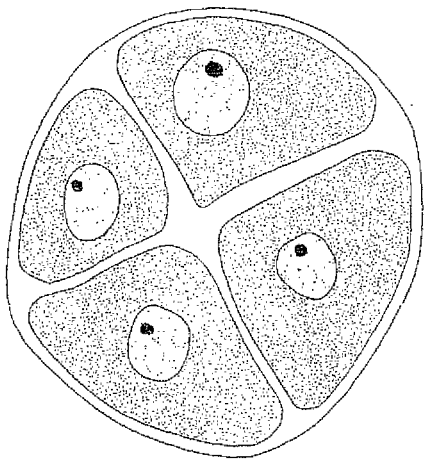

Fig. 15.

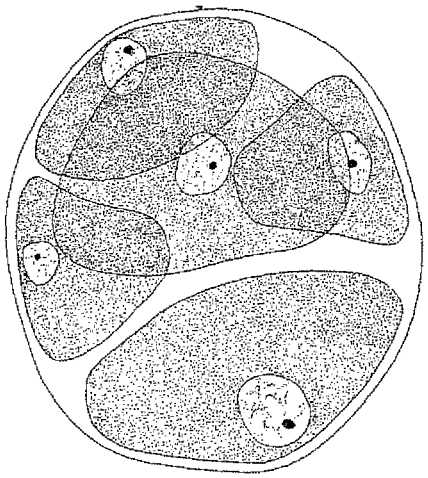

Fig. 16.

(c) Characters of the pollen grains

As a result of the great variability of the number of microspores formed, the size of the resulting pollen grains is also extremely variable. 200 grains were measured, their average diameter being $27{\cdot}58 \pm 6{\cdot}66\,\mu$ ($C = 24{\cdot}18\,\%$); they vary between 8 and $48\,\mu$.

In a previous paper (Krug, 1937) data were published on pollen size and variability of three varieties of *C. arabica*, one tetraploid, and the other two respectively hexa- and octoploid; in the following table the variability of their pollen size is compared with the one of the triploid:

C. arabica ($2n=44$): 28 to 44 μ C = 8 %
,, ($2n=66$): 28 to 60 μ C = 12·6 %
,, ($2n=88$): 24 to 56 μ C = 12 %
C. arabica × *C. canephora* ($2n=33$): 8 to 48 μ C = 24·18 %

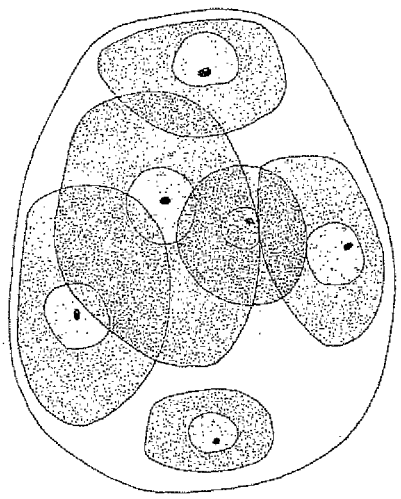

Fig. 17.

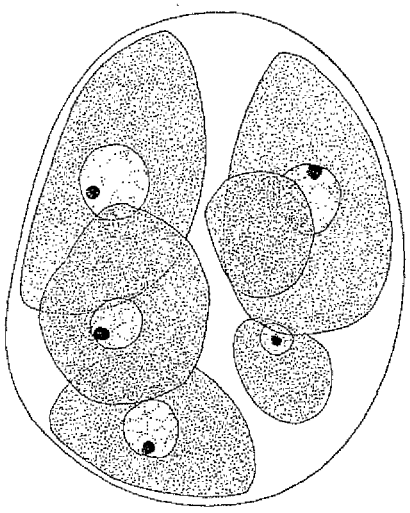

Fig. 18.

TABLE VI

Number of microspores formed by each "tetrad"

	No. of microspores						
	4	5	6	7	8	9	Total
Frequency	105	195	86	11	2	1	400
%	26·3	48·8	21·5	2·7	0·5	0·2	100

In spite of the fact that meiosis is also abnormal in the hexa- and octoploid, the variability of the pollen size is much higher in the triploid.

Germination tests with hybrid pollen grains indicated that they are almost completely sterile; pollen of normal tetraploid *C. arabica* plants germinated under identical conditions to more than 90 %.

IV. Results of selfing and crossing the triploid

A great number of flowers of the triploid plant were protected with bags to insure self pollination but only a minute number of fruits were

obtained; these were misshaped and only some of these contained a few irregular seeds with completely abnormal embryos; not a single one germinated.

Many flowers were also pollinated by *C. arabica* and *C. canephora* pollen, but no seeds were obtained.

As the triploid plants flowered abundantly, at the same time many other coffee species and varieties of varied cytological constitution flowered around them, a few fruits were formed on the triploid, yielding two apparently normal seeds. Two plants were obtained from them which revealed to have $2n=44$ *chromosomes*. Very few of their chromosomes seem to be of the *canephora* type.

V. Discussion and conclusions

As expected, the meiosis of the interspecific triploid hybrid *C. arabica* × *C. canephora* is completely abnormal, sterile pollen grains with varying number of chromosomes being formed. It is very probable that the same irregular behaviour of the chromosomes noted in microsporogenesis also occurs at megasporogenesis. The sterility of some of the interspecific hybrids grown in Java is therefore completely cleared up.

With respect to the behaviour of the chromosomes at meiosis a few interesting conclusions may be drawn. Up to the present date nothing is known regarding the genetical relations between the varied species of the genus *Coffea* and the origin of some of the cultivated types. The triploid hybrid above examined got one chromosome set of 11 elements from *C. canephora* and another of 22 from *C. arabica*; the fact that a pairing of chromosomes occurs at early prophase up to metaphase of meiosis, in average at a rate of respectively 61·5 and 56·5%, indicates that certain homology exists between many of the chromosomes; due to the fact that some of the *canephora* chromosomes are longer than the *arabica* ones, and that *C. arabica* is already considered as a tetraploid species, it is probable that in most of the cases autosynapsis occurred between *arabica* chromosomes. No data are available, however, to deny the occurrence of alosynapsis.

If the *arabica* species should be an autotetraploid, the existence of characters determined by duplicate genes should be frequent; however genetical studies which are in course revealed that most of the main characters, analysed up to the present, are determined by single pairs of genes. If the hypothesis of an autotetraploid origin should be confirmed by later cytological and genetical analysis, it must be assumed that the duplication occurred at a very remote date.

The loose association of a third member to some of the chromosome pairs in prophase and metaphase suggests that this third element belongs to the *canephora* set of chromosomes, as the occurrence of neither an association of three *arabica* chromosomes nor the pairing of *canephora* elements *inter se* is probable. It may be that certain regions of some of the *canephora* chromosomes are analogous to some existing in certain *arabica* elements; or that the association of a third chromosome to a group of two is merely caused by the attraction of inert regions (Kostoff & Arutiunian, 1938). The data obtained are as yet too incomplete to make any considerations regarding the possible genetical relations between the two species crossed.

Regarding the cytological constitution of the two progeny plants of one of the triploid hybrids the following considerations can be made. Both have $2n=44$ chromosomes and it is apparent that some of the chromosomes derived from *C. canephora*. The plants are still in the seedling stage and therefore nothing can be said yet about their morphological characters. As stated above, they originated from seeds of open pollinated flowers. If one analyses Table V, one concludes that these plants did not originate through self pollination, as it was observed that only one nucleus out of 74 was formed at second telophase with 21 chromosomes; the association, through self pollination, of two gametes summing up 44 is therefore highly improbable. Considering cross pollination, pollen of 11, 22 and 33 chromosomes was available from neighbouring plants. 11-chromosome pollen did not fertilize the triploid, as it does not produce 33-chromosome gametes. From the other two types of pollen, the one with 22 chromosomes is more probable to have been the pollinating agent, as it was available around the triploid plant in great majority. The only hexaploid plant, which furnished a small percentage of viable 33-chromosome pollen grains, flowered very little in the neighbourhood. The future study of the meiosis of these two tetraploid progeny plants will probably clear up its cytological constitution.

VI. Summary

1. A short description is given of the main morphological characters of two interspecific triploid hybrids (*C. arabica* × *C. canephora*); their growth habit is normal and leaf and flower characters are intermediate in shape and size when compared with their parents.

2. The meiotic behaviour of the chromosomes at microsporogenesis is given in detail; the expected abnormalities were observed at the distribution of the chromosomes at first and second divisions, resulting

in the formation of sterile pollen grains which are extremely variable in size.

3. The megasporogenesis in this triploid is believed to show the same abnormalities as observed in microsporogenesis.

4. The sterility of some interspecific hybrids grown in Java, as suggested in previous articles, is therefore confirmed and cleared up.

5. The intimate pairing of some chromosomes at early prophase up to metaphase suggests the possible autotetraploid origin of *Coffea arabica*.

6. The occasional association of a third chromosome at prophase and metaphase to groups of two suggests that some of the *canephora* chromosomes may have regions analogous to ones of some *arabica* elements; nothing can be stated, however, regarding a possible genetical relationship between *C. arabica* and *C. canephora*.

7. Two progeny plants of a triploid, obtained from open pollinated flowers, revealed to have $2n = 44$; it is suggested that they derived from cross pollination, 22 chromosome pollen from normal *arabica* plants in the neighbourhood having fertilized two occasional 22 chromosome egg cells of the triploid.

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