STUDIES IN THE CAPPARIDACEÆ

VIII. The Cytology of *Capparis Zeylanica* Linn., and Related Genera

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I. Introduction

THIS is the genus after which the family is named. It is representative of the sub-family Capparidioideæ. Cytological work on the family is very meagre indeed and that pertaining to this sub-family is confined to chromosome counts in five species of this genus, one of which was by the senior author on *Capparis sepiaria* (Raghavan, 1938). Recently we described the cytology of *Cratæva religiosa*, another member of the Capparidioideæ (Raghavan and Venkatasubban, 1939) and recorded the presence of secondary association. Besides these, there is no cytological work on the Capparidioideæ.

In the present paper haploid chromosome counts have been made of three members of the sub-family for the first time, *Capparis Zeylanica*,

Cadaba indica and *Mærua arenaria*. In *Capparis Zeylanica*, meiosis has been described in some detail especially as regards secondary association and in *Cadaba indica* material of which was not available in sufficient quantities for a more detailed study, only the meiotic chromosome number has been reported. So also in the other genus.

In all these, it must be said that securing good cytological preparations was a matter of extreme difficulty. It is difficult enough in the Capparidaceæ as a whole, but it would appear to be especially so in this sub-family.

Material for this study was obtained from plants grown in the University Botanical Garden. *Capparis Zeylanica* Linn. which is synonymous with *Capparis horrida* Linn., is a woody climber with prominent recurved thorns which are homologous with stipules. The presence of accessory buds and consequent occurrence of extra axillary flower buds is a feature worthy of note.

Anthers of the right stage of development previously determined by acetocarmine examination, were fixed in Navashin's fluid after prefixation in Carnoy. Fixation of entire flower buds even after the removal of the calyx would not yield any good results. The deletion of prefixation was attended by certain failure to get any preparation worth the name. Embedding was done in paraffin in the usual way after the chloroform technique and Newton's Iodine gentian violet was exclusively used for staining. The average thickness of the sections was about ten microns.

II. Observations

(*a*) *Capparis Zeylanica* Linn.—Stages earlier than First Metaphase were not studied in any detail on account of the extremely small size of the chromosomes. But even at diakinesis it could be clearly seen that the bivalents approximated closely to one another into separate groups. The most prominent feature of the First Metaphase was the occurrence of secondary association. As a matter of fact this phenomenon was so strongly in evidence that hardly a plate existed but exhibited secondary pairing. This is also evidenced by a glance at the accompanying table giving an analysis of the various associations which would reveal that the range of variation of the number of associations is so small and between such high numbers as 7 and 13. The number of bivalents at M I is 20. Figures 1–10 and Pl. IX, Figs. 1–3 show various M I plates showing different degrees of secondary association. Table A gives a summary of the various associations met with.

TABLE A

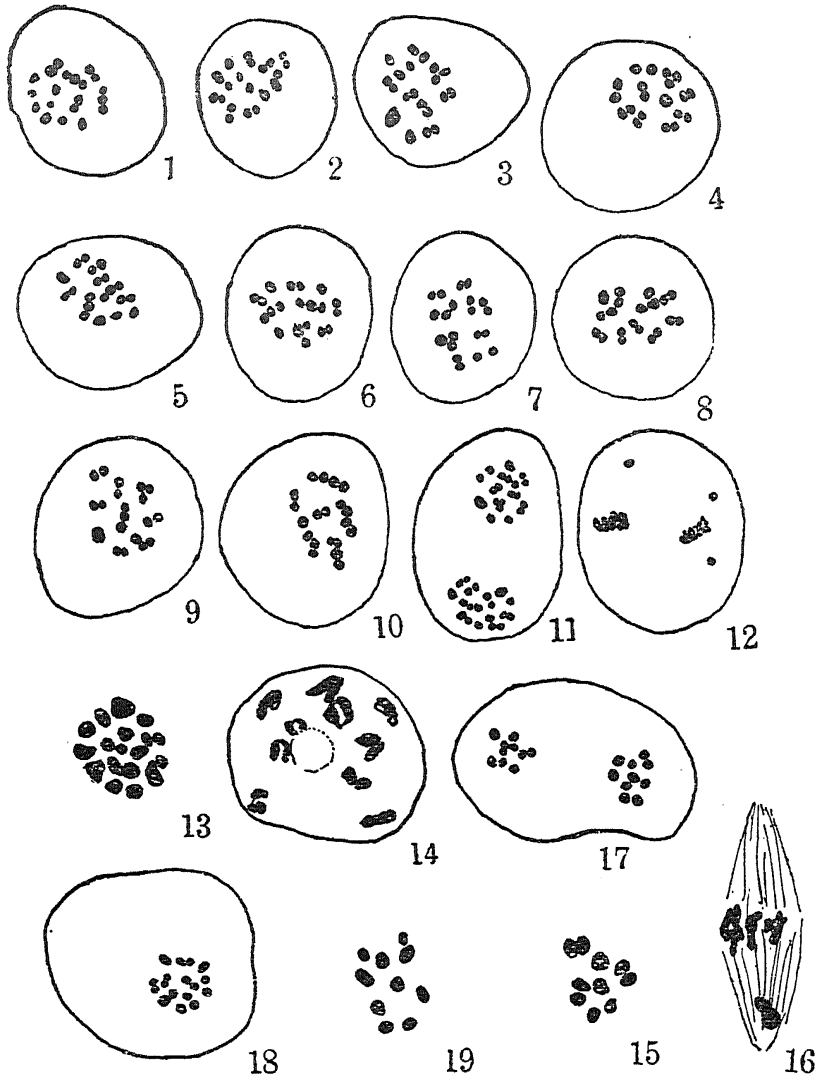
No. of associations	No. of bivalents in association				No. of cases	Total
	1	2	3	4		
7	8	3	2	..	1	2
	7	5	1	..	1	
8	4	8	..	..	1	8
	6	5	2	..	2	
	5	6	1	..	4	
	7	2	3	..	1	
9	3	7	1	..	2	6
	4	5	2	..	2	
	5	3	3	..	1	
	2	9	..	..	1	
10	2	6	2	..	4	8
	3	5	1	1	2	
	4	3	2	1	1	
	3	4	3	..	1	
11	1	5	3	..	4	6
	2	5	..	2	1	
	2	4	2	1	1	
13	1	2	1	3	1	1
					TOTAL ..	31

The maximum association is 3 (4), 1 (3), 2 (2), and 1 (1); and this was met with only once (Fig. 10 and Pl. IX, Fig. 1).

Secondary pairing is solely dependent on the diakinesis position of the bivalents relative to one another, and therefore all bivalents that lie adjacent at diakinesis and which are capable of secondary pairing are so paired at M I. As already indicated at diakinesis itself the bivalents could be seen to be closely approximated and it is these that constitute the secondary groupings at M I.

An interesting feature of this genus not recorded either in *Crataeva* or *Gynandropsis* is that associations of four are very common. Another feature is the association between bivalents of dissimilar sizes (Figs. 6, 10, and Pl. IX, Fig. 1). This would indicate an affinity between non-homologous chromosomes which have some segments in common.

First division is not followed by wall formation. There is an interkinesis when the nuclear membrane is quite in evidence and the chromosomes are distributed more or less peripherally. Interkinesis is of very short



duration and is immediately followed by Second Metaphase when the chromosomes lose their peripheral distribution and arrange themselves as a flat plate. The nuclear membrane has disappeared by this time. Secondary association between chromosomes still persists. Figs. 11 and Pl. IX, Fig. 4, represent the chromosome groupings. The same considerations that apply for the First Metaphase approximation of bivalents would appear to govern the groupings of M II chromosomes also. Enough M II plates were not available to make a comparative study of the groupings at this stage with those of M I. But Catcheside (1937) has made such a statistical study and found that M II plates show various degrees of secondary association covering the same range of types as at M I and in similar proportion.

During Second Metaphase there is not infrequently seen extrusion of variable number of chromosomes into the cytoplasm. Fig. 12 shows three having been extruded. Extrusion of four such bodies is also frequently seen. Presumably these will not be included in the tetrads to be formed and

therefore they be will deficient. The pollen grains, some proportion of them would degenerate. Though division is on the whole normal, tetrads were seen to degenerate in a number of cases. Obviously these degenerations are a result of the deletions referred to.

In a plant growing in a locality close by, degenerations are extensive. There is practically no seed formation and when we investigated the plant it was found that pollen formation was very scarce. Degenerations set in during all stages of pollen development. Even as early as the differentiation of the microsporogenous cells whole anther loculi were seen to degenerate *en masse*. It would therefore appear that in this species there is a tendency towards extensive degeneration.

(*b*) *Cadaba indica* Lamk., *Mærua arenaria* Hook. f. and Thomp., *etc.*— In addition to *Capparis Zeylanica* dealt with in this paper, chromosome numbers of two important genera have been determined for the first time. Fig. 13 shows the p.m.c. of *Cadaba indica* in M I. The haploid number is 18. An interesting feature in this is the varying size of the bivalents indicating that in the somatic complements there should be chromosomes of different sizes.

In *Mærua arenaria* the other genus, the haploid number is 10. Fig. 14 shows a p.m.c. in diakinesis. The 10 bivalents are distributed peripherally. Most of these are of the rodtype. Fig. 15 is an M I plate. Disjunction is normal. Occasionally cases of non-disjunction occur. In Fig. 16 a bivalent is seen to reach the pole earlier without having undergone separation. At M II the 10/10 distribution is almost the rule (Fig. 17) except in rare cases where slight variations are met with obviously due to the non-disjunction mentioned above.

In a previous paper (Raghavan, 1937) the chromosome number of *Cleome Chelidonii*, based upon aceto-carmine smears, was tentatively given as ten. It was not possible then to confirm it by further fixation. Now extensive fixation of the anthers of this species was rendered possible on account of a plentiful availability of material and it is seen that the haploid number is 17 (Fig. 18). It is quite likely that in the previously examined aceto-carmine preparation, the full number could not be counted obviously because of secondary association of bivalents, a phenomenon which has been found to be very widely prevalent in almost all the members of the Capparidaceæ examined so far. It is of interest also to record here that in the closely related *Gynandropsis pentaphylla*, where also the haploid chromosome number is 17, the frequency of occurrence of secondary paired bivalents in groups of 10, was found to be almost at the region of the mode. It is not therefore

unlikely that the few plates examined in aceto-carmine showed the modal groupings and the approximation is so close that groups can easily be mistaken for individual bivalents.

The haploid number of *Cleome viscosa* Linn. was confirmed to be 10 (Fig. 19).

It is interesting that though *Gynandropsis pentaphylla* and *Cleome viscosa* are so strikingly similar morphologically, their chromosome number is so different; whereas *Cleome Chelidonii* whose resemblance to *Gynandropsis* is very much less, shows the same chromosome number. Similarly, *Mærua* a member of the Capparidioideæ and *Cleome viscosa* of the Cleomoideæ exhibit the same chromosome number. This and other irregularities would make it very difficult to accept the primitivity of the arboreal Capparidioideæ. At any rate the evolution of these genera seems to have followed irregular lines.

III. Discussion

(*a*) *Capparis Zeylanica—a secondary polyploid.*—On the basis of maximum association the gametic constitution of the species may be represented by

aaaa
bbbb
cccc
ddd
ee
ff
g

It has already been indicated in the previous papers that seven is likely to be the primary basic number of the family and this would appear to be corroborated by the observations recorded herein. A natural cross between two seven chromosomed parents (one of which had presumably its chromosomes changed structurally by gene mutation, etc.), would lead to the ultimate formation of a tetraploid by amphidiploidy with the somatic constitution a a a′a′–g g g′g′, on the basis that the parental genomes that entered into the cross were represented by a–g × a′–g′. If the chromosomes a′–f′ undergo reduplication then we get a form with $2n = 40$. The same result could also be obtained if the original seven-chromosomed ancestor (a–g) had been fertilized by the diploid gamete of the a′–g′ sister plant and if this were followed by syndiploidy it is likely that a forty-chromosomed plant might have survived by the deletion of a pair of chromosomes through

otic or mitotic aberration. On this assumption the gametic genom of resulting forty-chromosomed species may be represented as:

a a′a′
b b′b′
c c′c′
d d′d′
e e′e′
f f′f′
g′g′ or gg′

evidenced by secondary pairing which is a strong indication of the estral homology between chromosomes a and a′, b and b′, etc., we should ect a maximum association of seven consisting of six threes and one two 3) and 1 (2)]. But such an association is not to be found and groups our are very common. This makes one infer that structural changes have yed a part in the evolution of the species in addition to polyploidy; this be explained by structural changes chiefly in the nature of reciprocal nslocation as having taken place between different chromosomes. The ected somatic constitution may be represented as:

a aa′a′a′a′
b bb′b′b′b′
c cc′ c′c′c′
d dd′d′d′d′
e ee′ e′e′e′
f ff′ f′f′f′
g′g′g′g′ or gg g′g′

egmental interchange takes place between a and e, b and f, and g and c omosomes, then the result will be:

a (ae) a′a′ a′a′
b (bf) b′b′ b′b′
c (cg) c′c′ c′c′
d d d′d′ d′d′
e (ea) e′e′ e′e′
f (fb) f′f′ f′f′
g (gc) g′g′

account of the new structural homology thus introduced, naturally bi- ents a (ae) and e (ea) will be secondarily associated. Similarly b (bf) d f (fb) and so on. The result of this would be three groups of four, e group of three and two groups of two and a single bivalent unassociated. is means that the affinity between a (ae) and a′a′ bivalents has not been

impaired by this structural change. The frequent association of bivalents of dissimilar size is a clear indication of an affinity between non-homologous chromosomes which have some segments in common.

As an alternative the following method of origin of the forty-chromosomed species may be considered:

Eight of the chromosomes of the tetraploid may be lost through deletion, say d′–g′. Supposing the constitution of this 20-type be X, gene mutation or structural change may modify this type to produce a species of the constitution X′. Amphidiploidy is likely to occur when X and X′ are crossed, so that a new type with 40 chromosomes arises. But here the maximum association should be 3 (4) and 4 (2), assuming as we did the deletion of the d′–g′ chromosomes. But instead we get 3 (4), 1 (3), 2 (2) and 1 (1). This can only be explained by reciprocal translocation as having taken place. Supposing the parental gametic genomes were of the following constitution:

X′		X	
—		—	
A_2A_2 A_3A_3		AA A_1A_1	
B_2B_2 B_3B_3		BB B_1B_1	
C_2C_2 C_3C_3		CC C_1C_1	
D_3D_3	X	D_1D_1	(D–G chromo-
(D_2–G_2 deleted) E_2E_3		E_1E_1	somes deleted)
F_3F_3		F_1F_1	
G_3G_3		G_1G_1	

A cross between these would result in a species having the following constitution:

AA A_1A_1	A_2A_2 A_2A_3
BB B_1B_1	B_2B_2 B_3B_3
CC C_1C_1	C_2C_2 C_3C_3
	D_1D_1 D_3D_3
	E_1E_1 E_3E_3
	F_1F_1 F_3F_3
	G_1G_1 G_3G_2

On this basis one would expect a maximum association of 3 (4) and 4 (2). If however reciprocal translocation takes place between D_1 and E_1 chromosomes, then we get, D_1 (D_1E_1) and E_1 (E_1D_1) and on the basis of homology between D_1 and D_3 chromosomes which we have assumed, we may get a group of three: D_3D_3, D_1 (D_1E_1), E_1 (E_1D_1). E_3E_3 would be left alone. F_1 and F_3, G_1 and G_3 will form a group of two.

We find therefore that on either of these assumptions, structural changes, chiefly reciprocal translocation, would appear to have played a prominent part in the evolution of the species. In the former almost all the chromosomes were involved except the D chromosomes. In the latter, only a few chromosomes, D_1 and E_1 chromosomes would appear to have been affected structurally. Since, however, it is very common we find in groups of four, association of bivalents of dissimilar size, it is likely that it is these b.f. chromosomes that have undergone structural changes and as such the first assumption is more tenable.

(*b*) *Secondary Association—its limitations as a guide to polyploidy.*—Evidence from secondary association alone cannot be regarded as conclusive in respect of ancestral homology and consequently of the basic number. One of the most common factors which would make secondary pairing unreliable, unaided by other evidence, is that structural changes of chromosomes may hamper this phenomenon. Structural changes of the homologous chromosomes may have taken place to a great extent in polyploids and as a consequence the degree of affinity required to cause attraction may not be present. Or translocations, simple or reciprocal, which are very common factors in the evolution of new species may give rise to higher associations, so that the basic number inferred from observed secondary pairing may be erroneous. Numerical differences cause changes in frequency; high numbers of chromosomes tend to reduce the chance of association between similar chromosomes. Large size of the chromosomes appears to inhibit secondary pairing since it is seen generally only in organisms with small chromosomes.

In the present paper we have explained the maximum association on the basis of segmental interchange. Unaided by any previous knowledge it may not be proper to conclude that the maximum grouping represented the basic number. But since chromosome behaviour in two other genera, representative of the two sub-families, has already been studied and ample evidence let in to show that seven was likely to be the primary basic number of the family, the maximum association seen in this important genus has been interpreted in the way it has been done. The noteworthy fact is that reciprocal translocation and other structural changes which have undoubtedly played an important rôle in the evolution of the species have taken place in such a manner as to keep up the original number, though the groupings have undergone corresponding changes.

If chromosome interchange had taken place, then the absence of ring formation is rather hard to explain. But since the chromosomes are very

small segmental homology manifests itself in an association of dissimilar chromosomes rather than in actual ring formation. Moreover it is not absolutely necessary that reciprocal translocation should be always followed by ring formation. A few cases have been reported where this has failed to occur. For instance, Clarke and Anderson (1935) have shown that in Maize chromosome interchange takes place without the external evidence of ring formation.

Heilborn (1936) considers that no credence should be placed on secondary association as indicative of ancestral homology and that it is a purely physical phenomenon, that chromosomes of equal size are associated or tend to be associated irrespective of their homology. According to him it is not a specific attraction or pairing between homologous parts of chromosomes but the parallelism of the associated chromosomes is mechanically induced through the polarity of the nuclei. Flowik (1938) has shown how this assumption is untenable so far as the genus *Carex* is concerned. In this paper additional evidence is to be found for not accepting the hypothesis. There is clear evidence of bivalents of different sizes associating. Primarily a result of ancestral homology, structural changes have also played a prominent part in this. There is also evidence of pairing of chromosomes of dissimilar size as could be seen from side views of metaphases. Observations of a similar nature have been made in other genera also. For instance, in *Cicer*, Iyengar (1939) has recorded the association between a short rod bivalent and a long one. Richharia (1937) has made a similar observation in the genus *Brassica*.

(*c*) *Phylogenetic Considerations.*—The chromosome numbers of the genera and the species so far investigated in the Capparidaceæ form an irregular series. Polyploidy, structural changes, meiotic and mitotic aberrations have undoubtedly played an important part in the evolution of the species. The numbers known so far are so few that generalizations at this stage may not be quite warranted. A few remarks can, however, be made regarding the distribution of the chromosome numbers in the various genera. The Capparidaceæ are almost entirely tropical, a few sub-tropical and rarely temperate in their distribution. From the chromosome list now available one finds that generally speaking, the sub-tropical and temperate genera have greater chromosome numbers than the tropical ones. This is in keeping with observations made previously in a few families. For instance, in the Cactaceæ, Stockwell (1935) found that the *Opuntias* which were the most northern of the *Cacti* had higher chromosome numbers than the rest. Even among the same genus the more northern species had a greater chromosome number than the southern ones. Similarly Hagerup (1928) observes "it is worth noting

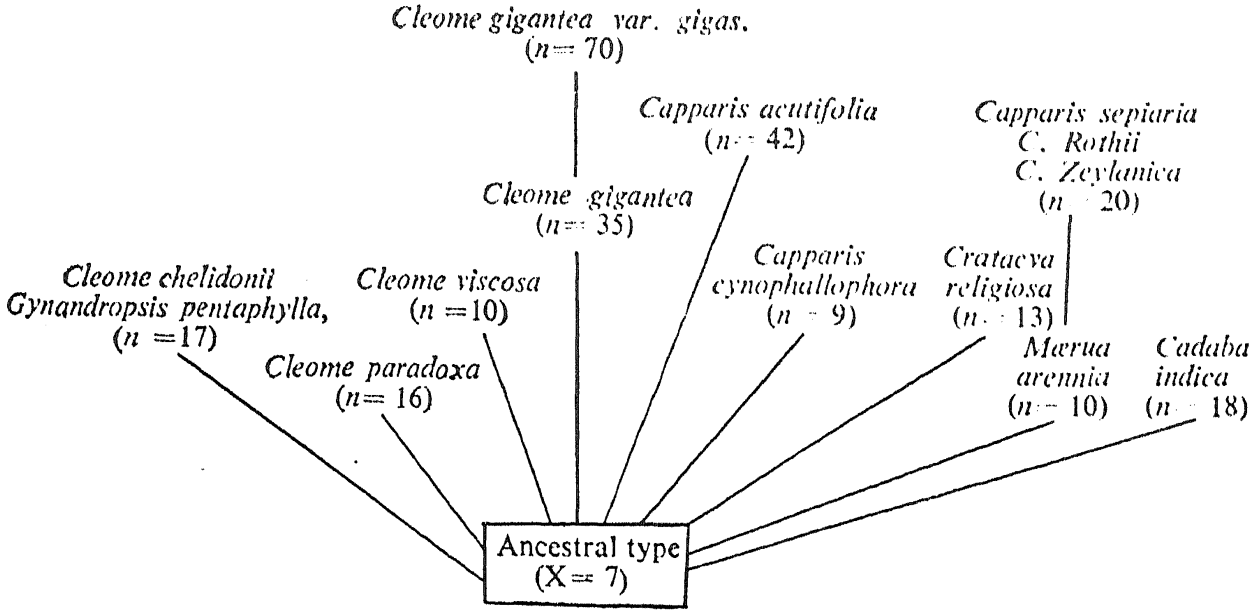
that among these four pairs of species (including *Empetrum nigrum* and *E. hermaphroditum*) those with the higher chromosome numbers are always the ones growing farther north ".

It is also likely that the original home of the Capparidaceæ was the Tropics. There would appear to be a correlation between distribution and polyploidy and it is quite conceivable that some of the sub-tropical species like *Cleome gigantea*, *C. g. var. gigas*, *Cleome spinosa*, etc., have essentially by polyploidy conquered new territories. Nawaschin (1929) says, " through changes in the rate of development a polyploid individual may acquire the ability of withstanding different climatic conditions and as a consequence penetrate into new territory." Hagerup (1933) also says, "polyploid forms may be ecologically changed so as to grow in other climates and formations where the diploid forms will not thrive ". Clearly the species mentioned above are polyploids.

From purely cytological evidence—and even that is very meagre in this family—it is difficult to assemble the genera phylogenetically, not only because chromosome bumbers are not absolutely diagnostic but also the numbers known so far are very irregular. But a few general remarks may not be out of place nonetheless. There is to be seen, though not very apparent, an increase in chromosome numbers as one passes from the taxonomically more primitive to the more advanced Capparidaceæ. Whether this is accompanied by any marked decrease in chromosome size, as it usually is the case, cannot be said. The chromosome numbers known up-to-date, more or less confirms the taxonomic evidence that the genus *Corparis* is comparatively primitive. It must be said that it is difficult to determine the exact relations of the more primitive genera with one another. The cytological data available may, however, be utilized for indicating the broad sectional relations rather than for the alignment of species.

It has already been suggested that seven is likely to be the primary basic number of the family and from this a number of secondary basic numbers have arisen and the various genera represent different balances of these numbers. In this allopolyploidy as indicated by secondary association has played a prominent part. A species of *Capparis* shows the lowest chromosome number known in the family ($2n = 18$) and conjoint with evidence available on morphological and taxonomical grounds, *Capparis* as representative of the sub-family Capparidioideæ is to be regarded as more primitive. We find, however, that almost the same secondary basic numbers are to be found in the two sub-families, Cleomoideæ and the Capparidioideæ and as such the evolution of the genera in the two tribes may be regarded

as representing parallelism at least so far as the chromosome numbers are concerned. This may be represented diagrammatically in a rough manner as follows:



IV. Summary

The haploid chromosome numbers of the following have been determined for the first time;—*Capparis Zeylanica* Linn. = Twenty (20); *Cadaba indica* Lamk. = Eighteen (18); *Mærua arenaria* Hook. f. and T. = ten (10). The chromosome number of *Cleome viscosa* Linn. is confirmed to be ten (10) and that of *Cleome Chelidonii* Linn. is seventeen (17) and not ten as previously reported.

Secondary association is reported in *Capparis Zeylanica* Linn. and the previous finding that the primary basic number of the family, 7, is supported by observations made herein.

Evidence is found to show that polyploidy as well as structural changes of chromosomes have played an important part in the evolution of the species. The association of 4 bivalents is interpreted on this basis.

Secondary association and its limitations as the sole factor in determining ancestral homology are discussed in the light of the present findings and of the data gathered previously on other genera of this family.

Some general remarks are made on the distribution of chromosome numbers in this family and a tentative scheme formulated to indicate phylogenetic evolution of some of the important genera.

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LEGEND TO TEXT-FIGS. 1–19

(All figures have been drawn at a magnification of ca 3,300.) Figs. 1–10. First Metaphase plates of *Capparis Zeylanica* Linn., showing varying degrees of secondary association.

FIG. 1	shows an association of	2 (3) 3 (2) 8 (1)
FIG. 2	,, ,,	8 (2) 4 (1)
FIG. 3	,, ,,	1 (3) 6 (2) 5 (1)
FIG. 4	,, ,,	1 (3) 7 (2) 3 (1)
FIG. 5	,, ,,	2 (3) 5 (2) 4 (1)
FIG. 6	,, ,,	3 (3) 4 (2) 3 (1)
FIG. 7	,, ,,	2 (3) 6 (2) 2 (1)
FIG. 8	,, ,,	2 (4) 5 (2) 2 (1)
FIG. 9	,, ,,	3 (3) 5 (2) 1 (1)
FIG. 10	,, ,,	3 (4) 1 (3) 2 (2) 1 (1) Maximum association.

FIG. 11.—M II showing the 20/20 distribution; note the persistence of secondary association

FIG. 12.—M II showing the extrusion of three chromosomes.

FIG. 13.—First Metaphase plate of *Cadaba indica* Lamk., showing 18 bivalents.

FIG. 14.—P. M. C. of *Mærua arenaria* Hook. f. and T. in diakinesis.

FIG. 15. ,, ,, ,, in first metaphase, 10 bivalents.

FIG. 16. ,, ,, ,, in first metaphase, note one of the bivalents reaching the pole earlier.

FIG. 17. ,, ,, ,, M II Note the 10/10 distribution.

FIG. 18.—P. M. C. of *Cleome Chelidonii* Linn. f., in first metaphase showing 17 bivalents.

FIG. 19. ,, *Cleome viscosa* Linn., in M I showing 10 bivalents.

EXPLANATION OF PLATE IX

FIG. 1.—Microphotograph of P.M.C. of *Capparis Zeylanica* Linn., showing maximum association. Same as Text-Fig. 10.

FIG. 2.—Microphotograph of P.M.C. of *Capparis Zeylanica* Linn., showing the same association as Text-Fig. 9.

FIG. 3.—Microphotograph of P.M.C. of *Capparis Zeylanica* Linn., showing an association of 3 (3) 5 (2) 1 (1).

FIG. 4.—Microphotograph of second metaphase showing secondary association persisting.

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