CYTOLOGICAL INVESTIGATION OF THE MANGO (*MANGIFERA INDICA* L.) AND THE ALLIED INDIAN SPECIES.

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INTRODUCTION.

The mango, which is one of the most important tropical fruits, has more than a thousand varieties in India, all of which belong to one species, *Mangifera indica* L. About two dozen of these are of much commercial importance, and are maintained in cultivation through vegetative propagation by grafting. They differ from one another mainly in fruit characters, and a few other minor features, e.g. colouration of the emerging leaves, colouration and pubescence on the panicle branches, etc. (Mukherjee, 1948 *b*). Of the 41 species included in the genus *Mangifera* distributed throughout Malaysia, only three are reported from India: (1) *M. indica* L., (2) *M. khasiana* Pierre (a species of doubtful occurrence), and (3) *M. sylvatica* Roxb. (Mukherjee, 1949).

Available information on the cytogenetics of species of Anacardiaceae and especially on the genus *Mangifera* L. is very meagre. Chromosome number has been reported so far only for *Mangifera indica*. Maheshwari (1934) first reported the number doubtfully as $n = 24$–26, and Roy (1939) published the haploid number of four varieties as ranging between $n = 6$–8. Recently Janaki Ammal (1945) has mentioned the number as $2n = 40$.

Due to want of knowledge on the cytogenetics of such an important tropical fruit tree, the present investigation was undertaken to elucidate the cytological basis of variation in the mangoes (*M. indica*) and the allied species, and to find out their mode of origin and correlate their inter-relationships.

MATERIALS AND METHODS.

The present investigation includes observations on three species—*M. indica* L. (including 23 cultivated varieties and 1 wild type), *M. sylvatica* Roxb., and *M. caloneura* Kz., which are available in India. Other species of the genus, occurring in the Malaysian region, could not be secured for examination during this investigation (1943–46), due to World War II. The materials of the cultivated varieties of mango were obtained from the Government Horticultural Research Stations in Bengal, Bihar and Madras, while the wild races of *M. indica* and *M. sylvatica* were collected from the forests of Chittagong Hill Tracts (near Burma border) and Assam respectively. Flower-buds of *M. caloneura* Kz., a Burmese species, had been collected from a planted tree in the Royal Botanic Garden, Calcutta. The seeds obtained from the plant did not germinate.

For a study of the morphology of somatic chromosomes, root-tips were collected from seedlings, raised in pots containing sand at bottom and soil above. The seeds

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TABLE I.

Comparative statement of chromosome number, number of satellites, secondary constrictions and nucleoli.

Name.	Chromosome number.		Number of satellites and secondary constrictions.				Total No. of SAT. and S.C.	Maximum number of nucleoli in each dyad nucleus.
			Definite.		Doubtful.			
	$2n$	n	SAT.	S.C.	SAT.	S.C.		
M. sylvatica	40	20	4	4	2	2	12	—
M. caloneura	—	,,	—	—	—	—	—	5
M. indica—								
(*a*) Wild type ..	—	,,	—	—	—	—	—	8
(*b*) Cultivated varieties:								
1. Shadwalla ..	40	,,	4	6		2	12	—
*2. Himsagar ..	,,	,,	2	8		4	14	8
*3. Kishenbhog ..	,,	,,	4	4			8	4
*4. Golapkhas ..	,,	,,	4	6		2	12	—
5. Rogni ..	,,	,,	4	4		2	10	5
*6. Langra ..	,,	,,	6	4		2	12	6
7. Anupam ..	,,	,,	4	6			10	5
8. Kalapahar ..	40–42	,,	4	8			12	—
9. Safdar Pasand ..	40-41	,,	4	6		2	12	—
10. Laskarshikhan ..	40	,,	2	6		4	12	8
11. Sah Pasand ..	—	,,	—	—	—	—	—	6
12. Jehanara ..	40	,,	4	4	2		10	—
13. Lakhna ..	,,	,,	4	4		4	12	—
14. Kohitur ..	,,	,,	4	4		2	10	5
*15. Bombai ..	,,	,,	4	6	2	2	14	4
*16. Fazli ..	,,	,,	4	4	2	4	14	—
*17. Alphonso ..	,,	,,	4	4			8	6
18. Panja Pasand ..	,,	,,	4	4		2	10	5
*19. Daseri (aman) ..	,,	,,	4	4			8	4
20. Kurukkan ..	,,	,,	4	4			8	9
*21. Pairie (Poona) ..	,,	,,	4	6			10	—
22. Latra (creeping)	40-41	,,	2	10		4	16	—
23. Baramassia ..	—	,,	—	—	—	—	—	8
24. Kaitki ..	—	,,	—	—	—	—	—	6

* indicates important commercial varieties.
— indicates undetermined.
SAT indicates satellited chromosomes.
S.C. indicates secondary constricted chromosomes.

of *M. sylvatica*, which did not germinate under usual conditions, were forced by alternate treatment with heat and cold.

Desired root-tip preparations were made from materials fixed at 9–11 a.m. in a mixture of 1% chromic acid and 10% formalin in a proportion of 6 : 4. Paraffin sections, 8–10μ thick, were stained by Newton's Iodine Crystal-Violet method.

Flower-buds of the same varieties and species were fixed in Belling's Nawashin fluid between 9–11 a.m. after a pretreatment in Carnoy's fluid. Paraffin sections, 16–18μ thick, were stained in Crystal Violet after premordanting in Nawashin's fluid.

The smear method for PMC did not prove successful, due to the rapid and successive division of the nuclei in the single fertile stamen. Moreover, the flower buds are so small and so much aggregated while young, that it becomes very difficult to dissect out the proper type of bud and the anther contained in it without the help of a dissecting microscope.

TABLE II.

Size variation in chromosomes of individual complement and their total length.

Name.	No. of chromosomes of different lengths and their total length.			Total length of metaphase chromosomes per nucleus.
	0·4–1μ (short).	1·0–1·5μ (medium).	1·5–2·0μ (long).	
M. sylvatica	30 22·5μ	10 12·5μ	.. ..	35μ
M. indica varieties—				
1. Shadwalla	22 16·5μ	12 15μ	6 10·5μ	42μ
2. Himsagar	24 18μ	12 15μ	4 7μ	40μ
3. Kishenbhog	30 22·5μ	6 7·5μ	4 7μ	37μ
4. Golapkhas	28 21·0μ	10 12·5μ	2 3·5μ	37μ
5. Rogni	24 18μ	14 17·5μ	2 3·5μ	39μ
6. Langra	28 21μ	10 12·5μ	2 3·5μ	37μ
7. Anupam	32 24μ	8 10μ	.. ..	34μ
8. Kalapahar	22 16·5μ	14 17·5μ	4 7μ	41μ
9. Safdar Pasand	24 18μ	12 15μ	4 7μ	40μ
10. Laskarshikhan	22 16·5μ	12 15μ	6 10·5μ	42μ
11. Jehanara	28 21μ	12 15μ	.. ..	36μ
12. Lakhna	28 21μ	12 15μ	.. ..	36μ
13. Kohitur	28 21μ	10 12·5μ	2 3·5μ	37μ
14. Bombai	24 18μ	12 15μ	4 7μ	40μ
15. Fazli	26 19·5μ	12 15μ	2 3·5μ	38μ
16. Alphonso	28 21μ	10 12·5μ	2 3·5μ	37μ
17. Panja Pasand	26 19·5μ	12 15μ	2 3·5μ	38μ
18. Daseri	28 21μ	10 12·5μ	2 3·5μ	37μ
19. Pairie	26 19·5μ	10 12·5μ	4 7μ	39μ
20. Latra	18 13·5μ	18 22·5μ	4 7μ	42μ

The above calculations are based on average length of the 'short' chromosomes as 0·75μ, 'medium' as 1·25μ and 'long' as 1·75μ, e.g. if there are 12 chromosomes of 'medium' length group, the total length has been put as 15μ.

Observations and drawings were made, using principally a Zeiss 2 mm. apochromatic objective 1·4 N.A., and an apochromatic condenser 1·4 N.A., with homogeneous immersion and compensating eyepieces ×10 and ×18, and a Zeiss drawing prism.

Observations.

Chromosome number.—The three species under investigation are morphologically very much allied; *M. sylvatica* being distinguished mainly by its glabrous panicle and acuminate beaked fruits, whereas *M. caloneura* differs from *M. indica* in bigger lax flowers and compressed round fruits. The leaves of the three species are almost similar, but in the cultivated varieties of *M. indica* the petiole is shorter, whereas in the wild type it is longer and almost of the same length as in *M. sylvatica*, with which it is more allied. The varieties of mango (*M. indica*) and the allied species examined, show a remarkable stability in their chromosome number, all possessing $2n = 40$ and $n = 20$ chromosomes. In three varieties (*Kalapahar, Safdar Pasand* and *Latra*) only, plates (Fig. 22) showing an aberration in their somatic chromosome number (41 and 42) have been found. The results are given in Table I.

Karyotype analysis.—A critical study of the chromosome morphology shows that *M. sylvatica* and the varieties of *M. indica* differ from one another in the size variation of the chromosomes (cf. Table II), and the total number of satellites and secondary constrictions in their complements (cf. Table I). The satellites in some of the chromosomes are extremely small. Due to the short length of the chromosomes, the secondary constrictions indicated by the unstained gaps, become obscure whenever there is a slight swelling or torsion of the chromosomes. Identity of some of the secondary constrictions, therefore, appears doubtful. Determination of the morphology of somatic chromosomes therefore becomes difficult, especially because few well spread-out plates are present in the small cells within the short cortical region of the root-tip. Moreover, the cell-inclusions, mainly of the nature of tannins in the outer cortical zone, interfere very much with the fixing and staining of the chromosomes. The number of satellites and secondary constrictions present in the different chromosomes of a complement, has therefore been determined after an examination of more than 10 good somatic plates.

The chromosomes varying in length between 0·4μ–2·0μ, have been classified under 3 main length-groups—*Short* (0·4–1·0μ), *Medium* (1·0–1·5μ) and *Long* (1·5–2·0μ). A comparative statement of chromosome morphology according to length-variation in the different types is given in Table II.

Apart from this broad grouping according to size-differences, the somatic chromosomes could be distinguished by their morphology into the following 11 categories:—

Type A. *Long* chromosomes with both *secondary constrictions* and *satellites*.

Type B. *Long* chromosomes with submedian primary constriction and a typical *satellite* at the end of the longer arm.

Type C. *Long* chromosomes with submedian primary constriction and subterminal or submedian *secondary constriction* on the longer arm.

Type D. *Medium* chromosomes with submedian primary constriction and a *satellite* with short or long threads at the end of the longer arm.

Type E. *Medium* chromosomes with a *satellite* at the end, but without any apparent primary constriction.

Type F. *Medium* chromosomes with submedian primary constriction and subterminal or median *secondary constriction* on the longer arm.

Type G. *Medium* chromosomes with median or submedian primary constriction.

Type H. *Short* chromosomes with submedian primary constriction and a fine *satellite* on the longer arm.

Type I. *Short* chromosomes with a satellite at one end and without any apparent primary constriction.
Type J. *Short* chromosomes with median or submedian primary constriction.
Type K. *Short* chromosomes without any apparent constriction.

Of the 11 chromosome types, mentioned above, all except G, J and K are nucleolar, i.e., possess satellites or secondary constrictions. An analysis of the somatic chromosome complement of the different species and varieties indicates the following idiogram:—

TABLE III.

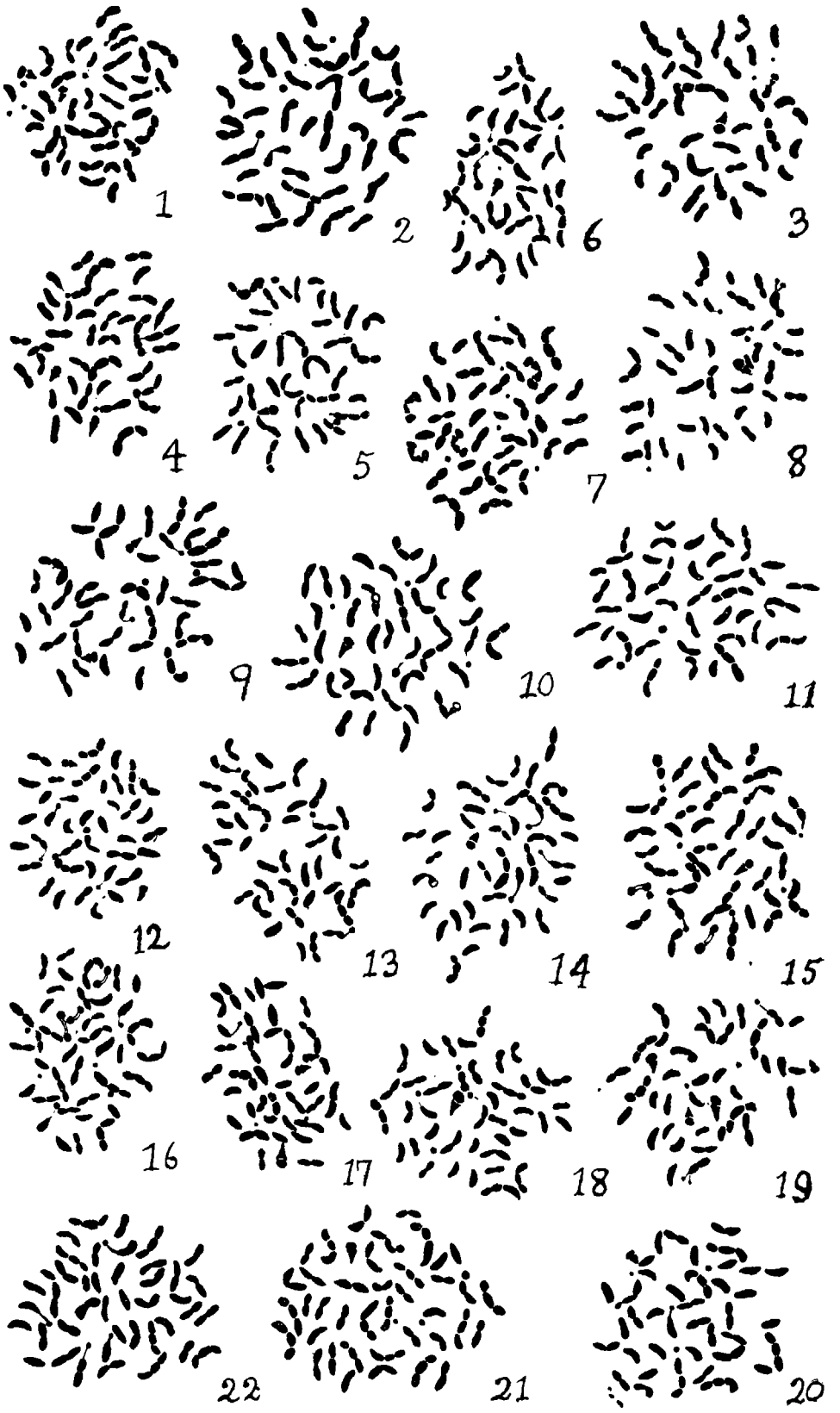
A comparative account of Karyotypes, observed in the mangoes and the allied species.

	Name of varieties and the number of nucleoli.	Chromosome types. A	B	C	D	E	F	G	H	I	J	K
												
(8)	1. Kishenbhog	..	..	4	2	2	..	2	..	..	18	12
	2. Alphonso	..	..	2	2	..	2	6	..	2	6	20
	3. Daseri	..	..	2	2	..	2	6	..	2	6	20
(10)	4. Rogni	..	..	2	2	2	4	6	..	..	12	12
	5. Kohitur	..	..	2	2	2	4	2	..	..	14	14
	6. Panja Pasand	..	..	2	2	2	4	4	..	..	10	16
	7. Pairie	..	..	4	..	4	2	4	..	..	14	12
	8. Anupam	..	..	..	2	..	6	..	..	2	18	12
	9. Jehanara	..	..	..	4	2	4	2	..	..	10	18
(12)	10. Shadwalla	..	2	4	2	..	4	6	..	..	16	6
	11. Golapkhas	..	..	2	2	2	6	..	..	..	20	8
	12. Langra	..	..	2	4	..	4	2	2	..	20	6
	13. Kalapahar	..	..	4	4	..	4	6	..	..	12	10
	14. Safdar Pasand	..	..	4	4	..	4	4	..	..	12	12
	15. Laskarshikhan	..	..	6	2	..	4	6	..	..	12	10
	16. Lakhna	..	..	..	4	..	8	..	..	..	12	16
	M. sylvatica	..	..	..	2	2	6	..	..	2	18	10
(14)	17. Himsagar	..	..	4	..	2	8	2	..	..	14	10
	18. Bombai	2	..	2	4	..	6	2	..	..	18	6
	19. Fazli	..	..	2	2	2	6	2	..	2	12	12
(16)	20. Latra	..	..	4	4	..	8	6	..	..	12	6

In the above table the species and the varieties have also been grouped under 5 classes according to the number of nucleolar chromosomes (8, 10, 12, 14 or 16). An examination of the table shows the following characteristic features.

Alphonso and *Daseri* (Figs. 17 and 19) have exactly similar chromosomes. They have the same total length of metaphase chromosomes as in *Kishenbhog*, *Rogni*, *Kohitur* and *Panja Pasand* (Figs. 6, 14 and 18) have the same number of C, D, E and F chromosomes. *Anupam* (Fig. 8) has the highest number (32) of 'short' chromosomes in the '10 nucleolar chromosome group'.

Shadwalla (Fig. 2) is conspicuous in having 2B chromosomes with prominent big SATs. *Golapkhas* (Fig. 5) has 2E chromosomes which are absent in other varieties of the '12 nucleolar chromosome' group. *Langra* (Fig. 7) is characteristic in having sharp primary constrictions in majority of the chromosomes, whereas in most of the other varieties much higher number of chromosomes are without any sharp primary constriction. *M. sylvatica* (Fig. 1) is characterized by the absence of



(For Explanation, *see* p. 293.)

'long' chromosomes and by the possession of a high number (30) of 'short' chromosomes.

Among the types having 14 nucleolar chromosomes, *Bombai* (Fig. 15) is characterized by a pair of A chromosomes, which have both satellites and secondary constrictions. *Latra* the mango with trailing branches has the highest number of nucleolar chromosomes (i.e., SATs and secondary constrictions) among the mangoes examined (Fig. 21).

Maximum number of nucleoli from Dyad nuclei.—The number of nucleoli in each dyad nucleus in PMC (Table I) varies between 4 and 9 (Figs. 45–57). They have definite size difference and may be recognized as *big, intermediate* and *small.* These three types of nucleoli are present in different numbers in different varieties, but they are found to be segregated into homomorphic pairs in the two nuclei in a dyad cell, indicating a regular disjunction of homologous pairs of satellited and secondary constricted chromosomes.

The number of satellited and secondary constricted chromosomes are generally found to be the same as the total number of nucleoli in the two nuclei of a dyad cell. There are some anomalies in this correlation, the number of nucleoli being less in *Bombai* (Fig. 51) and higher in *Himsagar, Laskarshikhan, Kurukkan* (Figs. 46, 47 and 57). In the former types, the anomaly in correlation may be due to the fusion of two or more nucleoli just after formation in early telophase, which stage passes off very quickly.

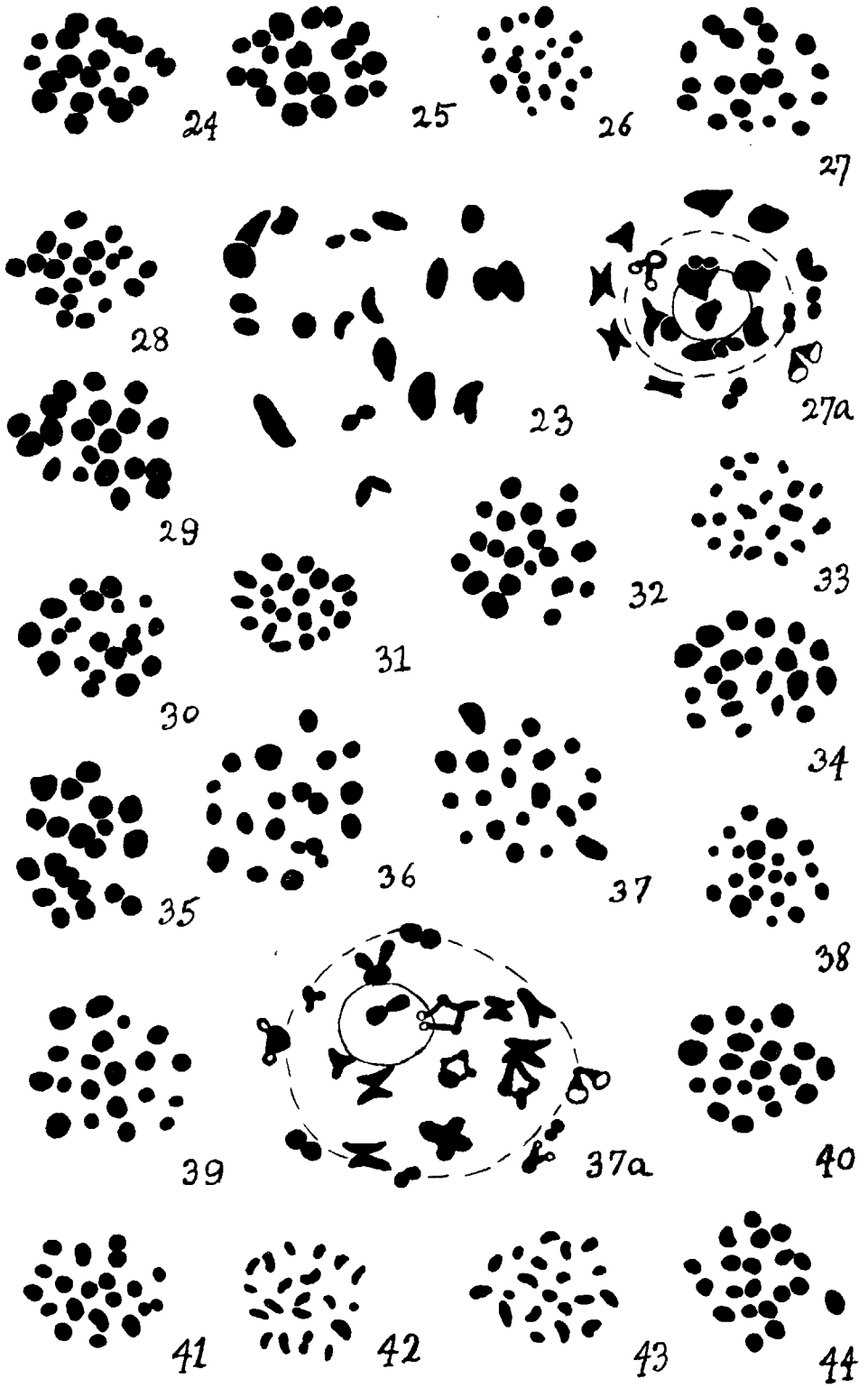
The early fusion of nucleoli is indicated by their occurrence in varying numbers in different dyad nuclei within the same slide, although the total nucleolar volume in those nuclei remains almost the same. On the other hand, the occurrence in some varieties of higher nucleolar number than the observed number of satellited and secondary constricted chromosomes is most probably due to the difficulty in tracing out all the SATs and secondary constrictions present, specially when these constrictions are super-numerary in nature, i.e., a single chromosome having more than one nucleolar constriction. However, in all cases the maximum number of nucleoli have been taken into consideration, as that is a surer guide to the determination of the number of nucleolar constrictions (Bhaduri 1944, 1947; Chakravorti 1948).

Meiosis in pollen mother cells.—The varieties of *M. indica* and the allied species, *M. sylvatica* Roxb. and *M. caloneura* Kz. are characterized by having well-developed anthers in only 1 of the 5 stamens.

Along with the development of the flower-bud the anther grows in size and follows a normal course of development leading to the differentiation of the pollen mother cells with a big nucleus. After usual changes in the PMC nuclei, the homologous chromosomes are found to pair into 20 clear bivalents, which arrange themselves mostly below the nuclear membrane and around the persisting nucleolus during diakinesis (Figs. 27*a* and 37*a*). The nuclear membrane and the nucleoli subsequently disappear along with the formation of a spindle, at the equator of which the bivalents are arranged. A polar view of the metaphase plates gives a clear picture of 20 bivalents, which are found to be aggregated into groups of 4, 3 and 2 due to secondary association (Figs. 24, 25, 29 and 35). Data regarding grouping of bivalents, which indicate secondary association of distant homologues, are given in Table IV. It clearly shows that the maximum association of bivalents is into 8 groups or units, and the most frequent association is 10.

FIGS. 1–22. Somatic chromosome plates during metaphase : (*see* p. 292).

1. *M. sylvatica.* 2–22. Varieties of *M. indica.* 2. Shadwalla. 3. Himsagar. 4. Kishenbhog. 5. Golapkhas. 6. Rogni. 7. Langra. 8. Anupam. 9. Kalapahar. 10. Safdar Pasand. 11. Laskarshikhan. 12. Jehanara. 13. Lakhna. 14. Kohitur. 15. Bombai. 16. Fazli. 17. Alphonso. 18. Panja Pasand. 19. Daseri. 20. Pairie. 21. Latra (creeping mango). 22. Kalapahar with 42 chromosomes. All drawings ×4,200.



(For Explanation, *see* p. 295.)

TABLE IV.

Comparative data regarding Secondary Association.

Names of Sp. and Vars.	Maximum Association.	Most frequent Association.
M. caloneura Kz. ..	*2(3)+7(2) = 9	2(3)+6(2)+2(1) = 10
M. indica Linn.—		
'wild' type	2(3)+7(2) = 9	2(3)+6(2)+2(1) = 10
Var. Shadwalla ..	2(3)+6(2)+2(1) = 10	2(3)+6(2)+2(1) = 10
,, Kishenbhog ..	4(3)+4(2) = 8	2(3)+6(2)+2(1) = 10
,, Langra	2(3)+6(2)+2(1) = 10	2(3)+6(2)+2(1) = 10
,, Anupam	2(3)+6(2)+2(1) = 10	2(3)+6(2)+2(1) = 10
,, Safdar Pasand ..	1(4)+2(3)+4(2)+2(1) = 9	3(3)+4(2)+3(1) = 10
,, Laskarshikhan ..	1(4)+3(3)+3(2)+1(1) = 8	1(4)+7(2)+2(1) = 10
,, Bombai	2(3)+6(2)+2(1) = 10	2(3)+6(2)+2(1) = 10
,, Fazli	1(4)+2(3)+4(2)+2(1) = 9	3(3)+4(2)+3(1) = 10
,, Alphonso ..	4(3)+4(2) = 8	2(3)+6(2)+2(1) = 10
,, Panja Pasand ..	2(3)+7(2) = 9	2(3)+6(2)+2(1) = 10
,, Daseri (aman) ..	4(3)+4(2) = 8	2(3)+6(2)+2(1) = 10
,, Kurukkan ..	3(3)+4(2)+3(1) = 10	3(3)+4(2)+3(1) = 10
,, Sah Pasand ..	3(3)+4(2)+3(1) = 10	2(3)+6(2)+2(1) = 10

* 2(3) means 2 groups of 3 bivalents in each group, etc.

After complete terminalization of the chiasmata in paired bivalents during metaphase, their regular disjunction takes place resulting in the separation of 20 homologous chromosomes towards the two poles. While moving to the two poles during anaphase, the chromosomes always appear at equal distances from the equator of the spindle on the two sides. During this process, no indication of multivalent formation or any other meiotic irregularity such as chromosome bridges, lagging chromosomes, etc., is seen.

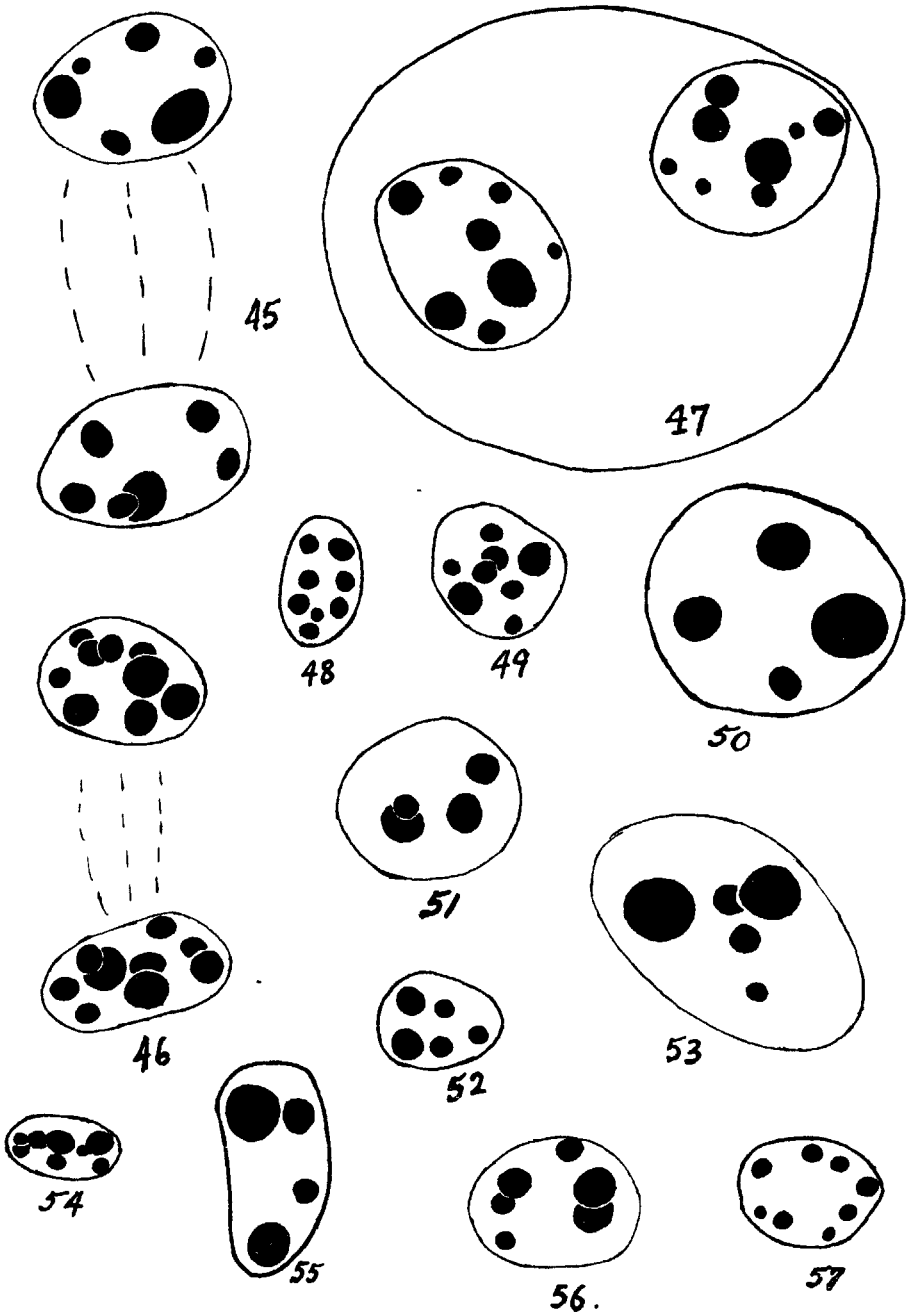
The chromosomes ultimately reach the two poles during telophase, and give rise to the nucleoli at the region of nucleolar organizers, i.e., SAT threads and secondary constrictions. Due to the small volume of the nucleus and the presence of a high number of satellited and secondary constricted chromosomes in it, the maximum number of nucleoli could not be clearly determined in every case. They become very clear during the short interphase stage when they take a bright stain. It is obvious therefore that the maximum number of nucleoli, present in a nucleus, can only be observed in preparations where such favourable stages are present (Bhaduri and Kar, 1948).

Second division and cytokinesis.—After a short period of rest during interphase succeeding meiotic division, the subsequent mitotic division of the chromosomes begins in the two nuclei within the single dyad cell. The configuration of the two spindles is not regular, and they appear parallel or at right angles to each other. After this second division 4 separate nuclei are formed, which remain enclosed within

FIGS. 23–44. Chromosome plates during meiosis: (*see* p. 294).

23. Diakinesis in *M. sylvatica* (scattered drawing). 24–44. Metaphase plates. 24. *M. caloneura*. 25. *M. indica* (wild race). 26–44. *M. indica* (horticultural varieties). 26. Shadwalla. 27. Himsagar. 27*a*. Himsagar (Diakinesis). 28. Kishenbhog. 29. Langra. 30. Anupam. 31. Kalapahar (Second Division). 32. Safdar Pasand. 33. Sah Pasand (Second Division). 34. Kohitur. 35. Laskarshikhan. 36. Bombai. 37. Kurukkan (polyembryonic variety). 37*a*. Kurukkan (Diakinesis). 38. Baramassia. 39. Latra. 40. Pairie. 41. Daseri. 42. Panja Pasand. 43. Alphonso. 44. Fazli (41–44. Second division metaphase plates).

Figs. 26, 33, 42 and 43 (×3,600); rest ×4,200.



FIGS. 45–57. Nucleoli during Telophase in Dyad and Tetrad cells in *M. indica* :

45. Kaitki. 46. Kurukkan. 47. Himsagar. 48. Wild race (Tetrad). 49. Kishenbhog (somatic cells). 50. Kishenbhog. 51. Bombai. 52. Anupam (Tetrad). 53. Rogni. 54. Baramassia (Tetrad). 55. Daseri. 56. Sah Pasand. 57. Laskarshikhan (Tetrad). All drawings × 4,200.

the common wall of the PMC. Subsequently the cytoplasm within the PMC divides by furrowing, and produces a wall around each of the 4 tetrad nuclei, leading to the formation of 4 microspores which are liberated after bursting of the anther when the flower opens.

Pollen grains.—The mature pollen grains in all the varieties show similar morphology in their elliptic shape and in having a closely pitted exine with 3 long, tapering sharply defined furrows (*tricolpate type*) containing a large germ-pore at the centre of each. They are also similar in size, varying generally between 24–30μ in average diameter.

The pollen grains are mostly normal and full of cytoplasm; only a few (1·8–12·0%) are crumpled or empty, indicating evident signs of negligible amount of male sterility. Experiments at Poona (Burns and Prayag, 1921), Sabour (Sen, 1943), and U.S.A. (Sturrock, 1944) have also shown that the varieties are readily intercrossable, indicating close compatibility among them.

The cytological findings indicate normal development of the grains and close compatibility between the varieties.

Discussion and General Conclusion.

Stability in chromosome number in Mangifera L.—A reference to the list of chromosome numbers of plants (Darlington and Ammal, 1945) shows that species comprising a genus have generally a varying series of chromosome numbers; rarely they possess the same chromosome number throughout a genus. Examples of such stability in chromosome number are found in *Antirrhinum* (14 sp. examined, $2n = 16$), *Aloe* (73 sp., $2n = 14$), *Quercus* (44 sp., $2n = 24$), *Ribes* (21 sp., $2n = 16$), *Ficus* (29 sp., $2n = 26$), *Erica* (6 sp., $2n = 24$), etc.

The present investigation shows that all the 3 sp. of *Mangifera* (*M. indica*, including the wild and cultivated varieties; *M. sylvatica* and *M. caloneura*) have the same chromosome number, $2n = 40$ and $n = 20$. The chromosome numbers of other species of the genus are not yet known.

The genus has been split into two sections, according to the presence or absence of the disc (Mukherjee, 1949). '*Section I*' having well-developed swollen disc contains 34 species, and '*Section II*' with disc reduced or absent contains 7 species. Only two species possess 10 stamens, of which 5 are fertile, 4 species have all the 5 stamens fertile, 1 species has 3 stamens fertile, while the remaining 34 species have only 1 of the 5 stemens fertile. The three species under investigation belong to the last group under '*Section I*'. The species belonging to the other section, or the taxonomically older species (having 5 stamens fertile) could not be examined as they occur in Malaysia. This lack of information is a great handicap in formulating any general theory on the phylogeny and origin of the genus on the basis of cytological data alone.

Although the present investigation suggests a stability in chromosome number in *Mangifera*, future observations, especially on the taxonomically older species (*M. Duperreana* Pierre, *M. pentandra* Hook f., and *M. lagenifera* Griff.) or other species of *Section II*, may show some lower chromosome number. The homogeneity in the range of floral structure in a majority of the species suggests, however, the possibility of the presence of the same chromosome number in most of them (Mukherjee, 1948*a*).

A study of the pollen morphology in 9 species of *Section I* and 4 species of *Section II* of the genus from herbarium specimens has shown that all of them have similar morphology and are of almost the same size (generally 23–32μ) as in *M. indica* (Mukherjee, 1950). Size difference in pollen grains of related species can be a test for polyploidy in a genus, as has been found in *Quercus*, *Allium*, *Triticum*, *Euphorbia*, *Rosa* and *Tradescantia*, etc. (Cf. Cain, 1944; Darlington and Ammal, 1945). The similarity in shape and size of the pollen grains in species of *Mangifera* therefore

substantiates the expectation of finding a stable chromosome number (viz. $2n = 40$) in a majority of the species belonging to 'one fertile stamen' group.

Furthermore, it has been established (Hagerup, 1932) that species with varying chromosome numbers in a genus have evolved from the original type in those regions of the globe, which are characterized by an extremely low temperature (such as arctic region or mountain peaks), or high temperature and low humidity such as deserts (Wulf, 1943). All the species of *Mangifera* grow under almost similar climatic conditions, none occurring on mountain tops above 3,000 ft. or in the deserts (Mukherjee, 1949). They are distributed throughout Malaysian region in the tropics, the flora of which has never been subjected to great climatic changes during the geological periods since the origin of the angiosperms in the Cretaceous (Wulf, 1943). The stability in chromosome number, anticipated in majority of the species, is also indicated by the phyto-geographical distribution of the genus.

Morphological variations correlated with chromosome morphology.—The karyotype of a species, based on the morphology of its chromosome complement, is usually characteristic for it. A comparison of the karyotypes of component species of a genus shows differences, which may be marked or inconspicuous. A similarity in the phenotype between two species is more or less expressed by a similarity in their karyotypes (Babcock and Cameron, 1934). The chromosome morphology, therefore, becomes an important guide in tracing taxonomic affinity.

Whereas in *Zea mays*, *Vicia faba*, *Crepis* sp., *Scilla* sp., etc., some prominent chromosomes in their karyotypes, characteristic for each type, have been found, there are a large number of species, e.g. in *Salix*, etc. (Wilkinson, 1944), where no such distinct chromosome in the karyotype could be found.

A reference to the drawings of the somatic complements of *M. sylvatica* and the different varieties of *M. indica* emphasizes remarkable uniformity in size and morphology of the chromosomes. The differences between the complements are inconspicuous, and indeed are detectable beyond reasonable doubt only after a critical study. In spite of the lack of any prominent feature, certain chromosome types such as those with secondary constrictions, those without any apparent constriction, and those which are satellited appear to have some taxonomic importance. The chromosomes have therefore been differentiated into 11 types on the basis of their morphology. Four of these types (H–K) belong to the short length group, 4 (D–G) to the medium length and the other 3 (A–C) to the long group (see Idiogram with Table III). Some of the chromosomes of the medium group merges into similar chromosomes in the short length group, e.g. D with H, E with I, G with J. Hence out of 11 chromosome types enumerated above, 8 main types can be clearly distinguished.

The varieties of *M. indica* and the allied species *M. sylvatica* differ from one another mainly in the possession of different assortments of these 11 chromosome types; the total number of satellited and secondary constricted chromosomes varying between 9–16, and the unconstricted short chromosomes varying between 6–20 (cf. Table III).

The morphology of the varieties also shows that there is no marked divergence among any of them. They differ from one another firstly in the fruit characters, and secondly in the different colour ranges of the emerging leaves and panicle branches (Mukherjee, 1948*b*). The range of variation is continuous with gradual merging of characters intergrading from one extreme to the other. The continuous range in the phenotypic differentiation of the varieties is also manifest in their chromosome morphology, which shows minute differences among the varietal complements. No sharp differentiation is seen in any of the complements.

The chromosome morphology of *M. sylvatica* is very much similar to that of the morphologically allied *M. indica*. The geographical distribution of the two species are also overlaping. This correlation between the phenotype and karyotype supports the view of Babcock and Cameron, that taxonomically allied species and

varieties have similar chromosome morphology (cf. Sax, 1930; Goodspeed, 1934; Resende, 1937; Babcock, 1942; Meyer, 1944).

A comparison of the karyotypes brings out some interesting correlations between pairs of varieties. *Shadwalla* and *Bombai* (an important commercial type), which are morphologically very much allied, possess almost similar karyotypes except that *Bombai* has one pair of chromosomes with both SAT and secondary constriction. Such close inter-relationship is also found between *Kohitur* and *Panja Pasand*, *Alphonso* and *Daseri*, *Himsagar* and *Pairie*, *Anupam* and *M. sylvatica*.

The morphological classification of the varieties into 3 groups (i.e., *Round-*, *Ovate-oblong-*, and *Long-fruited*) on the basis of fruit character (Mukherjee, 1948*b*), does not indicate any significant correlation with the grouping of the varieties into 5 classes according to the maximum number (8, 10, 12, 14 or 16) of nucleolar chromosomes in the nucleus.

The occurrence in *Latra*, one of the round-fruited varieties, of a higher number (16) of satellited and secondary constricted chromosomes in its complement indicates that this group of varieties is of later origin. A significant justification for this view is obtained from the evidence that the *wild* mangoes of the present day belong to the *Ovate-oblong* group. Examination of other round-fruited varieties, e.g. *Rumani*, *Nazimpasand*, *Sabsang* and *Dudho*, will verify whether the above statement is correct or not.

Polyploid Nature of the Mangoes.—Genetical and cytological studies have shown that from an evolutionary point of view, we can arrange our races of cultivated plants conveniently in the following four classes, according to their mode of origin (Crane, 1940):—

(1) By selection from gene-mutation within a single species.
(2) By simple autopolyploidy.
(3) By selection from products of interspecific hybridization, unaccompanied by chromosome duplication or aberration;
(4) By interspecific hybridization, accompanied by chromosome doubling (allopolyploidy) or other nuclear aberrations.

Investigation on the crops of temperate regions have shown that many of the cultivated species and races have originated by the last process (allopolyploidy) either in nature or under cultivation, e.g. *Dahlia variabilis*, *Prunus domestica* (European Plums), *Aesculus carnea*, *Rubus loganobaccus* (Crane, 1940). Amongst these, *Prunus domestica* is most interesting in view of the fact that the hypothesis of its origin in nature by hybridization between a diploid *Prunus divaricata* and a tetraploid *Prunus spinosa* followed by chromosome doubling, based on cytological and genetical observations (Crane and Lawrence, 1947) received confirmatory support from the investigations of Rybin (1936), who synthesized it from the above two species. Crane (1940) has also suggested that the tropical crops are likely to have originated along the same line.

Although no polyploid series of chromosome number has been found in the varieties of mango or in the allied species, the polyploid nature of the mangoes can be deduced from the following evidences:—

1. The diploid number, $2n = 40$, is itself sufficiently high to be the basic number for the genus and indicates its derivation from some plants with lower chromosome number through polyploidy.
2. Evidence for polyploidy is obtained from the presence of a high number (8–16) of satellited and secondary constricted chromosomes in the complement, which are nucleolar (Bhaduri, 1944; Bhaduri and Bose, 1947).

Since the establishment of De Mol's (1928) theory of numerical correlation between the number of nucleoli and the number of genomes present in a species,

suggesting the nucleolar number as an important guide to polyploidy, a large number of observations have accumulated (cf. Gates, 1942), on the basis of which Bhaduri came to the conclusion that higher nucleolar numbers have been evolved not through polyploidy alone, but hybridization and structural changes in chromosomes have also played an important rôle in the process (Bhaduri, 1942*a*, *b*; 1948). He further suggested that size difference in the nucleoli in a species is an important guide to its evolutionary history, and homomorphic pairs of nucleoli in the two poles of a dyad cell suggests the homozygous nature of the species and conversely heteromorphic pair suggests its heterozygous nature.

In addition to the above processes responsible for increase in nucleolar number, fragmentation of chromosomes at the regions of secondary constrictions has also been claimed recently (Bhaduri and Bose, 1947; Chakravorti, 1948) to play an important rôle in the process. Nucleolar number alone cannot therefore be used as a measure of the degree of polyploidy.

During the present investigation, *M. indica*, *M. sylvatica* and *M. caloneura* have been found to possess 8–16 nucleoli in a somatic cell, which are segregated into homomorphic pairs in the two nuclei within a dyad cell, suggesting their homozygous condition. Such a high number of nucleoli in the mangoes is due to their polyploid nature, and not due to segmental interchange or other structural changes in chromosomes, as evidenced by the striking regularity in pairing and disjunction of the bivalents during meiosis. It is worth pointing out here, that the large number of 'Prime types' found in *Datura stramonium* and *D. metel* each bearing the same chromosome number, $2n = 24$, and showing normal pairing and disjunction, differ from one another with respect to one or more interchange of segments of chromosomes (Bergner, Satina and Blakeslee, 1943; Blakeslee, *et al.*, 1940). This could only be detected by the formation of rings of four or more chromosomes during diakinesis in intervarietal crosses. It is difficult to state at the present stage whether such cytological differences exist between different varieties or species of *Mangifera*. It will therefore be an important line of investigation to examine cytologically the intervarietal crosses, which have already been produced at the Horticultural Research Stations in India and elsewhere.

3. During meiosis in the pollen-mother-cells the 40 chromosomes regularly pair into 20 bivalents in diakinesis, which separate ultimately into 20 homologous groups in anaphase. No multivalent formation or any other irregularity such as chromosome bridges or lagging chromosomes are seen. Such striking regularity in pairing and disjunction of chromosomes during meiosis indicates that the mangoes are not autopolyploids, but are allopolyploids. The good fertility in the varieties (the apparent sterility varying between 3–16%), further suggests that they may be amphidiploids.

Secondary association and Basic number.—A reference to Table IV shows that during meiotic metaphase, the 20 bivalents aggregate in groups of 4, 3, 2 or 1 to form a maximum secondary association into 8 units, indicating residual attraction between more distantly related chromosomes (Darlington and Moffet, 1930) and emphasizing the hybrid (allopolyploid) origin (Lawrence, 1931) of the mangoes. Recently Thomas and Revell (1946) have put forward the view that the characteristic secondary association between bivalents is due to the 'fusion between heterochromatic regions at pachytene' and gives 'little indication of homology'. Although 'secondary association shows no specificity' they, however, agree that 'there is a higher degree of association between the morphologically similar bivalents'. They moreover suggest that in the diploid, analysis of secondary association need not indicate chromosome relationship, whereas in the tetraploid it may do so, depending on '(*a*) the degree of prezygotene orientation, and (*b*) the amount and distribution of heterochromatin'. In the mangoes, the secondary association gives an additional evidence for their allopolyploid origin, and therefore is not in disagreement with the above view regarding secondary association in polyploids.

The maximum association of the bivalent groupings into 8 units indicates the basic number for the genus to be 8. Although no species with such low chromosome number is now known in *Mangifera* or any other genera of the family *Anacardiaceae*, future investigation may lead to the discovery of such a type. The occurrence of 8 distinct chromosome types in the complement of 40 is a significant corroboration for the view that 8 is the basic number (Nandi, 1936; Jacob, 1941).

The chromosome numbers, determined only in 4 genera of *Anacardiaceae*, show that 3 of them, *Rhus* (2 sp.), *Mangifera* (3 sp.) and *Semecarpus* (1 sp.), have respectively $2n = 30$, 40 and 60 chromosomes. The occurrence of 30 and 60 chromosome numbers in the allied genera suggests that they might have originated from a basic number of 5 or a multiple of 5; but *Mangifera* is indicated to have a basic number of 8. Whether there are two basic numbers in the family or that for *Mangifera* is a derived number can be determined definitely only by further observations.

Evolution of the varieties of mango.—Arguments put forward under previous headings have established beyond doubt the allopolyploid nature of the mangoes. Their amphidiploid origin is also strongly suggested by evidence from regular pairing and good fertility. It therefore appears that the primitive type or types, which subsequently gave rise to the mango varieties, originated through allopolyploidy and most probably amphidiploidy. The next problem is how so many varieties have originated ?

The morphology of the innumerable varieties shows a gradual continuous change in their characters, intergrading in range, as is expected in a polyploid (Crane, 1940). Significantly the chromosome morphology also shows minute intergrading differences in the varietal complement. The phenotypic and the genotypic characters therefore show an interesting parallelism in the slow, intergrading continuous changes in their diagnostic features. In view of these cytological evidences it is suggested that differentiation of the varieties from the original type or types has primarily taken place through gene mutations. The selected type has been preserved under cultivation through vegetative propagation by grafting. The compatibility between the varieties being very close, due to the close similarity in their chromosome morphology, intervarietal hybridization, occurring freely in nature, further induces the production of new varieties. The huge diversity in mango varieties in India is also to be explained as due to their cultivation for a long period, thereby giving ample opportunity for hybridization and forces of selection to operate. The occurrence of a large number of variations in mangoes in India supports the observations of Vavilov (cf. Darlington and Ammal, 1945) that a cultivated or wild species is expected to show the greatest diversity in the 'mountain-and-valley regions nearest the equator' as has been found by him in the case of various crop plants. The area of the maximum range of diversity is possibly the centre of origin of the species.

SUMMARY.

The present investigation has shown that *Mangifera sylvatica*, *M. caloneura* and *M. indica* (including 23 cultivated varieties and 1 wild race) have the same chromosome number $2n = 40$ and $n = 20$. The presence of same chromosome number also in majority of the remaining species of *Mangifera*, is suggested by the evidence of similarity in the pollen size and morphology, observed in 13 species belonging to both the sections of the genus, as also by their phytogeographical distribution.

The total number of satellites and secondary constrictions in the complements of different species and varieties varies between 8–16 with the size of the chromosomes ranging from 0·4 to 2·0μ. Another important characteristic is the presence of a large number of chromosomes without any apparent constriction in each complement. On the basis of their morphology the chromosomes have been distinguished into 11 types, of which 3 are intergrading and 8 distinct. An analysis of the karyotypes shows that the varieties of mango and the allied species differ from one another mainly in the possession of different assortments of these chromosome types, each complement showing slight intergrading difference without any sharp discontinuous change.

The phenotypic and the karyotypic characters show an interesting parallelism in the slow, intergrading continuous changes in their diagnostic features.

The number of nucleoli in a somatic cell varies between 8–16 and corresponds generally with the number of satellited and secondary constricted chromosomes. The varieties have been grouped into 5 classes according to the number of nucleolar chromosomes (8, 10, 12, 14 or 16). The morphological classification of varieties according to fruit-shape does not show any significant correlation with groupings according to nucleolar chromosome number.

A comparison of karyotypes, however, brings out some interesting correlations between pairs of varieties and species, viz. between *Shadwalla* and *Bombai*, *Kohitur* and *Panja Pasand*, *Alphonso* and *Daseri*, *Himsagar* and *Pairie*, and *Anupam* (*M. indica*) and *M. sylvatica*.

The nucleoli in each nucleus have definite size difference, viz. big, intermediate and small, and they form homomorphic pairs in the two nuclei in a dyad cell indicating the homozygous nature of the varieties.

Although no polyploid series of chromosome numbers has been found in the varieties of mango and in the allied species, their polyploid nature is indicated by the high number of somatic chromosomes and a correspondingly high number of nucleolar chromosomes.

A need for the examination of meiosis in intervarietal crosses has been stressed to find out if any segmental intercharge of chromosomes has taken place as in *Datura*.

During meiosis, the chromosomes show a regular pairing into 20 bivalents and subsequent regular disjunction. No multivalent formation or any other peculiarity is seen. At metaphase, the bivalents show a maximum secondary association into 8 units. This phenomenon suggests 8 as the basic number for *Mangifera*. It is also supported by the presence of 8 distinct chromosome types. The presence of $2n = 30$ and 60 chromosomes in the allied genera *Rhus* and *Semecarpus* respectively suggests that there may be two basic numbers in *Anacardiaceae* or that 8 for *Mangifera* might have been derived from 5 or multiple of 5.

From the evidences put forward it appears that the primitive type or types, which subsequently gave rise to the mango varieties, originated through allopolyploidy, most probably through amphidiploidy. The differentiation of the numerous varieties then took place primarily through gene mutations, the selected type being preserved under cultivation by grafting. The compatibility between the varieties being very close, intervarietal hybridization has been perhaps another important factor in the production of new varieties. The huge diversity in the mangoes in India is also to be explained as due to their cultivation here for a long period, thereby giving ample opportunity for hybridization and forces of selection to operate.

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