CYTOLOGY OF HEVEA

by

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INTRODUCTION

From a cytological point of view *Hevea* may be called a practically new subject. So far only two investigators have occupied themselves to a certain extent with the cytology of *Hevea*. The first of these was HEUSSER (1919) who, while carrying on investigations on the reproductive organs of *H. brasiliensis* devoted some attention to meiosis.

As HEUSSER intended to approach the improvement of the rubber-tree by means of artificial crossing, he rightly thought a preliminary study of the reproductive organs to be of importance.

In his description of meiosis, however, HEUSSER does not enter into particulars; starting from a few simple drawings he mentions the most striking stages with a brief discussion of their connection with the phenomena of heredity. For this investigation young buds from random seedlings of ordinary estate-rubber were used.

Some years later there appeared a note on the number of chromosomes of some species of *Hevea* by BANGHAM (1931), in which the number n = 8 reported for *H. brasiliensis* by HEUSSER was corrected. For all the species investigated BANGHAM finds 2n = 34 and, moreover, n = 17 for *H. brasiliensis*. His publication contains no further information as to the process of meiotic division.

In the meantime artificial cross-pollination has been generally applied to rubber-selection in the Netherlands East Indies. I have also been engaged in this work for some years, during which I made a number of observations which induced me to an investigation into the germinative power of *Hevea* pollen (RAMAER 1932).

In the course of this investigation I found, besides a number of normally fertile forms, a few types with degenerated pollen, which invited cytological investigation.

After a preliminary test of normal *H. brasiliensis* I came to doubt the correctness of the number of chromosomes n = 17 as stated by BANGHAM.

A comparison with other tropical cultivated plants, such as *Saccharum*, *Nicotiana*, *Gossypium*, etc., emphasized the paucity of our knowledge of the cytology of *Hevea*.

It therefore appeared to me, that a thorough cytological investigation was fully justified.

The results of this investigation, which was for the greater part carried on in the Botanical Laboratory at Utrecht, are recorded in the following pages.

CHAPTER I

DESCRIPTION AND PECULIARITIES OF THE MATERIAL INVESTIGATED

§ 1. *Material.*

For the investigation of meiosis in *Hevea brasiliensis* MUELL. ARG. three clones, which were in every respect normal, were selected from the observation collection of the „West Java" Experimental Station at Buitenzorg. Two of them, BR_1 and BR_2 are of Sumatra origin being obtained from the Bogor Redjoh estate in South Sumatra. The third, Tjir. I is one of the best known clones in Java and has originally been found at the rubber-estate Tjirandji.

Five clones were further investigated which show symptoms of sterility: PR 104 and C.R.S. 24 from the „West Java" Experimental Station, Ct. 88 from the Culture Garden of the Department of Agriculture at Buitenzorg, KN 251 and KN 220 from the Klapanoenggal rubber-estate near Buitenzorg.

The study of meiosis prompted a desire to include the somatic chromosomes in my investigation. No material being at hand a number of viable seeds of *H. brasiliensis* were obtained from the Experimental Station for Agriculture in Surinam. To the director Prof. Dr. G. Stahel I wish to express my profound gratitude for the sending of the seeds and to Prof. Dr. L. P. de Bussy for his kindness in acting as intermediary.

The seeds were germinated for the greater part in the hothouse of the Utrecht botanical gardens, after which the roots of the young seedlings were used for further investigation.

Material of *Hevea Spruceana* MUELL. ARG., of *H. guianensis* AUBL. and of *H. collina* HUBER was collected as well as of *H. brasiliensis*. Of these species a number of trees exist in the Culture Garden at Buitenzorg. I am greatly indebted to Messrs. H. de Veer and Ir. G. G. Bolhuis for information concerning the origin of these forms and for other valuable assistance.

H. Spruceana and *H. collina* were originally obtained from the Selection Station at Santarem (Brazil) and *H. guianensis* from the Selection Station in Surinam.

Finally the Culture Garden at Buitenzorg is in the possession of some peculiar forms of *Hevea*, which are considered to be species-hybrids of *H. collina* × *H. brasiliensis* (D2—78) and of *H. Spruceana* × *H. brasiliensis* (D2—49). From these trees material was also collected.

§ 2. *Taxonomical.*

That the rubber clones cultivated at present belong to the species *H. brasiliensis* MUELL. ARG. is hardly ever doubted. They originated from ordinary estate rubber in the Netherlands East Indies which in its turn sprang from seeds gathered by WICKHAM in Brazil in 1876. In connection with the numerous variations of the characters of *H. brasiliensis* in the Netherlands East Indies the question of the purity of this species has already been repeatedly discussed.

As is communicated by CRAMER (1914) the possibility that among the seed material of WICKHAM, besides *H. brasiliensis*, *H. collina* may have occurred, is admitted by HUBER, so that in the Netherlands East Indies *H. brasiliensis* might have been mixed with *H. collina*.

According to HEUSSER (1919), however, the fact that among the great number of flowers investigated, he has never met one of the *collina* type, is an argument in favour of the contrary.

In my opinion it is doubtful whether as much reliance should be put on this argument as HEUSSER does, in view of the fact that absolutely nothing is known about cross-fertilization of the various species of *Hevea*.

As I have already said the variation of the characters of cultivated *H. brasiliensis* is very considerable. There is no characteristic that does not vary to a very great extent. This has even been utilised for the drawing up of tables of identification for young trees of the best known cultivated clones (FREY—WYSSLING, HEUSSER and OSTENDORF 1932).

In Brazil similar variation is found, as is reported by DUCKE (1933) who paid much attention to the classification of *Hevea*, and made observations in various parts of Brazil.

The difficulties involved by the classification of the genus *Hevea*,

are evidently very great. In reviewing my material I think I cannot do better than take the classification of DUCKE for my guide, as the latter is considered the greatest authority in the field of the taxonomics of *Hevea.*

The original classification of the genus into the subgenera *Euhevea* and *Bisiphonia,* according to the existence of either one or two (complete or incomplete) whorls of stamens, is made by MUELLER ARGOVIENSIS (1873) and also used by HUBER (1905) but recently modified by DUCKE (1930). The latter distinguishes 5 groups, the first of which approximately corresponds with the subgenus *Euhevea,* which includes the species *H. guianensis* AUBL. and *H. collina* HUBER. They are chiefly characterised by one regular whorl of 5 stamens, the practically entire absence of a disc in the male flower, and small, hard, leathery leaflets.

To this I may add that the forms of *H. guianensis* and *H. collina* I investigated, when compared with *H. brasiliensis,* are very slowly growing trees and produce extremely small seeds. The seeds are half the volume of those of *H. brasiliensis,* and are triangular in shape.

When the trees begin to shed their leaves the latter assume brilliant red and yellow colours; their latex is yellow and resinous.

The great similarity of these two species induced DUCKE (1933) to consider the species *collina* HUBER a variety of *guianensis* AUBL. For conveniences sake, however, I shall go on speaking of *H. collina.*

The 2nd, 3rd and 4th groups include five species which possess an irregular number of stamens in two indistinctly separated and incomplete whorls (See below Ch. 3). They belong to the subgenus *Bisiphonia* of MUELLER ARGOVIENSIS.

In the 5th and largest group DUCKE includes *H. brasiliensis* MUELL. ARG. and *H. Spruceana* MUELL. ARG. Both species are characterised by two complete and regular whorls of 5 stamens and therefore also belong to the *Bisphonia* type of MUELLER ARGOVIENSIS. Besides in the number of stamens they differ from the first group by the possession of a disc in the male flower, large leaves which are scarcely, or not at all, leathery, more vigorous growth, larger seeds, and white latex. The leaves, at the moment of falling, are pale yellow or pink.

H. brasiliensis and *H. Spruceana,* however, are markedly different from one another.

The most striking difference is in the colour of the flowers. Whereas

H. brasiliensis always has yellow flowers, *H. Spruceana* is, as far as I have been able to ascertain, the only *Hevea* with purple flowers. Each of the 3 outsides of the pericarp is concave with *H. brasiliensis*, convex with *H. Spruceana*. The fruit of *H. brasiliensis* bursts open spontaneously, the seeds and parts of the pericarp being hurled away; the fruit of *H. Spruceana* opens slowly, the seeds dropping vertically and the parts of the pericarp remain hanging. The fruit, moreover, is oblong and the seeds are approximately twice as long as those of *H. brasiliensis*, whose fruit is just as long as it is wide.

The shape and place of the disc-lobes of the male flowers differ

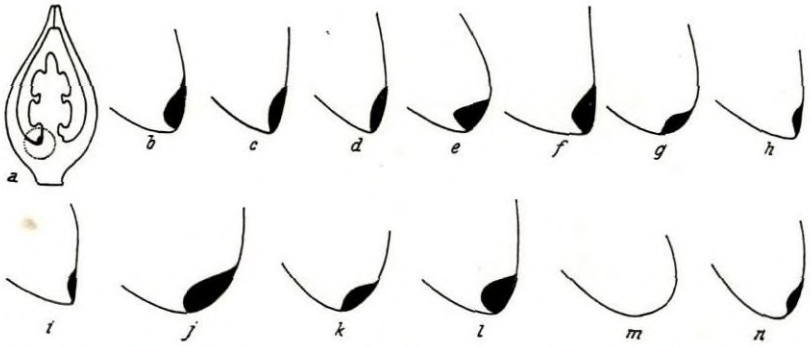

FIG. 1. Variation in shape of the disc-lobes in longitudinal sections of male buds of *Hevea*.

a: Diagram of a longitudinal section. b—n represent magnifications of the marked part of a. b—i: Several clones of *H. brasiliensis*. j: *H. Spruceana*. k,l: *H. Spruceana* × *brasiliensis*. m: *H. collina* HUBER. n: *H. collina* × *brasiliensis*. 85 ×.

in the two species (fig. 1); the flowers of *H. Spruceana* are long-haired, those of *H. brasiliensis* are short-haired. The buds of *H. brasiliensis* taper, those of *H. Spruceana* are blunt, and finally the leaves of the former are glabrous, those of the latter hairy.

The differences between the two species are much more considerable than the variations within the species *brasiliensis*.

As regards the specimens I investigated they entirely agree with DUCKE's description, so that I can be quite sure about their identity.

Of the five sterile forms of *H. brasiliensis* it can also be stated that they show all the characteristics of *H. brasiliensis* with the always present variation.

Fig. 1 shows the various shapes of the disc-lobes in male buds of all forms mentioned; they are found either against the foot of the column (shown to the left) or more on the receptacle.

As to the *H. brasiliensis* seeds obtained from Surinam, they had all the characteristics of *brasiliensis* seeds, and their seedlings resembled also those of *H. brasiliensis*, but more cannot be said.

In § 1 a few *Heveas* were already mentioned which might be looked upon as species-hybrids. Up till now however, these trees have not been investigated, so that a short description follows below.

D2—78 is looked upon as a hybrid of *H. collina* × *H. brasiliensis*. Its growth is satisfactory and it has leaves which are fairly large and not leathery, therefore corresponding rather with those of *H. brasiliensis*. In the male flowers a disc is found (fig. 1n) whose 5 lobes are smaller than those of *H. brasiliensis*. The flowers are of an intermediate size, form and colour, the seeds resemble more those of *H. brasiliensis*.

The number of stamens varies from 5 to 10. Moreover they are mostly very irregularly inserted in two whorls which are not markedly separated. Half- or badly developed stamens also occur.

Of this three I have investigated 151 flowers; table I shows the numbers of stamens occurring in the flowers examined. Half stamens have been counted as complete ones.

TABLE I

Number of stamens	Number of flowers
5	10
6	19
7	30
8	**48**
9	34
10	10

Taken as a whole, therefore, the number of stamens is intermediate though tending in the direction of the greater number. The same can be said about the other characteristics, which are nearly all intermediate with a slight approach to the *brasiliensis* type.

D2—49 is considered a hybrid between *H. Spruceana* × *H. bra-*

siliensis. The coloration of the flowers is striking, these showing a purple basis with yellow lobes.

The shape of the fruit is intermediate with a slight approach to the *brasiliensis* type. I have not been able to observe their bursting; from the state of some dry fruits received from Buitenzorg, however, the conclusion that this characteristic is also intermediate is justified; it is supposed, however, that the seeds are not hurled any considerable distance as the valves of the fruit remain partly together and attached to the stalk. The seeds are very big, being almost the size of *Spruceana* seeds, but somewhat thicker, not broader.

Markings and colour of the seeds are intermediate. The shape of the disc of the male buds is intermediate but varying in several flowers (fig. 1k, l), the hair of the flowers and the shape of the buds are intermediate, the leaves are practically glabrous.

Further it can be stated that the Herbarium at Utrecht possesses herbarium material of a tree collected by DUCKE in Amazonas, which is thought by him to be a hybrid between *H. Spruceana* × *H. brasiliensis*. The flowers of this plant are also predominantly purple with yellow lobes, while the leaves are glabrous. The description of these „species-hybrids" brings up the question of hybridization.

§ 3. *Hybridization.*

Experience in the field of the hybridization of *Hevea* is limited to the species *brasiliensis*. The results of artificial cross- and self-fertilization enable us to form an idea of the fertility of cultivated *H. brasiliensis*. Literature on this subject can be found with S' JACOB (1931).

It appears then that, just as all other characters, fertility varies a great deal.

As a rule cross-fertilization is considerably more successful than self-fertilization. During the years 1928—1931 I myself carried out 40609 cross-fertilizations with an average success of 6.7% against 3250 self-fertilizations of which 0.9% were successful. In 1931 I began observations and experiments on interspecific hybridization, induced by the presence of the „species hybrids" in the Culture Garden of Buitenzorg, as described in § 2. These plants were not obtained by artificial cross-pollination but according to my informant, Mr. H. DE VEER, they originated as follows.

A number of seeds were collected from two trees, of *H. collina* and *H. Spruceana* respectively, at the Bogor Redjoh estate and were germinated in two separate beds. The seedlings of each of the beds soon separated into two entirely different groups, one showing a striking resemblance with the mother tree, the other group showing *brasiliensis* influence.

It was reasoned that the seeds of the first group resulted from self-fertilization and those of the second from cross-fertilization by pollen from the surrounding *H. brasiliensis;* so the latter group would, in this case, be species-hybrids. A number of these plants planted out in the Culture Garden at Buitenzorg, grew up well. In 1931 they were 6 years old, flowered and produced fruits and viable seeds.

In the same year I myself repeated this experiment for check. Of each of the trees described in § 2: *H. guianensis*, *H. collina* and *H. Spruceana* seeds were collected and germinated in three separate beds. The progeny of *H. guianensis* for the most part died but that of the two other forms grew up well. After a few months striking differences were visible between the plants in both of the beds.

Two groups were clearly distinguishable. In the *collina*-bed part of the plants dropped considerably behind the other part in growth. After six months the latter were as much as from 2 to 3 times as tall as the former. The smaller plants showed, in their leaves, typical characteristics of *H. collina;* the taller more of *H. brasiliensis*.

With the *Spruceana*-group the same was observable, although the growth differences were less striking than in the progeny of *H. collina*. Although the differences in the appearance of the foliage and other habit characters of immature plants have not been discussed in the preceding sections, they are great enough to enable the above-mentioned grouping.

Finally I reciprocally crossed *H. brasiliensis* with *H. Spruceana*. *H. brasiliensis* × *Spruceana* proved very successful, better even than many crosses between clones of *H. brasiliensis*.

H. Spruceana × *brasiliensis*, however, did not succeed.

Fertilization took place, but the young fruits were all shed, a phenomenon which also frequently occurs with *H. brasiliensis* and is attributed to the physiological condition of the plant.

I have not been able to make any experiments in the crossfertili-

zation of *H. guianensis* and *H. collina* as flowers of neither species were available during the flowering period of *H. brasiliensis*.

§ 4. *Sterile forms.*

During an investigation of the germinative power of pollen of *H. brasiliensis* (RAMAER 1932) I found with clone P.R. 104 a great deal of dead pollen besides a small quantity of full pollen grains, whose size varied considerably; only a few of them germinated on sugar-agar.

The male flowers and stamens were externally normal but at closer investigation most anthers were filled with dead pollen. One single loculus had almost exclusively full pollen grains. Degeneration of pollen appeared to take place after the tetrads had fallen apart.

R.P. 104 produces exceedingly little fruit so that this clone can almost be called sterile.

During experiments concerning artificial pollination on the Klapanoenggal estate two clones KN 220 and KN 251 were found, the male flowers of which did not attain to full development but were shed in the budding stage. When the buds were opened there proved to be, on the place of the column with the 10 anthers, a hard little pin, which often lay loose on the receptacle.

A closer examination of the male buds revealed that here not only degeneration of the young pollen takes place but also of the whole stamens. It was remarkable that a few male flowers could be induced to open by removing all other buds from the panicle.

As to the fruiting of these clones, KN 220 never produced any fruit; KN 251, on the contrary, produced a great many fruits and viable seeds.

On going through the observation collection of the „West Java" Experimental Station I discovered 2 more clones, which behaved in every respect like KN 251.

These three clones therefore may be looked upon as female plants as pollen is never produced. As I already remarked in my publication above mentioned, such forms are eminently fit for experiments on natural insect-and wind-pollination.

Finally, during the cytological investigation described in Ch. 2, it moreover appeared that the „species hybrid" *H. Spruceana* × *H. brasiliensis* also produces dead pollen and is consequently male sterile.

CHAPTER II

CYTOLOGY

§ 1. *Method.*

Meiosis was almost exclusively investigated in pollen mother-cells; the technical details mentioned in this section consequently refer to male bud preparations. Some additional observations, however, were made on somatic chromosomes. Root tips of young seedlings were fixed in Bouin-Allen B—15 or in La Cour's 2 BE (LA COUR 1931) and stained with Newton's gentian violet.

The young male buds were collected in the neighbourhood of Buitenzorg, in the flowering season of 1932. As a rule the moment of fixation ranged from 9 to 11.30 a.m. For fixing Carnoys fluid (acetic-alcohol) was always used. The fixed material was carried to Europe in paraffin and handled in the Botanical Laboratory at Utrecht.

Being fixed with Carnoy the one stain was Heidenhain's haematoxylin. A preliminary test had already proved this stain to be satisfactory. Yet the results obtained later on appeared very unequal, and on the whole only moderately satisfactory. E.g. *Hevea Spruceana* caused great difficulties with this stain and I have not really succeeded in obtaining a completely succesful preparation, the plasma almost invariably retaining too dense a colour. Other types proved to be very variable in this respect. Staining with haematoxylin with subsequent differentiation with a saturated solution of picric acid in water (HSU CHUAN TUAN 1930) brought no improvement.

Gentian violet by Newton's method stained the chromosomes very unsatisfactorily. Previous treatment with chromic acid (CLAUSEN 1929, SKOVSTED 1934), occasionally yielded better results, but Heidenhain's haematoxylin remained the superior method so that eventually it was applied throughout the investigation.

Although the cytoplasma generally remained deeply stained and the chromosomes sometimes too lightly, the nucleolus was often very faintly stained and not infrequently entirely transparent. It was remarkable that with some types (e.g. *Hevea guianensis*) the nucleolus always remained pitch black.

At first sections of 15 μ thickness were made, but these were afterwards proved to be too thin for prophase nuclei. 18 μ is the most suitable thickness for *Hevea*.

Longitudinal sections are to be preferred to cross ones as the contents of the anthers are better surveyable, first in differentiating and afterwards in studying the meiotic stages. In the cross section of a loculus two or three pollen mother-cells at most are found whereas in longitudinal sections often much more, sometimes as many as from 12 to 16.

A macroscopic expedient for the selection of buds in which the meiotic divisions are in progress, as e.g. the „bendera" stage of *Saccharum* (BREMER 1921) and the macroscopically measurable length of the buds of *Oenothera* (GEERTS 1909), is not known for *Hevea*. Nor do HEUSSER (1919) and BANGHAM (1931) supply any indications.

The fixed material therefore consisted of buds of much varying lengths; for which reason a great number of longitudinal sections were made first, for purposes of orientation. With the aid of ocular- and stage-micrometers the lengths were determined of those buds in which stages of meiosis occurred.

These lengths appeared to show considerable differences for the various forms. The figures of Table II will serve to illustrate this.

TABLE II

H.	brasiliensis	clone	BR 1	2.04—2.21 mm.
,,	,,	,,	BR 2	2.04—2.21
,,	,,	,,	Tjir. 1	1.79—1.96
,,	,,	,,	PR 104	1.70—1.87
,,	,,	,,	KN 251	1.70—1.87
,,	,,	,,	Ct 88	1.70—1.87
,,	,,	,,	C.R.S. 24	1.70—1.87
,,	,,	,,	KN 220	1.70—1.87

H. Spruceana	1.11—1.28
H. guianensis	1.11—1.28
H. collina	1.11—1.28
H. collina × brasiliensis . . .	1.11—1.28
H. Spruceana × brasiliensis .	1.28—1.45

At the time that meiotic division is taking place the female buds are much bigger than the male, one female bud of Ct 88 in which pachytene, diakinesis and telophase were found, having a length of more than 3 mm.

The drawings were made in the usual way with the aid of a drawing apparatus. Those chromosomes lying in low focus are indicated by a lighter shade in the drawings when distinction is necessary.

§ 2. *The somatic chromosomes.*

The metaphase-plates shown in fig. 2 give an idea of the somatic

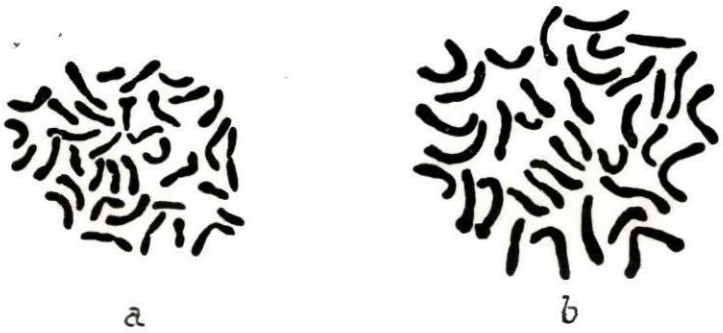

FIG. 2. Somatic divisions from root tips of *Hevea brasiliensis*.
a. Fixation with La Cour's 2 BE, b. with Bouin-Allen B-15. 4000 ×.

chromosomes. If the magnification of about 4000 × is taken into consideration, they appear to be very small. The drawings were first made at a magnification of 2700 × and afterwards enlarged $1^1/_2$ times in copying.

Plate 2a is from a root tip fixed with La Cour's 2 BE, plate 2b from a root tip of another seedling and fixed with Bouin-Allen B—15. The difference in size between the chromosomes is considerable, those of b are more than $1^1/_2$ times as long as those of a. Whether the latter is due to different fixation or to different nutritional conditions of the root tips, cannot be said.

As regards the structure of the chromosomes, 2a is more satisfactory than 2b. A constriction is clearly visible in various chromosomes, in b, however, not at all. This is a result of the different influence of the two liquids used for fixation. It is emphasized by HUSKINS and SMITH (1935) that the structure of chromosomes appears unsatisfactory, when fixing liquids are used which contain ureum.

Slides from growing points have also been made and stained with Newton's iodine gentian violet; good division figures however were not found.

Frequently, somatic chromosomes in the tapetal cells of the anthers could be observed in which a constriction was visible, even with Heidenhain's staining. The tapetal chromosomes are evidently bigger than those in the root tips. Counts, however, were not possible, no regular metaphase plates occurring.

§ 3. *Normal meiosis.*

A. Short description.

The pollen mother-cells in various anthers of a *Hevea* flower are often in different stages of meiotic division. In differentiating this is a disadvantage, but a convenience in the study of transitions. According as a stage is of longer duration it is found in a greater number of stamens at the same time.

Neither is the same stage always found, in the 4 loculi of the same anther and it even occurs that in the upper part of a compartment pollen mother-cells have reached a more advanced stage than those in the lower part. This happens at the end of diakinesis and also occasionally at the beginning of diplotene.

After the resting stage following the last somatic division, the prophase of meiosis begins with a leptotene stage, which is indistinct and very complicated.

The pollen mother-cells are still condensed, and fill the loculi almost completely. (For the structure of the flowers and stamens see HEUSSER 1919). Either simultaneously with, or possibly after the beginning of zygotene, which is just as indistinct as the leptotene, synizesis sets in. Cells with a synizetic knot are already detaching themselves from each other and from the tapetum, while the angles of the cell-walls are rounding off.

This continues during the subsequent pachytene stage, the first distinct stage of prophase.

Pachytene now passes into diplotene, which like leptotene consists of a complicated mass of very thin threads and loops. The cells have now become entirely rounded off and lie just touching, in a row in the middle of the loculi.

In the diplotene stage the contraction of the chromosomes sets in, which process continues until metaphase. Diplotene gradually passes into diakinesis, when the contents of the nucleus spread over the nuclear-membrane. After the latter and the nucleolus have disappeared a contraction of the chromosome group occurs. Next the chromosomes spread among the spindle fibres (which in the meantime have made their appearance) and then range themselves in the metaphase plate.

Metaphase and anaphase show a fairly normal course and pass off rapidly. In the telophase groups alveolisation takes place and soon two additional nuclear-membranes are formed.

This is followed by interphase (interkinesis) in which each separate chromosome becomes clearly visible, while one or several nucleoli appear.

Between the two nuclei no cell-wall is formed. After the disappearance of the two nuclear-membranes metaphase, anaphase and telophase of the second meiotic division take place.

After the telophase-II follows a stage which greatly resembles the interphase. The now very small chromosomes are again separately visible until the pollen mother-cells break up into tetrads of young uni-nucleate pollengrains, which soon round off.

B. Details.

LEPTOTENE, ZYGOTENE AND SYNIZESIS. Very gradually the granular contents of the resting nucleus (fig. 3) change into an intricate network of very fine threads in which the chromomeres are sometimes clearly visible as small granules; the threads, however, cannot be traced (fig. 4).

Anastomoses soon appear, but it is not possible to find any indications of pairing having begun. The chromatin is in the form of thin and thick threads intertwined. That the thicker parts have a double nature is probable but has not been proved. It is impossible to make out a distinct zygotene stage in *Hevea*.

Moreover at this stage the contents of the nucleus usually show contraction; the chromatin in the form of a dense tangle, together with the nucleolus, lies against the nuclear-membrane. This synizesis was repeatedly met with in the preparations, as in most plants where the fixation has not been done with the most painstaking care.

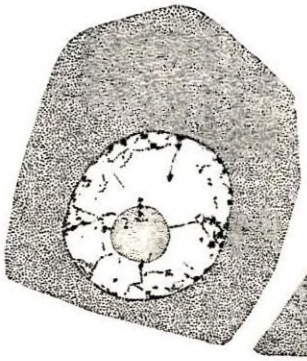

FIG. 3. Resting stage. 1800 ×.

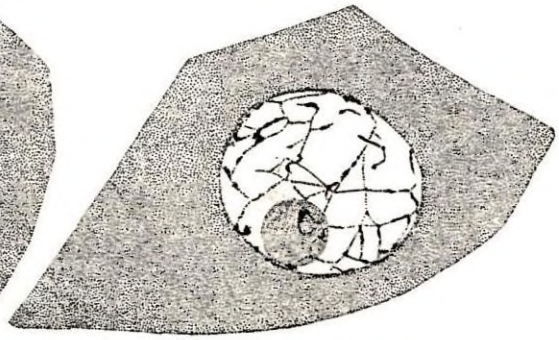

FIG. 4. Leptotene. 1800 ×.

Most cytologists [see BELAR (1928), DARLINGTON (1932), SHARP (1934)] at present assume synizesis to be an artefact which may occur at late leptotene as well as at zygotene or early pachytene, the aspect of the knot varying in each of these cases.

In *Hevea* synizesis usually occurs at zygotene. Fig. 5 illustrates a case in which two threads that are just pairing project from the main mass; thus it is evident that in *Hevea* parasynapsis takes place. Four pairs of chromomeres are clearly visible. The nucleolus in this nucleus is practically colourless.

Sometimes the early pachytene shows contraction when thick ends of thread are projected from the knot.

Often the degree of contraction varies in the nuclei of a single loculus, some nuclei showing no synizetic contraction at all, others to a certain amount, others again showing complete contraction.

On the whole it seems improbable to me that the dense synizetic knot should offer a favourable opportunity for the side-by-side pairing of the chromosomes which are very long at this stage. The assumption that synizesis is an artefact has every probability on its side.

PACHYTENE. Although this stage is much clearer than the prece-

ding I have not yet succeeded in analyzing any pachytene nucleus. The nucleus is filled with a confused mass of thick chromatin threads which cross and recross in every direction (Fig. 22a). There is no question, however, of a continuous spireme for, although it is only rarely possible to trace any one chromosome completely, a large

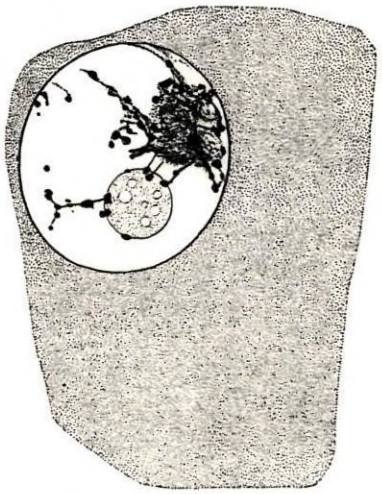

FIG. 5. Synizesis; note parasynaptic pairing of threads. 1800 ×.

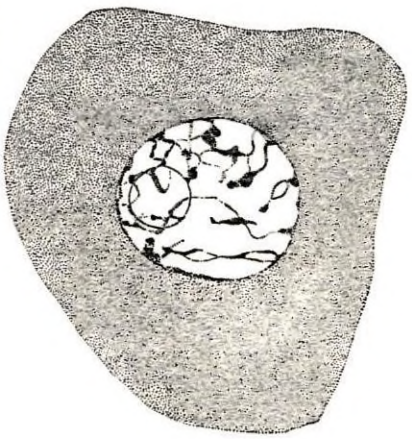

FIG. 7. General appearance of middle diplotene. 1800 ×.

number of free ends may be observed. In one case I counted as many as 18; the chromosomes are doubtless present in the haploid number, but mostly stick together. Often a chromosome seems to be attached with one end to another chromosome in an arbitrary place; it also happens at times that the thread appears broken and, especially at late pachytene, that very often ends of threads lie exactly opposite each other. These peculiarities, however, cannot possibly be solved with the available material.

DIPLOTENE. The double nature of pachytene threads does not become recognizable again until the transition into diplotene. The two partners begin to separate in several places; at first thickenings show in these points, but soon after openings become visible. These by extending approach each other, until the double chromosome consists of a succession of loops, connected by means of the chiasmata.

This process does not start simultaneously in all chromosomes nor does the separation of the double thread in all points begin at exactly the same moment. Sometimes the first opening becomes visible in the middle of the unseparated thread, but frequently at the end. This is about all I could observe with the staining applied. Only a very few chromosomes were to be traced throughout (fig. 6).

FIG. 6. Chromosomes in early diplotene. 2700 ×.

Although diplotene is just as complicated as leptotene, the appearance of the threads is entirely different. Chiasmata are present in large numbers (fig. 7, p. 209) but the maximum number per chromosome I have not been able to ascertain. The diplotene is also very sensitive to Carnoy-fixation, some contraction of the contents of the

FIG. 8. Bivalents in late diplotene. 1800. ×

FIG. 9. Diakinesis with 18 bivalents. 1800 ×.

nucleus is frequently found so that the thin threads stick together in a great many places, and it is impossible to trace them.

At diplotene contraction of the chromosomes sets in which is accompanied by a shifting of the chiasmata. It is not until contraction has shortened and thickened the chromosomes, that they become at all clearly visible in their entirety. Yet I have been able to see even at late diplotene only a few separate chromosomes clearly.

After the transition to diakinesis there is a definite improvement. Fig. 8 shows the cut portion of a nucleus in which the chromosomes, according to my estimate, have been shortened to about half their original length. It is not yet possible to count them at this stage.

DIAKINESIS. Fig. 9 represents diakinesis in *H. guianensis*. Here 18 pairs of chromosomes can be counted with certainty. The nucleolus which is actually pitch black, had to be left white for the sake of clearness. The chromosomes are already in an advanced stage of contraction, but the greatest contraction is reached after diakinesis.

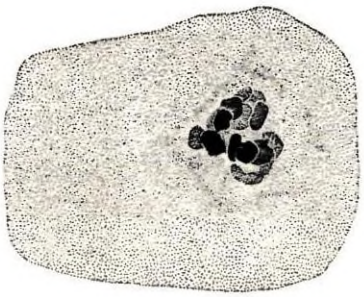

FIG. 10. *Contraction after* diakinesis. 1800. ×

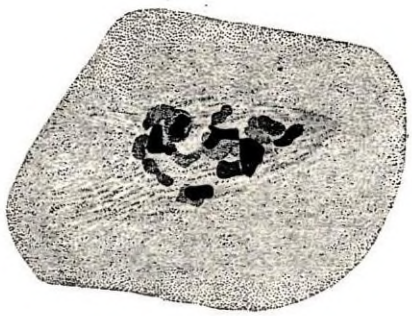

FIG. 12. Chromosomes scattered through the spindle. 1800 ×.

The nuclear-membrane disappears just before the nucleolus. On a few occasions I have noticed instead of one large nucleolus several small ones which are never observed at diakinesis proper.

Moreover a certain contraction of the contents of the nucleus takes place in consequence of which the 18 bivalents cluster together (fig. 10).

TERMINALISATION OF CHIASMATA. Although in this first investigation I have not been able to tell the chromosomes of *Hevea* apart and to describe them separately, yet I have made a number of observations which may be recorded on their form and their transformation from pachytene to diakinesis.

As was already shown in fig. 6 a number of loops and chiasmata make their appearance at early diplotene, occuring at fairly regular distances in the double thread. The number in this case including

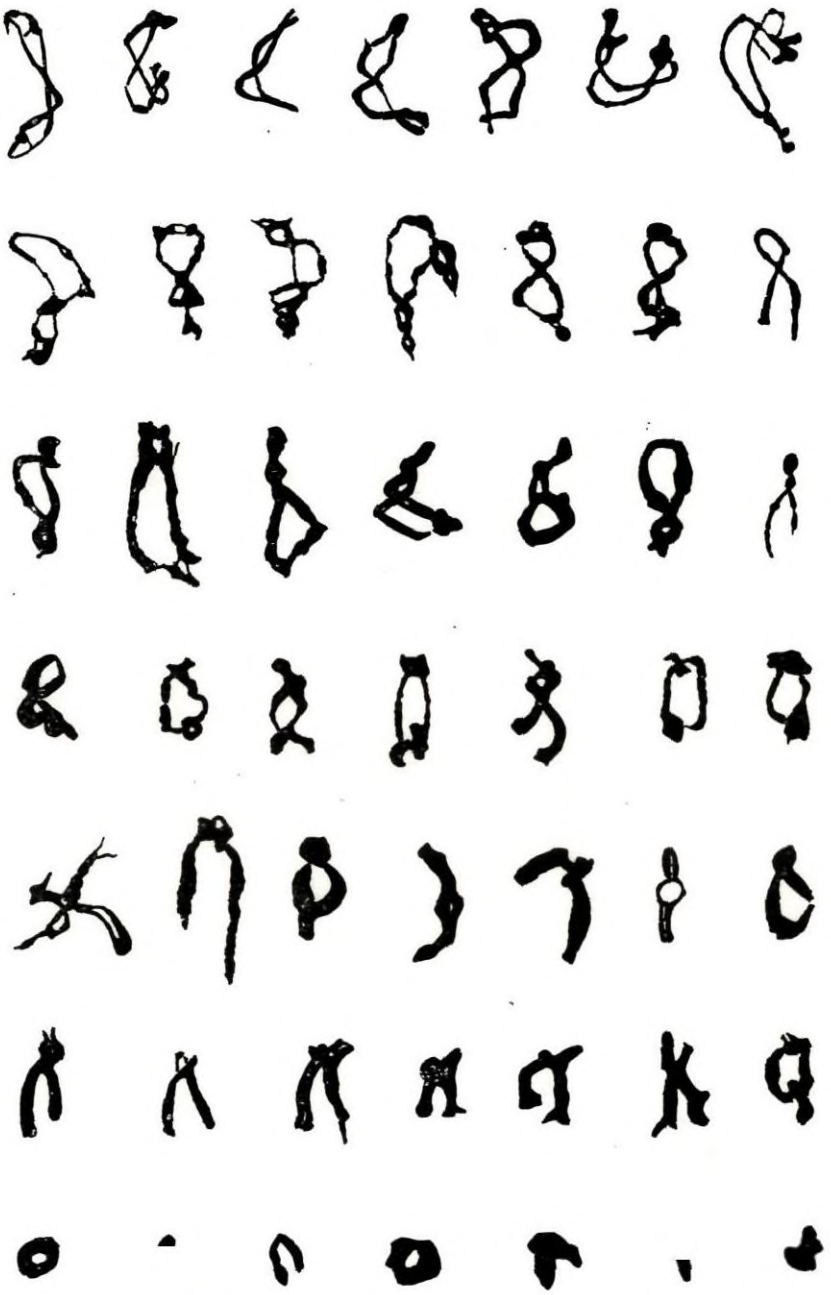

FIG. 11. Chromosomes of *Hevea* from diplotene to late diakinesis. 2700×.

the terminal chiasmata is four, but in late diplotene the chiasmata no longer occur at regular intervals.

One (sometimes two) of the openings widens at the expense of the others. Owing to the widening of these openings the chiasmata are shifted along the chromosome and become crowded together, whilst they gradually move towards one or both ends of the chromosome. This process can take place in various manners as is shown in fig. 11. In some cases a terminal opening widens, in others a more central one. In the former case the chiasmata are all shifted in the same direction; in the latter in both directions.

This difference is possibly connected with the first appearance of the openings at early diplotene. The opening which become visible first, might continue till diakinesis.

The shifting chiasmata, after becoming aggregated, finally are resolved at the ends of the chromosomes with the result that the number per chromosome decreases. This process of terminalisation (DARLINGTON, 1929) continues until diakinesis when the bivalent, retain a minimum of chiasmata. At diakinesis ringshaped and cross- and V-shaped bivalents occur. The former have 2 chiasmata, the latter one.

The number of 2-chiasmata-[1]) bivalents amounts to four or five, the number of 1-ch-bivalents to fourteen or thirteen. I have been unable to ascertain this exactly, but I got the impression that this proportion is constant, at least in the case of *H. brasiliensis*. Of the other species too little suitable material was available.

The 2-ch-bivalents may contain two terminal chiasmata, or one terminal chiasma and one „interstitial". The chiasma of the 1-ch-bivalents may be completely terminalized but it is more frequently interstitial, however, not median.

Only one or two 1-ch-bivalents have a median chiasma. At middle diakinesis there are always some bivalents which have already become so contracted that their shapes can no longer be ascertained. Contraction apparently does not proceed at the same rate in every chromosome, and possibly the same applies to terminalisation. This makes it very difficult to decide whether the terminalisation of

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[1]) Which in future will be indicated by ch.

a 2-ch-bivalent is already finished at diakinesis, and consequently whether another 1-ch-bivalent might arise from it.

On the question of the splitting of the two paired chromosomes in the prophase, so that each chiasma is constituted by the crossing of two of the four chromatids so formed, I have made the following observations.

At diplotene I have been unable to observe this split but the appearance of the chiasmata indicates its existence, as the upper and lower angles of a chiasma frequently are slightly rounded off and not distinctly acute, to be the case if the chiasmata were formed simply by the crossing of two threads, without any others being present.

At early diakinesis, however, a fissure is clearly visible in many cases. Some of these cases are shown in fig. 11. Moreover the structure of a chiasma is more or less visible here and there.

METAPHASE-I. After the contraction following diakinesis, the chromosomes scatter through the spindle, which has meanwhile

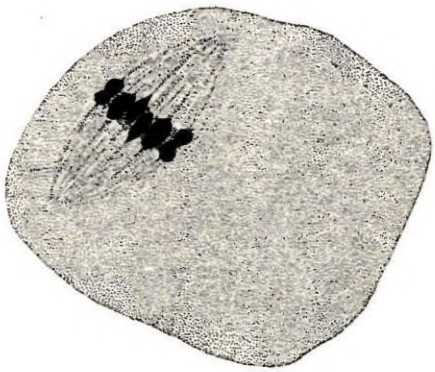

FIG. 13. Metaphase-I, side view. 1800 ×.

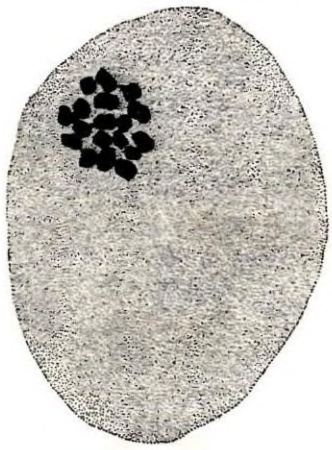

FIG. 14. Metaphase-I, polar view. 1800 ×.

made its appearance (fig. 12, p. 211) and afterwards range themselves in the equatorial plane.

The side view of the metaphase plate is very regular (fig. 13), but is of short duration as pure metaphase is comparatively seldom

found, some chromosomes having usually passed into anaphase. The spindle is frequently found close to the cell-wall.

Metaphase also shows that only a few bivalents occur with more than one chiasma. Most of the bivalents are bar-shaped with a slight constriction in the middle and only a few are diamond-shaped or oval.

A polar view of the first metaphase (fig. 14) often affords a favourable opportunity for counting. With BR_1 and Tjir. I (*H. brasiliensis*) I have repeatedly, with certainty, found the haploid number to be 18; with BR_2 I have never obtained a clear metaphase, on the other hand many diakineses and interkineses allowed accurate counting. The metaphases of *H. Spruceana* and *H. guianensis* too were hardly distinguishable. Whilst the cytoplasma was densely stained the chromosomes lay very close together. The number of chromosomes of these species has also been determined in diakinesis. Of *H. collina* good metaphases with 18 chromosomes were found. So the haploid number of 18 occurs in all the species investigated.

Considerable variation in the size of the chromosomes was noticed in different metaphase plates, even with flowers from the same tree.

ANAPHASE-I. This stage is not always perfectly regular. Some

FIG. 15. Anaphase-I, side view. 1800 ×.

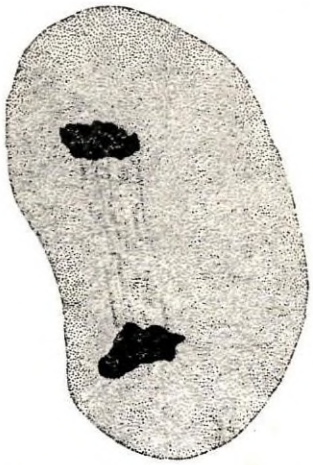

FIG. 17. Telophase-I. 1800 ×.

chromosomes may be early, others late (fig. 15). The polar view of a few anaphases allowed both groups to be counted at different foci

Two cases of this kind are shown in fig. 16. The similarity of the grouping of the chromosomes is clearly visible. At anaphase-I the chromosomes are square, many show signs of the longitudinal split

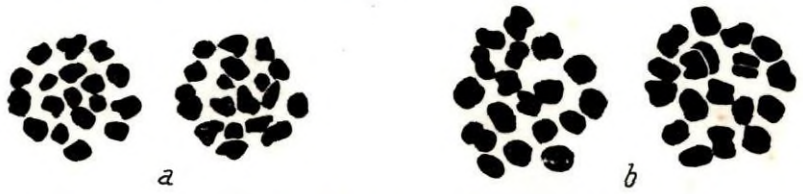

FIG. 16. Anaphase-I, polar view.
a. *H. brasiliensis*. b. *H. collina*. 2700×.

(which apparently occurred in the prophase) but the halves remain in very close contact and pass in this condition into telophase.

The chromosomes in fig. 16b show a striking difference in size from those in fig. 16a (here the species are different and the stages not quite identical).

TELOPHASE (fig. 17, p. 215). Of this stage there is little to be said. Owing to alveolisation the chromosomes themselves entirely disappear, to reappear at interphase.

INTERPHASE. For the counting of the chromosomes, interphase (interkinesis) is at least as important as the metaphases of the two divisions. In the first place the chromosomes may appear so clearly separated that even with a small magnification it is possible to count them. Not infrequently counting has been possible at interphase when metaphases were useless or lacking.

Moreover interphase affords an opportunity to check the distribution of the chromosomes at anaphase-I which is of very great importance especially in the forms with irregular meiosis.

Fig. 18 shows an interphase of BR_2. At this stage the chromosomes have often a deeply cleft appearance; at the same time they show a certain similarity of form with some bivalents at diakinesis. Besides, the whole stage recalls diakinesis in many respects and may better be called prophase-II than interphase.

Not infrequently more than one nucleolus is found in the interphase nucleus and once I found nuclei with 5 and 6 nucleoli.

With *H. guianensis*, *H. collina* and *H. Spruceana* the nucleoli were always perfectly black.

The transition to metaphase-II is also accompanied by some contraction of the nuclear contents just as in the first division.

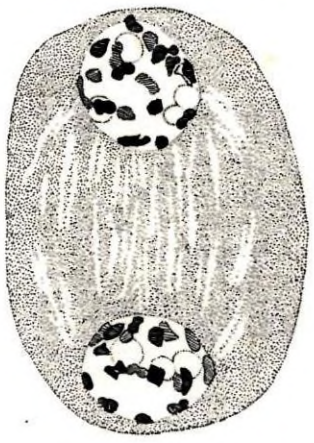

FIG. 18. Interphase. 1800×.

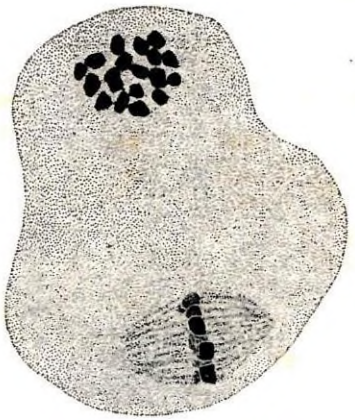

FIG. 19. Metaphase-II. 1800×.

A stage during which the chromosomes spread through the spindle has not been observed.

METAPHASE-II. This metaphase is distinguished from -I, by the smaller size and the more angular shape of the chromosomes (fig. 19). Splitting, as is observed at interphase, only remains visible here and there by an inward bended outline of the ends.

The direction of the two spindles with respect to each other varies considerably. They sometimes lie parallel and in a few cases a polar view allowed the two to be counted.

Again in metaphase-II many countings were done which always yielded the same result, n = 18.

ANAPHASE-II. Only in one single case were countings made at this stage. Anaphase-II proceeds more regularly than anaphase-I and is soon followed by telophase.

Telophase is followed by another stage in which the chromosomes become separately visible, and although they are very small, counting is occasionally possible. This stage resembles interphase very

closely (except that the chromosomes show no split) and precedes the formation of tetrads. Tetrads result from a constriction of the protoplasm of the pollen mother-cell.

§ 4. *Abnormal meiosis in sterile forms.*

As stated in Ch. I, meiosis has been investigated in six forms showing male sterility. PR 104 is almost sterile for both sexes; KN 251, Ct 88, C.R.S. 24 and *H. Spruceana* × *brasiliensis* (D2—49) are male sterile and KN 220 is entirely sterile for both sexes.

PR 104. The appearance of the loculi in the longitudinal sections is already abnormal at the time of the pollen mother-cells. Whereas in normal cases (Ch. II, § 2) pollen mother-cells are already fairly considerably rounded off during pachytene, and separated from the tapetum, ranking themselves in a line in the middle of the loculus, the pollen mother-cells in this case fill up the compartment entirely until pollen formation and are also more angular. This gives the loculus the appearance of growing too slowly. Or, as is reported by ROSENBERG (1917) of a similar case with *Hieracium:* The divisions of the pollen mother-cells may begin at a very early stage in the development of the loculus.

Further the most divergent stages of meiosis may be found in the pollen mother-cells of one loculus. E.g. diakineses and interphases are often found lying side by side; sometimes pollen mother-cells with 4 nuclei, second anaphases and pachytene occur in one and the same compartment. Nor is meiosis itself normal. The deviations which occur are also found in the following forms and will be described together with the latter.

KN 251, Ct 88, and C.R.S. 24. In contradistinction from PR 104 the loculi with the pollen mother-cells of these clones present an almost normal appearance until the formation of uninucleate pollen-grains. Occasionally, however, a flower-bud is found in which the stages of meiosis occur, but whose tissue has an unhealthy appearance and is obviously already degenerating. The young pollen of the „species-hybrid" *H. Spruceana* × *brasiliensis* (D2—49) also degenerates but the stamens do not.

Metaphase-I is irregular in the 4 clones and the „species-hybrid" mentioned above. The chromosomes do not always arrange them-

selves into an equatorial plate but frequently separate in various places in the spindle. Fig. 20 shows a number of such irregular metaphases; a and b are metaphases of PR 104, c of C.R.S. 24, d and e of KN 251, f and g of Ct 88 and h of the hybrid *H. Spruceana* × *brasi-*

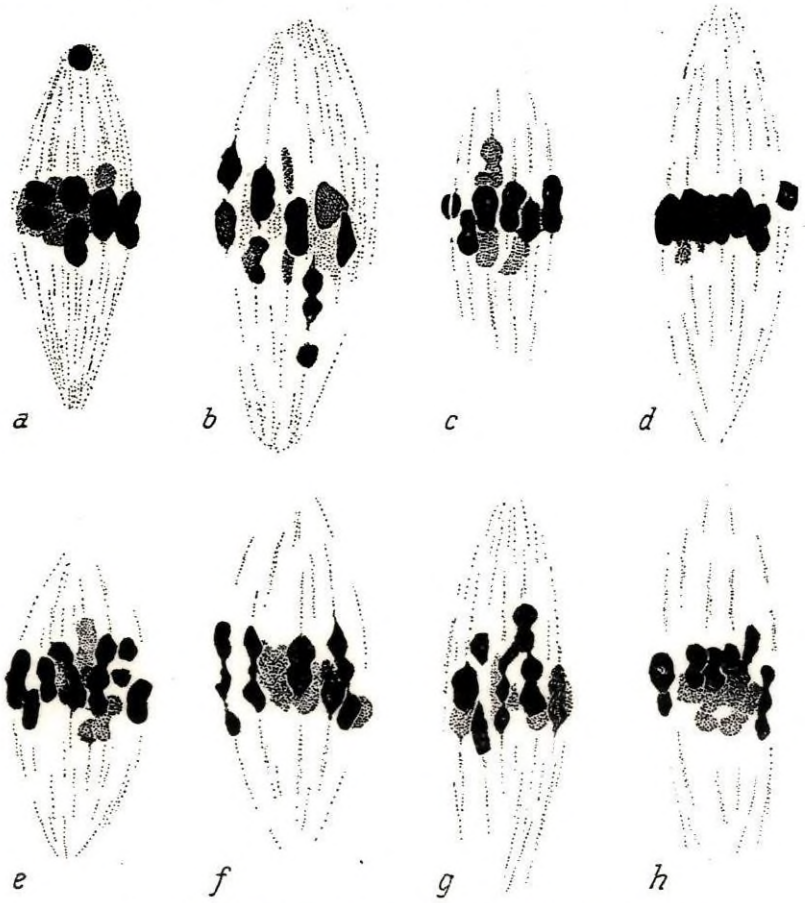

FIG. 20. Metaphase-I from male-sterile forms of *Hevea*.
a, b. PR104. c. C.R.S. 24. d, e. KN 251. f, g. Ct 88. h. *H. Spruceana* × *brasiliensis*. 2700 ×.

liensis (D2—49). In these figures only the important and clearly visible parts of the metaphase groups are drawn jet black (which here does not indicate the chromosomes at highest focus).

Apparently regular metaphases also occur, but then a small chromo-

some is often seen lying near the edge of the metaphase-plate (fig. 20 c and d), which is never found at metaphase in normal clones. The assumption that it is a univalent is obvious and investigation of diakinesis has proved this assumption to be correct; splitting of the univalent has repeatedly been observed. (fig. 20 c). The univalent chromosome also occurs sometimes at one of the poles while the other chromosomes are still arranged in the equatorial plate (fig. 20a).

Besides univalents and bivalents, trivalents are also found at metaphase, although the opacity of the mass of the chromosomes often causes doubt. Yet I am sure that like univalents they occur frequently, if not regularly. In fig. 20b (below) is a trivalent in early anaphase, in fig. 20 e, f and h are trivalents in metaphase.

Further it is probable that per metaphase more than one univalent and more than one trivalent occur (fig. 20 e and f). Fig. 20 g shows what appears to be a 7-valent group, but may merely a case of the chromosomes sticking together (fixation artefact?). Figs. g, h and i are all drawn from PR 104-slides.

At diakinesis in these sterile types the univalents are easy to

FIG. 21. Trivalents and quadrivalents from diakinesis in male-sterile clones of *Hevea brasiliensis*. 2700 ×.

recognize but it is often impossible to distinguish multivalents with certainty from bivalents. Fig. 21 shows a number of multivalents at diakinesis; a, b and c are trivalents of KN 251, PR 104 and Ct 88 respectively; d is a multivalent of Ct 88 from the same nucleus as c, but it cannot be said with certainty whether this is a trivalent or a quadrivalent. The same applies to e and f, multivalents of PR 104; g and h are very probably quadrivalents and i certainly is. Figs. g, h and i are all drawn from PR 104-slides.

In side views of metaphase I have not been able to recognize any quadrivalents. Presumably they are of less frequent occurrence than either trivalents or univalents.

The distribution of the chromosomes at the anaphase cannot always be regular. If there are 1 univalent and 1 trivalent, which may occur rather frequently, the following cases are possible:

a. the univalent and $^1/_3$ of the trivalent go to the same pole; the distribution then becomes normal (18—18).

b. the univalent and $^2/_3$ of the trivalent go to the same pole; the distribution then becomes 17—19.

c. the univalent divides itself longitudinally, the halves each go to a pole, making the distribution 18—19.

Investigation of a number of interphases mostly indicated 18—18, twice 17—19, and only once 18—19. The number of suitable interphase nuclei was too small to determine the frequency of the various types of distribution which occurred.

The determination of the number of chromosomes in the interphase nuclei is unimportant except where a clone produces good (i.e. viable) pollen, but only PR 104 yields, besides a considerable quantity of dead pollen, a few full grains; after artificial pollination with this pollen, however, no fructification occurs. The anthers of PR 104 and *H. Spruceana* × *brasiliensis* (D2—49) further remain normal, but do not open spontaneously. The „species-hybrid" has only dead pollen.

The stamens of KN 251, C.R.S. 24, and Ct 88 also degenerate. The anthers, together with the column, become one hard shapeless pin, which often lies loose on the receptacle.

In the megaspore mother-cells of these clones I have only found, of all the stages which are of importance, one early diakinesis. In this a chromosome occurs which may be a trivalent.

KN 220. An entirely different kind of deviation from normal meiosis is found in the pollen mother-cells of this completely sterile clone. KN 220 is an instance of an asynaptic *Hevea* with a meiosis in which practically only univalents occur.

The earliest stage I have acquired is synizesis of which nothing can be said. A typical pachytene stage does not follow, the contents of the nucleus after synizesis consisting of thin u n p a i r e d threads. Fig. 22 shows the principal stages of this asynaptic meiosis. Compare the asynaptic „pachytene" of 22 b with a normal pachytene as represented in 22 a. Though homologous chromosomes lie side by side in various places no pairing takes place.

This stage is sensitive to Carnoy fixation, a few threads always remaining clustered together at the nucleolus. Nothing is visible but a mass of threads partly contracted and sticking together. From pachytene to diakinesis the nucleus has proved unsuitable for critical observation.

Not until diakinesis does a great number of univalents become distinguishable. One diakinesis is shown in fig. 22 c, where 33 distinct chromosomes are drawn.

One of the black chromosomes might be taken for a bivalent and a few chromosomes cover each other exactly. Moreover the nucleolus has been left white in the drawing, but is actually completely black. As a whole this stage did not prove suitable for careful counting. Diakinesis is the only stage in which chromosomes occur which might be bivalent. Their number, however, is extremely small in this case, and certainly does not include ring-shaped bivalents.

After diakinesis follows a contraction of the set of chromosomes and after this metaphase. Spindle-fibres were seen in a few cases but no typical metaphase was found.

After clustering together the chromosomes gradually disperse until eventually they run like a band through the whole cell. This dispersion takes place in many different ways. Often, as in fig. 22 d, some of the chromosomes remain together, while the others disperse, but equally often dispersion takes place evenly. In this stage, the „anaphase" of the first meiotic division, the chromosomes are generally easy to count. Often 2 or 3 chromosomes lie close together or a few may cover each other but there are no typical bivalents among them.

This renders counting occasionally uncertain but still I counted mostly 36 and only a few cases were doubtful (35 or 36).

So I consider in this clone also 36 to be the normal somatic number. This anaphase is very frequent in preparations and consequently appears to take more time than anaphase in normal clones.

Spindle fibres sometimes exist. At late anaphase counting becomes more difficult (fig. 22e); the angle in the anaphase-figure I frequently observed, it proceeds the formation of telophase groups.

At telophase the chromosomes are always divided into more than two groups often of very different numbers. Formation of both three and four groups occurs constantly. The latter case is shown in fig. 22f. The most frequently occurring situation is: one large group, two

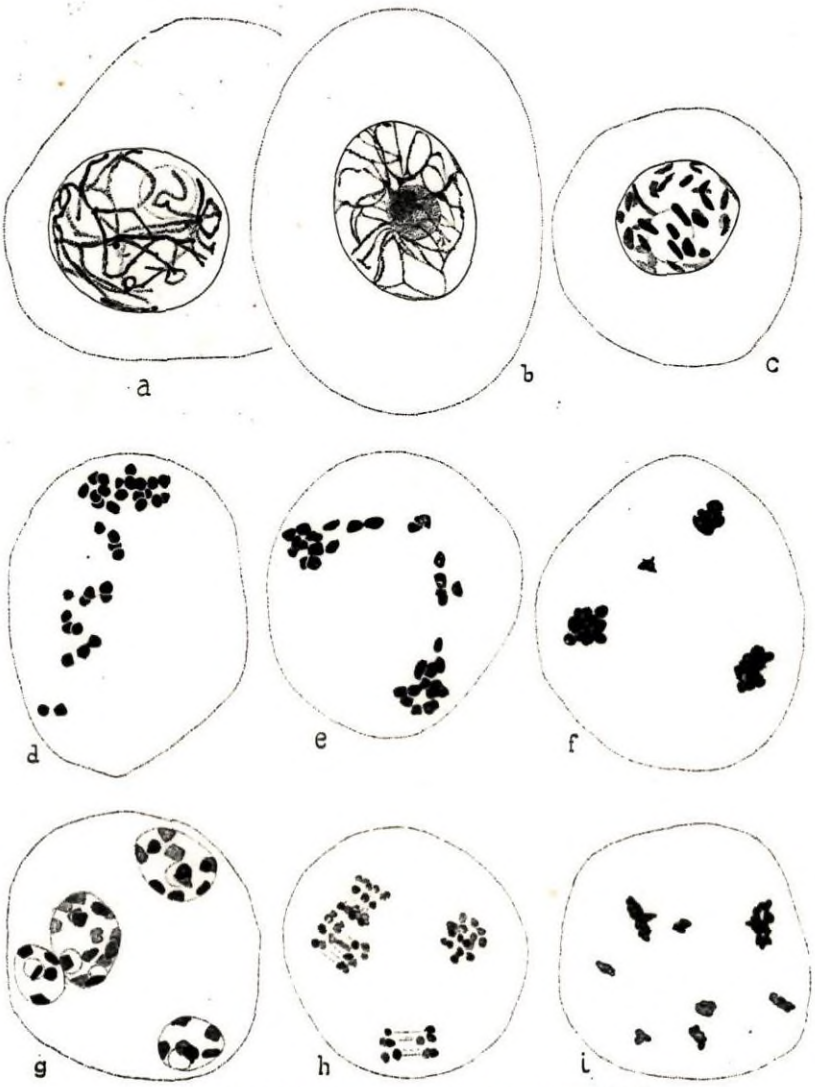

FIG. 22. Meiosis in asynaptic *Hevea* (KN 220).
a. Normal pachytene. b. Asynaptic pachytene. c. Diakinesis with univalents. d. Anaphase-I. e. Late anaphase. f. Telophase-I. g. Interphase. h. Anaphase-II. i. Telophase-II. 1500 ×.

groups of average size and one small group. Each of the groups forms a daughter-nucleus with one or more nucleoli (fig. 22g). At interphase therefore, 3 or 4 nuclei of various sizes may be found in each pollen mother-cell. Bi-nuclear interphases I have never found, nor any case of all the chromosomes being combined into one restitution-nucleus.

At interphase the chromosomes are separately visible again and can sometimes easely be counted. The interphase of fig. 22g shows altogether 37 chromosomes. I suppose that one of the univalents has

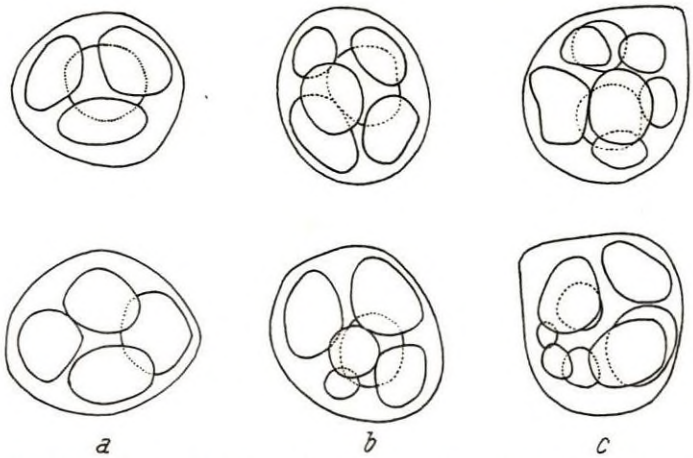

FIG. 23. „Tetrads". a. Normal tetrads. b. „Hexads" and c. „octads" from asynaptic KN 220. 650 ×.

been split at anaphase. Some of the chromosomes are also strikingly smaller than others.

Interphase is followed by the second division which, rather curiously, progresses quite regularly, apart from an abnormal number of spindles. The spindle-fibres of three or four spindles are mostly clearly visible. In fig. 22 h a pollen mother-cell with 4 anaphases of the second division is shown. The chromosomes have become too small to be counted at this stage, but it is easy to determine that even groups of 2 chromosomes still enter into anaphase, and presumably even separate chromosomes which occasionally are found.

The number of grand-daughter-nuclei which is finally produced depends upon the number of telophase groups produced by the first

division. If there are 3 groups, 6 nuclei are formed whilst from 4 telophase groups 8 grand-daughter-nuclei are produced.

These nuclei envelope themselves with a quantity of cytoplasm which may correspond nearly with the number of chromosomes included, thus instead of normal tetrads, „hexads" and „octads" are formed (fig. 23b and c).

With KN 220 I have never found normal tetrads.

Young pollen-grains may be produced but they always degenerate together with the stamens.

CHAPTER III

DISCUSSION

Before subjecting the species of a certain genus and various forms of a certain species to a cytological investigation the identity of the material to be investigated should first be carefully ascertained.

Hevea occupies in this respect a peculiar position. On the one hand the plant has become very well-known owing to the enormous culture plantations of *H. brasiliensis*, on the other hand its classification, as I shall try to show, rests partly on a very insecure foundation.

There are a few species, it is true, that show very marked distinctions from others, as *H. guianensis* AUBL. and *H. Spruceana* MUELL. ARG., but there are also „species" which might as well be varieties of another species, as products of hybridization.

For reasons stated above, I have used DUCKE's classification; but I think, with reference to the description of the supposed species-hybrid *H. collina* × *brasiliensis* (Ch. 1, § 2), some criticism to be justified. Of the 5 groups into which DUCKE (1930) divides the genus *Hevea* the 2nd, 3rd and 4th are in the first place characterized by a number of stamens which varies from 5 to 10 in incomplete or rudimentary whorls. Also the species-hybrid *H. collina* × *brasiliensis* has, as described in Ch. 1, § 2, flowers with a number of stamens varying from 5 to 10.

Though the origin of this plant by interspecific hybridization is not entirely certain, the probability of this origin is very great, and in this case I think it is also very probable that species of *Hevea* that are characterized by an irregular number of stamens have arisen by hybridization of other species.

It will not be easy to supply conclusive evidence on this point but crossing experiments with a number of species of *Hevea* would be very helpful in testing the truth of my supposition.

Besides DUCKE himself is also of opinion that natural species-hybrids of *Hevea* actually occur, which is apparent from the hybrid between *H. Spruceana* × *brasiliensis*, sent by him to the Utrecht Herbarium.

BANGHAM (1931) quotes a remark by T.F.C. (anonymus) (1920) to the effect that: „Experience has shown that cross fertilization between *H. confusa* and *H. brasiliensis* readily takes place".

BANGHAM further is of opinion that the fact that all the species investigated by him possess the same number of chromosomes: „would suggest that fertile hybrids might be formed in some cases".

In the opinion of these investigators, therefore, interspecific hybridization of *Hevea* occurs.

The variation of the characters of a species like *H. brasiliensis* is very great; that the same holds good for other species appears from the following remarks by DUCKE (1933 p. 50):

„...de nombreux échantillons florifères et fructifères provenant de plus d'un arbre et de plus d'une localité, pour chacune des espèces plus fréquentes du Rio Solimoes et Rio Negro (*guianensis*, *lutea*, *Benthamiana*, *brasiliensis*, *membranacea* et *Spruceana*) permettent de connaître la variabilité très forte de ces espèces, même dans des caractères que l'on avait jugés suffisants pour établir des sections du genre..."

As a consequence DUCKE, rightly in my opinion, considers *Hevea collina* HUBER a variety of *H. guianensis* AUBL. (DUCKE, 1930, 1933), but at the same time this again justifies doubt as to the existence of a number of *Hevea* species, as the differences between the species are often not much more considerable than those which appear within one species like *H. brasiliensis*.

The systematic classification of the genus *Hevea*, therefore, must be regarded as uncertain.

The general progress of normal meiosis is, as is to be expected, exactly the same with the various species.

Although fixations with Carnoy 3 : 1 and staining with Heidenhain's haematoxylin were only moderately successful, still the study of meiosis has not resulted merely in a determination of the number of chromosomes. The staining does not allow of a precise analysis of early prophase; but that the number of chiasmata decreases from diplotene till diakinesis, owing to terminalization and resolution, I

think may be accepted with certainty. At early diplotene I observed 4 chiasmata, but that bivalents with more than 4 chiasmata (5 and 6) occur is probable in view of the appearance of some already contracting bivalents at middle diplotene (fig. 11).

At diakinesis I could not find more than 2 chiasmata and even then in only 4 or 5 out of 18 bivalents. The remainder have only 1 chiasma, the situation of which varies from median interstitial (cross-shape) to quite terminal (V-shape). Now it is a question whether these interstitial chiasmata shift to the ends before metaphase.

The steadily progressing contraction of the chromosomes would tempt to this assumption. The same applies to the bivalents which have 2 chiasmata at middle diakinesis, one of them being terminal the other subterminal. The differentiation in structure of the bivalents after diakinesis, however, is not great enough in the available material to allow of an accurate verification, but when a bivalent at late diakinesis is still cross-shaped further terminalization is not probable.

In accordance with the behaviour of chromosomes and chiasmata from diplotene till metaphase, 2 groups of plants may, roughly speaking, be distinguished. In the first group little if any terminalization takes place, as is e.g. the case with *Fritillaria* (DARLINGTON 1930); in the second group terminalization is complete as shown in *Primula sinensis* (DARLINGTON 1931).

There are other forms whose behaviour is intermediary between the two types mentioned (MOFFETT 1933).

It may be stated with certainty that *Hevea* does not belong to the first group, for bivalents with interstitial chiasmata as found at metaphase in *Fritillaria*, decidedly do not accur in *Hevea*. That terminalization in all bivalents of *Hevea* is complete may, however, be doubted. Presumably *Hevea* will have to be included in the intermediary type.

Although for the object of a closer study of the chromosomes separately, the number n = 18 is fairly large, and the chromosomes themselves are fairly small, differences in size do exist. This is clearly to be seen in the somatic metaphase-plates shown in fig. 2. The largest chromosomes are at least twice as long as the smallest.

Added to this, „attachment constrictions" are clearly visible in a number of the chromosomes fixed with La Cour's 2BE, while they do not occur in the same places in the different chromosomes.

In this investigation only a few somatic metaphase-plates were inspected but an extensive investigation will perhaps render it possible to group the chromosomes and to characterize them by means of the size and place of constrictions.

The differences in size are of such a nature that at meiosis the numbers of chiasmata will vary, because „the mean number of chiasmata per bivalent is approximately proportional to the length of the paired chromosomes" (DARLINGTON 1932).

The present cytological investigation of *Hevea* has not yet opened up new aspects with regard to taxonomy, as the species investigated so far have all the same number of chromosomes. BANGHAM (1931) already stated this but the number n = 17 mentioned by him is wrong. Both in the various clones of *H. brasiliensis* and in the species *Spruceana* and *guianensis* (besides the variety *collina*) I found n = 18 during meiosis in the pollen mother-cells.

Countings were done at diakinesis, metaphase-I, anaphase-I, interphase and metaphase-II, leaving no room for doubt as more than 100 countings have been made. Moreover I found in root tips of material from Surinam and of Sumatra seedlings 2n = 36.

Though the number of species investigated is still limited the number 18 is found in all cases, so that 18 might be taken as the „basic number" of *Hevea*. 18 seems a fairly high basic number. Only a few plants have a similar basic number, e.g. 19 in *Salix* and 17 in *Pyrus*.

With a number like 18, however, one is inclined to think of tetraploidy or triploidy, but a *Hevea* with 9 or 12 chromosomes has not yet been found. Of other Euphorbiaceae, *Euphorbia* is the only one which has had a great number of species investigated (HARRISON 1930). Here the numbers 6 and 9 fairly often occur but other numbers have been found as well. There are nowhere indications that 18 could be a secondary basic number for *Hevea*.

Because HEUSSER (1919) in his figures of various meiotic stages persistently drew 8 chromosomes the thought has occurred to me that HEUSSER may have been working with a form with 9 chromosomes. The absence, so far, of deviations from the fixed number 18, however, renders this unlikely.

BRUUN (1931) has succeeded in classifying 161 species of *Primula* on the ground of morphological characteristics of the chromosomes.

„Average size and shape" are the principal characters and further account has been taken of „the size and number of the chromosomes in relation to each other, basic number and appearance of the constrictions".

The groups coincide with the sections, which facts prove the taxonomic value of karyology. This might be of importance for *Hevea*. A comparative karyological study of a great number of species might open new perspectives for taxonomy.

Of much importance may be the occurrence of abnormal meiosis in some male sterile and completely sterile clones of *Hevea brasiliensis*.

Typical male sterility in *Viola Orphanidis* has been described by CLAUSEN (1930): „Pollen sterile plants preferably are segregated from plants with somewhat irregular meiosis, with either monosomic, trisomic of tetrasomic behaviour of the chromosomes and the sterility of the pollen may be caused by absence of a certain chromosome, lost by trisomic or tetrasomic distribution". Pollen sterility, however, was also found with plants showing the normal chromosome number.

Somewhat abnormal meiotic behaviour is also reported by WINGE (1924) for speltoid aberrants of *Triticum* and by HUSKINS for fatuoid *Avena* (1927) and for speltoid *Triticum* (1928). Here univalents and trivalents occur constantly and especially in hybrids lacking a chromosome, but are also found in hybrids showing the normal chromosome number with partly abnormal combinations.

Typical male sterility, however, is not found where both sexes are sterile. None of the above mentioned cases agrees completely with the pollensterile *Hevea* clones. In the latter irregular meiosis with the occurrence of uni- and multivalents always is followed by degeneration of the cells. This happens after formation of tetrads or uninucleate pollen-grains.

With *V. Orphanidis* the pollen mother-cells degenerate during the first meiotic prophase as a result of an incompatible set of chromosomes originating in meiotic irregularities in the previous generation. In fatuoid *Avena* and speltoid *Triticum* some meiotic irregularities occur but without typical male sterility.

When discussing whether pollen sterility in *Hevea* may be caused by the lack of a chromosome or by gene action, the second explanation seems to be more probable than the first, as in interkinesis 2×18 chromosomes are found most frequently.

Appearance of univalents and multivalents often is connected with polyploidy. An extreme condition in this respect is shown e.g. by MEURMAN (1929) in *Prunus Laurocerasus*. We do not know anything about polyploidy in *Hevea* but the occurence of multivalents might be an indication of it.

About the asynaptic clone KN 220 a few things may be said. Asynapsis has been found in several plants. Well known are the investigations of ROSENBERG (1917, 1927) on the semi-heterotypic division in parthenogenetic *Hieracium*. In the meiosis of the „Levigatum type" only univalents occur, in the „Boreale type" univalents and a varying number of bivalents.

In genus- and species-hybrids partial or total asynapsis is repeatedly found.

LJUNGDAHL (1922) reported total asynapsis in hybrids of *Papaver atlanticum* × *dubium*, KARPECHENKO (1924, 1927, 1928) in hybrids of *Raphanus sativus* × *Brassica oleracea*, GOODSPEED and CLAUSEN (1927) in *Nicotiana Bigelovii* × *glutinosa*. Partial asynapsis occurs frequently in species hybrids; a number of such cases are given by DARLINGTON (1932). Further asynapsis has been described for dwarf oats and wheat by HUSKINS (1927) and HUSKINS and HEARNE (1933) and for *Primula kewensis* by NEWTON and PELLEW (1929).

In the above mentioned cases of asynapsis, the direct cause is not always the same. In the Levigatum type of *Hieracium* asynapsis may be caused by hybrid nature. In the cases of *Raphanus* × *Brassica*, *Papaver* and *Nicotiana* the hybrids show the same somatic chromosome-number of the parents, but the chromosomes fail to pair, which may be due to low affinity. When the parents possess an unequal number of chromosomes, the hybrids show mostly bivalents and the rest univalents.

Total, partial and varying „asynapsis" is sometimes caused by the lack of a chromosome as is the case with dwarf oats, *Primula kewensis* and occasionally in pollen mother-cells of *Viola Orphanidis* (CLAUSEN, 1930).

That the parents of KN 220 would have belonged to different species, must be excluded; consequently asynapsis as a direct result of species hybridization is out of the question.

Some similarity exists with the Levigatum type of *Hieracium*,

the latter however being parthenogenetic and KN 220 complete sterile.

Asynapsis might further be due to the lack of a chromosome; in KN 220 I found mostly 36 chromosomes in the anaphase-I and once 37 in the interkinesis; so the lack of a chromosome is not probable.

Finally asynapsis may be caused by the action of an „asynaptic gene" (BEADLE 1933) but on this possibility no information as to *Hevea* can be obtained.

The course, as well as the cause, of asynaptic meiosis differs in many plants. A different course may be taken in different pollen mother-cells of the same plant. In *Viola Orphanidis* it is almost normal, apart from the occurrence of quadrivalents.

With the Levigatum type of *Hieracium* diakinesis is followed by interkinesis with the omission of the first meiotic division, this leading to the formation of dyads and of gametes with an abnormally high chromosome number. A similar process is found in *Raphanus-Brassica* hybrids, in hybrids of *Nicotiana tabacum* × *Rusbyi* (BRIEGER, 1928) and in dwarf oats.

With KN 220 I never observed formation of dyads. The irregular first division is always followed by a regular second division and always results in the formation of „hexads" and „octads".

I have never met a similar constantly occurring deviation in the literature on this subject.

Besides dyads and tetrads, groups consisting of three to seven cells are formed in *Raphanus-Brassica* hybrids; in *Musa* (TISCHLER 1910 and CHEESMAN 1932) „tetrads" are formed which also contain varying numbers of cells, usually more than four. In such forms which also show irregular divisions, WHITE (1928) found univalents.

Much resemblance with KN 220 exists in hybrids of *Nicotiana Bigelovii* × *glutinosa*. With the latter, however, formation of abnormal tetrads takes place, especially as a result of irregular anaphase-II, whereas in KN 220 it is due to irregular anaphase-I.

As is shown by BEADLE (1933) in asynaptic maize, the existence of unpaired chromosomes in metaphase, diakinesis and even in diplotene does not always mean complete failure of pairing in zygotene. Synapsis may be followed by separation during pachytene without chiasma formation.

So the lack of a typical pachytene in our „asynaptic" *Hevea* does not exclude the possibility of zygotene pairing. With many plants it is possible to check the cytological behaviour of the chromosomes by genetical experiments on crossing-over. With *Hevea*, however, this is practically impossible as the offspring of a tree, when the latter is artificially pollinated, require five years before the next cross or selfing can be made.

The cytology of *Hevea* will be thrown on its own as is the case with many cultivated plants.

From the present investigation it is evident that the chromosomes of *Hevea* are worthy of a further study both in normal meiosis and irregular meiosis as well as in somatic divisions. So I hope to be able to continue the investigations on this subject.

SUMMARY

1. A study has been made on the cytology of the following *Hevea* species: *H. brasiliensis* MUELL. ARG., *H. Spruceana* MUELL. ARG., *H. guianensis* AUBL. *H. collina* HUBER. and of two species-hybrids: *H. Spruceana* × *brasiliensis* and *H. collina* × *brasiliensis*.

In connection with the description of the species-hybrids and with the great variation of characteristics in *Hevea* the value of the recent taxonomy of *Hevea* is discussed.

2. As far as possible the behaviour of the chromosomes in meiosis has been investigated. From early diplotene to diakinesis terminalisation of chiasmata and a decrease of the number of chiasmata from 2—6 to 1—2 takes place. Terminalisation is not entirely complete, the one or two chiasmata in late diakinesis being terminal or interstitial and even central (cross-shape).

3. The haploid number of chromosomes has been proved to be 18 in the pollen mother-cells of all the species and forms investigated. Countings were made in diakinesis, metaphase-I, anaphase-I, interphase and metaphase-II, the total number amounting to more than hundred meiotic stages, leaving no room for any doubt.

In metaphase plates of somatic divisions in root tips 36 chromosomes were found.

4. In some cultivated clones of *Hevea brasiliensis* showing partial or total male sterility, irregular meiosis takes place which is coupled with the occurrence of univalents and multivalents; of the latter trivalents are most common. The same irregularities were found in the hybrid of *H. Spruceana* × *brasiliensis*.

5. A case of asynapsis in a clone of *H. brasiliensis*, showing complete female and male sterility, has been described and compared with asynaptic meiosis in other plants. There is an almost complete lack of chromosome pairing in the prophase. A typical metaphase-I has not been found, the chromosomes after prophase being scattered in the cytoplasm. Three or four daughter-nuclei of different size are always formed, the chromosomes of which, curiously enough, show a very regular second division, resulting in the formation of „hexads" and „octads".

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THE ORIGINATION OF CHROMATIN DEFICIENCIES AS MINUTE DELETIONS SUBJECT TO INSERTION ELSEWHERE

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I. EARLIER WORK ON THE NATURE OF INTERSTITIAL „DEFICIENCIES"

The phenomenon of so-called „deficiency", that is, genetic deficiency of a minute, interstitial (i.e., non-terminal) region of a chromosome map, was first met with by BRIDGES in the case of the forked-Bar region of the X-chromosome of *Drosophila*. It appeared as a spontaneous reversion of Bar to non-Bar accompanied by a lethal effect, and at the suggestion of the present author that the cause might lie in the loss of a piece of the chromosome, which would allow recessive mutant genes in the homologous chromosome to manifest themselves and would prevent crossing-over, the necessary tests were made by BRIDGES and showed that these effects were in fact produced. Unexpectedly, however, it appeared that the terminal (right-hand) region of the chromosome was present and normal, so that a simple breakage would not explain the results. As the phenomenon of deletion was not yet known, and seemed at the time a rather special assumption, it was deemed doubtful whether the region that appeared lost had really been physically lost, or had been inactivated or caused to undergo simultaneous mutation of all of its genes, chain-wise, to recessive mutant allelomorphs. At the same

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