# CYTOTAXONOMIC INVESTIGATION WITH THE AID OF AN IMPROVED METHOD ON THE FAMILY VERBENACEAE WITH SPECIAL REFERENCE TO THE LINES OF EVOLUTION

By ARUN KUMAR SHARMA and SATYABRATA MUKHOPADHYAY
*Cytogenetics Laboratory, Botany Department, Calcutta University, Calcutta, India.*

## Introduction

The family Verbenaceae, a constituent of the bicarpellate complex, is characterised by a large number of genera occurring mainly in the tropics. In the plains of Bengal alone, fortyeight species occur as wild, and a large number of them are cultivated as well. Taxonomic interest is provided by the family, its variety of forms ranging from herbaceous to woody. One of the most commonly occurring shrubby forms is exhibited by the species of *Clerodendron*. Further, in *C. infortunatum* Gaertn., the most frequent species of this genus, phenotypic differences between individuals are commonly seen. Cytological reports in this genus show a wide range of chromosome numbers from 24 to 184. The possibility of existence of individuals with different chromosome numbers in the same species has been indicated by the discrepant reports of Nishiyama and Kondo (1942) and Bowden (1945), one reporting fortysix and the other fortyeight chromosomes in the somatic cells respectively. In India a large number of species of this genus still remains unexplored and no detailed cytological work has yet been done on *C. infortunatum* with its wide range of phenotypes.

In addition to *Clerodendron*, cytological interest is provided by other genera in the fact that a wide range of chromosome numbers has been found in different genera of this family, starting from $n=5$ in *Verbena* to even $n=23$ in *Clerodendron*. Leaving aside the occurrence of polyploidy in the same species (Sugiura, 1936b) aneuploid variations at an intraspecific level are recorded in *Callicarpa japonica* by Sugiura (1936b) and Patermann (1938) and *Lantana camara* (Schnack, 1947; Tjio, 1948; Singh, 1951) too. Interspecific aneuploidy is also common in the genera *Lippia* and *Lantana* (Sharma and Mukherjee, unpublished; Natarajan and Ahuja, 1957; etc.). All these facts taken together suggest that the family provides ideal material for cytologists who are interested in working out the basis of origin of different chromosome numbers, and interrelationships and affinities between genera and species as far as can be interpreted from cytological data. With these ends in view the present investigation was undertaken on thirtyfive different species and varieties with special reference to the genus *Clerodendron*.

## Materials & Methods

The present work includes twentynine species belonging to thirteen genera and covering four tribes of Verbenaceae. One species, *Clerodendron infortunatum*, shows the presence of different horticultural types which can be distinguished from each other by the pigmentation in flowers and flower stalks. Six types have been included in the present study. A list of the names, identified and verified from the Indian Botanical Gardens at Sibpur, is given below:

1. *Clerodendron thomsonae* Balf. var. I.
2. *C. thomsonae* Balf. var. II.
3. *C. macrosiphon* Hook.
4. *C. squamatum* Vahl.
5. *C. inerme* Garten, Fruct.
6. *C. nutans* Wall.
7. *C. siphonanthus* Ait.
8. *C. fragrans* Vent.
9. *C. splendens* G. Don.
10. *C. minahassae* Teijsm & Binn.
11. *C. ugandense* Prain.
12. *C. infortunatum* Gaertn. Type I.
13. *C. infortunatum* Gaertn. Type II.
14. *C. infortunatum* Gaertn. Type III.
15. *C. infortunatum* Gaertn. Type IV.
16. *C. infortunatum* Gaertn. Type V.
17. *C. infortunatum* Gaertn. Type VI.
18. *Vitex negundo* Linn.
19. *V. agnus-castus* Linn.
20. *V. trifolia* Linn.
21. *Tectona grandis* Linn.
22. *Stachytarpheta indica* Vahl.
23. *Duranta plumieri* Jacqu. (blue flower).
24. *D. plumieri* Jacqu. var. alba.
25. *D. macrophylla* (Horticultural variety).
26. *Petrea volubilis* Linn.
27. *Gmelina hystrix* Schult.
28. *Holmskioldia sanguinea* Retz.
29. *Caryopteris mastacanthus* Schau. (blue).
30. *Caryopteris mastacanthus* Schau. (pink).
31. *Callicarpa macrophylla* Vahl.
32. *Congea tomentosa* Roxb.
33. *Verbena erinoides* Lam. (mauve).
34. *V. aubletia* Jacq. (red).
35. *Nyctanthes arbortristis* Linn.

A few of the species were collected from the Calcutta University Botanical Gardens and a few others, specially types of *Clerodendron infortunatum*, *Duranta plumieri* (blue flowers), *Vitex negundo*, etc. were collected from the suburbs of Calcutta. The horticultural varieties were mainly collected from the Imperial Nursery, Calcutta, and Royal Agri-Horticultural Gardens in Calcutta and the Indian Botanical Gardens at Sibpur. Plants were grown in earthenware pots containing a suitable mixture of sand and soil. Very healthy roots were obtained mainly in the rainy season, but the period extends up to the end of October. In most cases, the root tips were collected at noon.

For temporary squash preparations, a number of pretreatment chemicals were tried with different concentrations and of varying periods of treatment. Of these, p-dichlorobenzene of different concentrations with varying periods has yielded good results in a majority of cases. In a few species a good result was obtained when p-dichlorobenzene was mixed with a ·002 M solution of oxyquinoline. A mixture of p-dichlorobenzene and aesculine (1: 1) also yielded very well scattered plates in some species.

After pretreatment the root tips were hydrolysed and stained in orcein-acid mixture (2% aceto-orcein: (N)HCl : : 9 : 1) by heating over a flame for a few seconds. They were then squashed in 1% aceto-orcein solution.

Permanent preparations were made from this temporary squash preparation by inverting the slides in normal butyl alcohol, when the squashed material became dried. When a coverslip became detached both the slide and the coverslip were mounted in euparal.

For meiotic study temporary squash preparations were made following Belling's 1% aceto-carmine squash technique.

The figures were drawn at a table magnification of approximately × 2400 using a Leitz microscope with compensating eyepiece of × 20, an apochromat objective of 1·3 and an aplanatic condenser of 1·4 N.A.

On the drawings, the chromosomes with secondary constrictions or satellites are represented by outlines only.

## Observations

Chromosome numbers so far recorded in the family show wide variation. It is as low as ten in the species of *Verbena* and may be as high as one hundred and eightyfour in *Clerodendron ugandense*. In *Clerodendron* an interesting series of $2n$ numbers ranging from 30, 46, 52 to 184 has been found, and variation nuclei have also been noted in a few cases. The majority of the genera studied show $2n=34$.

In species where meiosis has not been studied, the number which occurs in the highest frequency has been taken as the normal number of the species.

In general chromosomes are short sized. Comparatively long chromosomes have been noted in *Verbena* and the shortest ones in *Clerodendron nutans*. Complements with medium sized chromosomes have been found in most cases.

### Genus—*Clerodendron*

This genus is placed under the tribe Viticeae. Eleven species and the six types of *Clerodendron infortunatum* have been worked out. In *Clerodendron macrosiphon* $2n=30$, in *C. thomsonae* *C* *inerme* and in *C. splendens*, $2n=46$, in one species of *Clerodendron*, *C.*

*thomsonae* var. II $2n=48$, and in the rest of the species $2n=52$ have been observed. Somatic nuclei with variation numbers have been noted in *C. thomsonae* var. II, *C. siphonanthus*, *C. fragrans*, and in *C. minahassae*. The highest chromosome number of 186 has been recorded in *C. ugandense*, and ten chromosomes, the highest number, have been found with secondary constrictions in *C. siphonanthus*. A thorough study of the karyotypes reveals that a number of chromosomes in different species shows a gross similarity among them. In spite of this fact, further study in detail reveals the difference between the species which will be dealt with separately later. Ten types of chromosomes have been selected out of the different somatic complements studied in the species. A general description of the types, based on their morphological characters, the sizes and positions of the primary and secondary constrictions, is given below.

Type *A*—Comparatively long chromosome with two constrictions, primary and secondary, one is nearly median in position and the other is located in the submedian to nearly subterminal position at the end of one arm.

Type *A′*—Comparatively long chromosome, but shorter than A with a nearly median primary constriction and a satellite at the distal end of one arm.

Type *B*—Comparatively long chromosome with nearly submedian to submedian primary constriction.

Type *C*—Comparatively long chromosome with two constrictions, primary and secondary, both in the nearly submedian position. The three segments are nearly equal in length.

Type *D*—Comparatively long chromosome, with two constrictions, primary and secondary, both in the submedian position at opposite ends. The median segment is considerably longer than the two end segments.

Type *D′*—Comparatively long chromosome with primary and secondary constrictions both located in the nearly subterminal positions. This type is shorter than D type.

Type *E*—Comparatively long chromosome with median to nearly median primary constriction.

Type *F*—Medium sized to nearly short chromosome with median to nearly submedian primary constriction.

Type *F′*—Nearly short to medium sized chromosome with submedian to nearly subterminal primary constriction.

Type *G*—Short chromosome with median to nearly median primary constriction.

1. *Clerodendron thomsonae* Balf. var I. ($2n=46=A_2+C_2+E_2+F_{34}+G_6$)

The somatic complement contains fortysix chromosomes. Chromosomes form a graded series according to which size varies from 0·9 $\mu$ to 2·5 $\mu$. Four chromosomes in the complement bear secondary constrictions.

The somatic complement has been seen to consist of one pair of *A*, one pair of *C*, one pair of *E*, seventeen pairs of *F* and three pairs of *G* type chromosomes. Six pairs of *F* type bear nearly submedian constriction and the rest bear median constrictions. One pair of *F* type is formed of nearly short chromosomes (Figs. 1 and 1a).

Twentythree clear bivalents have been observed in Metaphase I.

2. *C. thomsonae* Balf. var. II. ($2n=48=A_2+B_2+E_6+F_{26}+G_{12}$)

Fortyeight chromosomes have been found to occur in the normal somatic complement. Size ranges from 0·9 $\mu$ to 3·2 $\mu$. Chromosomes when arranged in order of size form a graded series. Only two chromosomes in the complement bear secondary constrictions.

The somatic complement is composed of one pair of *A*, one pair of *B*, three pairs of *E*, thirteen pairs of *F* and six pairs of *G* chromosomes. One pair of *E* type is longer than other pairs. Two pairs of *F* are with nearly submedian constrictions and longer than others (Figs. 2 and 2a). In addition to the normal complement somatic nuclei with variation numbers 42, 50, 46 (Figs. 3, 4 and 5) have been worked out.

Meiotic study reveals twentyfour clear chromosomes in the two poles of Metaphase II.

3. *C. macrosiphon* Hook. ($2n=30=A_4+B_2+F_{24}$)

In the normal somatic complement thirty chromosomes occur. Chromosomes differ in size and the size varies from 1·5 $\mu$ to 2·5 $\mu$. When arranged according to size a graded series is formed. Four chromosomes in the complement have been found to bear secondary constrictions.

The complement on analysis shows the presence of two pairs of *A*, one pair of *B* and twelve pairs of *F*. Four pairs of *F* bear nearly submedian constrictions, three pairs of which are longer than the fourth one. Two pairs of the rest of the *F* types are shorter than others and all bear median constrictions (Figs. 6 and 6a).

Fifteen clear bivalents have been found in Metaphase I and fifteen chromosomes are seen in the two poles at Metaphase II.

4. *C. squamatum* Vahl. ($2n=52=C_2+E_8+F_{34}+G_8$)

Fiftytwo chromosomes form the somatic complement of the species. They are, on an average, very small. Size difference amongst them is found but is not marked, and size varies from 0·9 $\mu$ to 1·6 $\mu$. One pair of chromosomes has been found to bear secondary constrictions.

Somatic complement consists of a pair of *C*, four pairs of *E*, seventeen pairs of *F* and four pairs of *G* type chromosomes (Figs. 7 and 7a). Six pairs of *F* type are nearly short in size. In addition to the normal complement a somatic nucleus with a variation number of fortyeight has been worked out (Fig. 8).

In the meiotic study, twentysix clear bivalents have been observed in Metaphase I.

5. *C. inerme* Garten, Fruct. ($2n=46=A_2+B_6+C_2+E_6+F_{24}+G_6$)

Fortysix chromosomes are present in the normal complement. Chromosome size varies from 1·4 μ to 2·3 μ. Four chromosomes possess secondary constrictions in the complement.

Karyotype analysis reveals the presence of one pair of $A$, three pairs of $B$, one pair of $C$, three pairs of $E$, twelve pairs of $F$ and three pairs of $G$ type chromosomes. Four pairs of $F$ type are longer than the other pairs and bear nearly submedian constrictions (Figs. 9 and 9a).

6. *C. nutans* Wall. ($2n=52=A'_6+E_2+F_{32}+G_{12}$)

The normal somatic complement contains fiftytwo chromosomes. Chromosomes form a graded series and size varies from 1·1 μ to 2·1 μ. Three pairs of chromosomes in the complement contain secondary constrictions.

A critical study reveals that the somatic complement is formed of three pairs of $A'$, one pair of $E$, sixteen pairs of $F$ and six pairs of $G$ type. Two pairs of $F$ type are longer than other pairs and the rest form a series of nearly short chromosomes (Figs. 10 and 10a).

7. *C. siphonanthus* Ait. ($2n=52=A_2+A'_6+D'_2+F_{26}+F'_4+G_{12}$)

Fiftytwo chromosomes form the normal somatic complement of the species. Chromosomes are on an average rather small and the size varies from 1·2 μ to 2·0 μ. Ten chromosomes in the complement possess secondary constrictions.

The following types are present in the chromosome set of the normal complement: one pair of $A$, three pairs of $A'$, one pair of $D'$, thirteen pairs of $F$, two pairs of $F'$ and six pairs of $G$. One pair of $F$ is shorter than the other (Figs. 11 and 11a). Besides the normal set, a variation number of 46 has also been recorded (Fig. 12).

Twentysix clear chromosomes have been observed in each pole of Metaphase II.

8. *C. fragrans* Vent. ($2n=52=A_2+A'_2+B_4+C_4+E_8+F_{26}+F'_6$)

The normal somatic complement of the species contains fiftytwo chromosomes, which in order of size, form a graded series. The size ranges from 1·2 μ to 2·5 μ. Eight chromosomes in the complement have been found to bear secondary constrictions.

A detailed analysis of the karyotype shows one pair of $A$, one pair of $A'$, two pairs of $B$, two pairs of $C$, four pairs of $E$, thirteen pairs of $F$ and three pairs of $F'$ chromosomes. Two pairs of $F$ are slightly shorter than the rest (Figs. 13 and 13a).

In addition to the normal complement, somatic nuclei with variation numbers, 30, 19 and 46 have been recorded (Figs. 14, 15 and 16).

9. *C. splendens* G. Don. ($2n=46=A_4+B_2+C_2+D_2+E_6+F_{16}+G_{14}$)

Fortysix chromosomes occur in the somatic complement of the species. Size varies from 1·2 μ to 2·8 μ. Chromosomes are of graded nature. Eight chromosomes in the complement with secondary constrictions have been worked out.

The complement is composed of two pairs of $A$, one pair of $B$, one pair of $C$, one pair of $D$, three pairs of $E$, eight pairs of $F$ and seven pairs of $G$ type. One pair of $A$ is shorter than the other and the $D$ type here is considerably longer than the $A$, $B$ and

*C* pairs, being the longest pair in the set. Three pairs of *F* type are slightly shorter than the other pairs (Figs. 17 and 17a).

Meiotic study reveals twentythree clear bivalents in metaphase I and twentythree chromosomes in each pole of metaphase II.

10. *C. minahassae* Teijsm & Binn. ($2n=52=A'_6+E_2+F_{22}+F'_{10}+G_{12}$)

The normal somatic complement contains fiftytwo chromosomes. On an average, chromosomes are small. Size difference among them is present but not marked, ranging from 1·2 μ to 1·7 μ. Chromosomes are graded. Three pairs of chromosomes bear secondary constrictions.

A critical study of karyotype reveals the presence of three pairs of *A'*, one pair of *E*, eleven pairs of *F*, five pairs of *F'*, and six pairs of *G* type. Two pairs of *F* are longer than the other pairs and have nearly median constrictions (Figs. 18 and 18a). In addition, somatic nuclei with fortysix chromosomes have also been worked out (Fig. 19).

11. *C. ugandense* Prain ($2n=184$)

Somatic complement in this species contains one hundred and eightyfour chromosomes (Fig. 20). Besides this number, somatic nuclei with one hundred and fiftyfour chromosomes (Fig. 21), fortythree chromosomes (Fig. 22) and one hundred and ninetyfive chromosomes have been also recorded (Fig. 23).

12. *C. infortunatum* Gaertn. Type I. ($2n=52=A'_4+B_4+F_{34}+F'_2+G_8$)

Fiftytwo chromosomes are present in the normal complement. Size varies from 1·1 μ to 1·6 μ. Four chromosomes contain secondary constrictions.

The normal complement is composed of two pairs of *A'*, two pairs of *B*, seventeen pairs of *F*, one pair of *F'* and four pairs of *G* (Figs. 24 and 24a).

Twentysix clear chromosomes have been observed in the two poles of Metaphase II.

13. *C. infortunatum* Gaertn. Type II. ($2n=52=A_2+E_2+F_{40}+G_8$)

The somatic complement is composed of fiftytwo chromosomes. Chromosome length varies from 1·1 μ to 1·9 μ. One pair of chromosomes bears secondary constrictions.

In the complement, there occur one pair of *A*, one pair of *E*, twenty pairs of *F* and four pairs of *G* type chromosomes (Figs. 25 and 25a). Twentysix clear bivalents have been noted in Metaphase I.

14. *C. infortunatum* Gaertn. Type III. ($2n=52=A'_4+E_2+F_{38}+G_8$)

The complement is formed of fiftytwo chromosomes. Chromosome size varies from 1·0 μ to 2·1 μ. The normal somatic complement consists of two pairs of *A'*, one pair of *E*, nineteen pairs of *F* and four pairs of *G* (Figs. 26 and 26a). Twentysix clear bivalents have been found in Metaphase I.

15. *C. infortunatum* Gaertn. Type IV. ($2n=52=A_4+E_6+F_{40}+G_2$)

Fiftytwo chromosomes are present in the somatic complement. Size of the chromosomes varies from 1·2 $\mu$ to 2·1 $\mu$. Four chromosomes bear secondary constrictions.

The following types have been recorded in the chromosome set of the normal complement—two pairs of *A*, three pairs of *E*, twenty pairs of *F* and one pair of *G* (Figs. 27 and 27a). Twentysix clear chromosomes have been found in each pole of Metaphase II.

16. *C. infortunatum* Gaertn. Type V. ($2n=52=A_2+A'_2+F_{46}+G_2$)

Fiftytwo chromosomes are present in the somatic complement. Chromosome size varies from 1·1 $\mu$ to 1·8 $\mu$. Four chromosomes bear secondary constrictions.

In the complement, there occur one pair of *A*, one pair of *A'*, twentythree pairs of *F* and one pair of *G* type (Figs. 28 and 28a).

17. *C. infortunatum* Gaertn. Type VI. ($2n=52=A'_4+E_8+F_{40}$)

The normal somatic complement contains fiftytwo chromosomes. Their size varies from 1·1 $\mu$ to 1·8 $\mu$. Two pairs of chromosomes contain secondary constrictions.

The normal complement consists of two pairs of *A'*, four pairs of *E* and twenty pairs of *F* type chromosomes (Figs. 29 and 29a).

## Genus—*Vitex*

Three species of this genus have been investigated and the $2n$ numbers found are 32 and 34. Karyotype analysis reveals that morphological similarity is found in the members of the complement of the species and a number of types is common amongst them. In all nine different types have been found, considering the size and location of the primary and secondary constrictions. A brief description of the types is given below:

Type *A*—Comparatively long chromosome with submedian to nearly submedian primary constriction.

Type *B*—Comparatively long chromosome with median primary constriction.

Type *C*—Comparatively long chromosome with two constrictions, primary and secondary, one is nearly median and the other is located in the submedian position at the end of the longer arm.

Type *C'*—Similar to *C* but shorter in size, and distal shortest arm almost a satellite.

Type *D*—Comparatively long chromosome with two constrictions, primary and secondary, both in the nearly submedian positions dividing the entire chromosome into three almost equal parts.

Type *E*—Comparatively long chromosome with nearly median primary constriction and a satellite at the distal end of the short arm.

Type *F*—Medium sized chromosome with median to nearly submedian primary constriction.

Type *G*—Short sized chromosome with nearly submedian primary constriction.

Type *H*—Short chromosome with median primary constriction.

1. *Vitex negundo* Linn. ($2n=34=A_2+B_2+C_2+D_2+E_2+F_{14}+G_4+H_6$).

The normal somatic complement is formed of thirtyfour chromosomes. The chromosomes, when arranged in order of size, form a graded series. Size varies from 1·6 $\mu$ to 3·1 $\mu$. Six chromosomes with secondary constrictions have been worked out.

A thorough study of the karyotype shows the presence of one pair of each of the types *A*, *B*, *C*, *D* and *E*, seven pairs of *F*, two pairs of *G* and three pairs of *H* type chromosomes. *B* type is longer than *A* and *E* is longer than *C* and *D* in this complement. *F* types show size difference, and three pairs possess nearly submedian constriction while in the rest it is median (Figs. 30 and 30a).

Seventeen clear bivalents have been found in Metaphase I and seventeen chromosomes in the two poles of Metaphase II.

2. *V. agnus-castus* Linn. ($2n=32=A_2+C_2+C'_2+F_{20}+G_2+H_4$)

Thirtytwo chromosomes occur in the normal somatic complement of the species. Size varies from 1·2 $\mu$ to 2·7 $\mu$. Chromosomes form a graded series. Four chromosomes bear secondary constrictions in this complement.

The normal somatic complement consists of one pair of *A*, one pair of *C*, one pair of *C'*, ten pairs of *F*, one pair of *G* and two pairs of *H* type chromosomes. The *C* pair is longer than the *A*, and *F* pairs show size differences. Three pairs of *F* type possess nearly submedian constrictions (Figs. 31 and 31a).

3. *V. trifolia* Linn. ($2n=34$)

Only meiosis of this species has been completed. Seventeen clear bivalents have been found in Metaphase I of this species. Regular tetrad formation and no irregularity have been found.

Genus—*Tectona*

One species in this genus has been worked out.

1. *Tectona grandis* Linn. ($2n=36=A_2+B_{14}+C_{20}$)

Thirtysix chromosomes have been found in the somatic complement of the species. Chromosomes are in general short and form a graded series when arranged in order of size. Size ranges from 1·0 $\mu$ to 1·6 $\mu$. One pair of chromosomes bears secondary constriction. Karyotypic study reveals that the complement is formed of following types (Figs. 32 and 32a):

Type *A*—One pair of comparatively long chromosomes with two constrictions, primary and secondary, one in nearly median position and the other submedian at the distal end of the longer arm. The median segment is longer than other segments of the chromosome.

Type *B*—Medium sized chromosomes. Out of the seven pairs of this type two pairs possess nearly median primary constrictions and the rest median primary constrictions.

Type *C*—Ten pairs of short chromosomes with median primary constrictions.

Eighteen clear bivalents have been noted in Metaphase I.

Genus—*Stachytarpheta*

Somatic study of only one species in this genus has been done. *S. indica* Vahl. contains somatic nuclei with $2n=160$ chromosomes. Chromosomes are short in general, and the majority have median primary constrictions. Six chromosomes have been seen to bear secondary constrictions (Fig. 33).

Genus—*Duranta*

All the three species investigated in this genus contain $2n=34$. In *D. macrophylla* two chromosomes in the complement contain secondary constrictions. Considering the size of the chromosomes as well as the positions of the primary and secondary constrictions a number of types can be recognised in each complement, and it has been found that a number of types is common in different species. The gross morphological characters of the types are described below:

Type *A*—Comparatively long chromosome with two constrictions, primary and secondary, one nearly median and the other submedian in position.

Type *A'*—Comparatively long chromosome with nearly median primary constriction and a satellite at the distant end of one arm.

Type *B*—Comparatively long chromosome with two constrictions, primary and secondary, one nearly submedian and the other submedian in position. The median segment is considerably longer than the other segments.

Type *C*—Comparatively long chromosome with nearly median to nearly submedian primary constriction.

Type *D*—Medium to comparatively short chromosome with median to nearly median primary constriction.

Type *E*—Short chromosome with median primary constriction.

1. *Duranta plumieri* Jacqu. ($2n=34=A'_4+C_4+D_{22}+E_4$)

The normal somatic complement of the species contains thirtyfour chromosomes, forming a graded series. Size ranges from 0·9 $\mu$ to 2·1 $\mu$. Four chromosomes in the complement bear secondary constrictions.

A critical study of the karyotype shows the presence of two pairs of *A'*, two pairs of *C*, eleven pairs of *D* and two pairs of *E* type. One pair of *A'* is shorter than the other and among the two pairs of *C*, one contains median and the other nearly median constriction (Figs. 34 and 34a).

Seventeen clear bivalents have been observed in Metaphase I and seventeen chromosomes in each pole of Metaphase II.

2. *D. plumieri* Jacqu. var. alba ($2n=34=A_2+B_2+C_8+D_{18}+E_4$)

Thirtyfour chromosomes have been found in the normal somatic complement. Size difference is present ranging from 0·9 $\mu$ to 2·5 $\mu$. Secondary constrictions have been seen in four chromosomes.

The normal somatic complement consists of one pair of *A*, one pair of *B*, four pairs of *C*, nine pairs of *D* and two pairs of *E* type. Members of the *D* type show noticeable size difference. Three pairs possess nearly median constriction and are nearly short in size. One pair is considerably longer than other pairs and is nearly equal to the *C* type (Figs. 35 and 35a).

Seventeen clear bivalents have been observed in Metaphase I.

3. *D. macrophylla* ($2n=34=A_2+C_4+D_{26}+E_2$)

Somatic complement contains thirtyfour chromosomes. Chromosomes form a graded series and the size ranges from 1·2 $\mu$ to 1·7 $\mu$.

Karyotype analysis shows that the complement is composed of one pair of *A*, two pairs of *C*, thirteen pairs of *D* and one pair of *E* type. Five pairs of *D* type contain nearly median constrictions and the others median constrictions (Figs. 36 and 36a).

### Genus—*Petrea*

One species of this genus has been investigated.

1. *Petrea volubilis* Linn. ($2n=34=A_2+B_{14}+C_{18}$)

Thirtyfour chromosomes have been observed in the somatic complement of the species. Chromosomes are in general short and size difference is not marked, ranging from 1·2 $\mu$ to 1·7 $\mu$. They form a graded series, according to size, one type slowly merging into the other. One pair of chromosomes in the complement possesses secondary constrictions. Three types of chromosomes may be recognised considering the size and position of the constrictions. Description of the types is as follows (Figs. 37 and 37a):

Type *A*—A pair of comparatively long chromosomes each with a nearly median primary constriction and a satellite at the distal end of the arm.

Type *B*—Comparatively medium sized chromosomes with nearly median to median primary constrictions. Seven pairs of this type are present, two of which are slightly shorter than others.

Type *C*—Short chromosomes with a median primary constriction. Nine pairs are seen.

Besides the normal complement a somatic nucleus with 35 chromosomes has been worked out (Fig. 38).

Meiotic study reveals seventeen clear bivalents in Metaphase I and seventeen chromosomes in the two poles of Metaphase II.

Genus—*Gmelina*

One species of this genus has been investigated.

1. *Gmelina hystrix* Schult. ($2n=38=A_2+B_{10}+C_4+D_{22}$)

The normal somatic complement of the species contains thirtyeight chromosomes. Chromosomes are in general short and their size varies from 0·8 μ to 1·6 μ. Two chromosomes contain secondary constrictions. Four morphologically different types can be recognised, namely (Figs. 39 and 39a):

Type *A*—A pair of comparatively long chromosomes, each with a median primary constriction and a satellite at the distal end of one arm.

Type *B*—Five pairs of medium sized chromosomes with median to nearly median primary constrictions, grading in size.

Type *C*—Two pairs of very short chromosomes with nearly subterminal primary constrictions.

Type *D*—Eleven pairs of very short chromosomes with median primary constrictions.

In addition to the normal complement, a nucleus with thirtyfive chromosomes has been observed (Fig. 40).

Genus—*Holmskioldia*

One species of this genus has been worked out.

1. *Holmskioldia sanguinea* Retz. ($2n=32=A_2+B_2+C_{16}+D_8+E_4$)

Thirtytwo chromosomes form the normal complement. Chromosomes are nearly medium sized in general and their size varies from 1·2 μ to 2·5 μ. They form a graded series and on the basis of length they may be divided into three groups:

(*i*) Ten pairs of comparatively long chromosomes.

(*ii*) Four pairs of medium sized chromosomes.

(*iii*) Two pairs of short chromosomes.

Four chromosomes in the set contain secondary constrictions.

A critical study of the karyotype reveals that the complement is composed of five types of chromosomes (Figs. 41 and 41a) namely:

Type *A*—Comparatively long chromosome with two constrictions, primary and secondary, one median and the other submedian in position. One pair of this type has been found.

Type *B*—Comparatively long chromosome with nearly submedian primary constriction and a satellite at the distal end of the long arm. One pair is present.

Type *C*—Comparatively long chromosome with median to nearly median primary constriction. Eight pairs of this type are found.

Type *D*—Medium sized chromosome with nearly median primary constriction. Four pairs have been found.

Type *E*—Short chromosome with median to nearly median primary constriction. Two pairs are present.

Besides, a cell with thirtyfour chromosomes has been observed (Fig. 42).

## Genus—*Caryopteris*

In the two species investigated under this genus, $2n=40$ and 52 are seen. Chromosomes are in general medium sized. Depending on the gross morphological similarity a number of types has been found to be common between the species. In all, seven different types have been worked out and their general description is given below:

Type *A*—Long chromosome with two constrictions, primary and secondary, one nearly median and the other nearly subterminal in position.

Type *B*—Long chromosome with nearly subterminal primary constriction.

Type *C*—Long chromosome with median primary constriction and a satellite at the distal end of one arm.

Type *D*—Comparatively long chromosome with two constrictions, primary and secondary, both located in nearly submedian position, dividing the chromosome into three nearly equal segments.

Type *E*—Comparatively long chromosome with nearly subterminal primary constriction. This type is shorter than *B*.

Type *F*—Medium sized chromosome with median to nearly median primary constriction.

Type *G*—Short chromosomes with median to nearly median primary constriction.

1. *Caryopteris mastacanthus* Schau. (blue) ($2n=52=A_2+B_2+D_2+F_{14}+G_{32}$)

The somatic complement is composed of fiftytwo chromosomes. Their size varies from 1·1 $\mu$ to 2·5 $\mu$. Four chromosomes bear secondary constrictions.

The normal complement is formed of one pair of each of *A*, *B* and *D*, seven pairs of *F* and sixteen pairs of *G* type chromosomes. Two pairs of *F* are longer than the others (Figs. 43 and 43a). In addition to the normal number, somatic nuclei with thirtyeight chromosomes (Fig. 44) and fortyone chromosomes (Fig. 45) have also been recorded.

2. *C. mastacanthus* Schau. (pink) ($2n=40=A_2+B_6+C_4+E_8+F_8+G_{12}$)

Forty chromosomes form the somatic complement. Size varies from 1·6 $\mu$ to 4·0 $\mu$, and when arranged serially they form a graded series. Three pairs of chromosomes bear secondary constrictions.

The karyotype is formed of one pair of *A*, three pairs of *B*, two pairs of *C*, four pairs each of *E* and *F* and six pairs of *G* type chromosomes. *B*, *E* and *F* types show size difference. Two pairs of *G* type are longer than the remaining pairs (Figs. 46 and 46a).

Besides, somatic nuclei with thirtynine (Fig. 47) and fortyone (Fig. 48) chromosomes have been seen.

## Genus—*Callicarpa*

One species of this genus has been worked out.

1. *Callicarpa macrophylla* ($2n=34=A_4+B_2+C_{20}+D_2+E_6$)

The normal somatic complement of the species contains thirtyfour chromosomes. Most of the chromosomes are medium sized. Size varies from 0·9 $\mu$ to 2·5 $\mu$ and chromosomes form a graded series. Four chromosomes in the set bear secondary constrictions.

Karyotype study reveals that the complement is formed of five different types of chromosomes—distinguished from one another by their size and location of the constrictions, namely (Figs. 49 and 49a):

Type *A*—Comparatively long chromosome with nearly median primary constriction and a satellite at the distal end of one arm. Two pairs are present.

Type *B*—One pair of comparatively long chromosomes with median primary constriction.

Type *C*—Long to medium-sized chromosome with median to nearly median primary constriction. Ten pairs have been found.

Type *D*—One pair of medium sized chromosomes with nearly subterminal primary constriction.

Type *E*—Three pairs of short chromosomes with median primary constrictions.

In addition to the normal complement, a variation number of 32 chromosomes has been recorded (Fig. 50).

## Genus—*Congea*

One species of this genus has been worked out.

1. *Congea tomentosa* ($2n=34=A_2+B_6+C_{14}+D_{12}$)

The normal somatic complement of the species contains thirtyfour chromosomes. Chromosomes are in general medium sized, graded and show size differences. Size ranges from 1·4 $\mu$ to 2·1 $\mu$. Two chromosomes with secondary constrictions have been seen.

Karyotype analysis reveals that the complement is formed of four types of chromosomes (Figs. 51 and 51a) namely:—

Type *A*—Comparatively long chromosome, with two constrictions, primary and secondary, one median and the other submedian. One pair of chromosomes is present.

Type *B*—Comparatively long chromosome with nearly submedian primary constrictions, represented by three pairs in the complement.

Type *C*—Comparatively medium sized chromosome with median to nearly median primary constrictions. Seven pairs are present.

Type *D*—Comparatively short chromosome with median primary constrictions. Six pairs have been worked out.

In addition to the normal complement a variation plate with thirtyone chromosomes has been recorded (Fig. 52).

Seventeen clear bivalents have been observed in diakinesis and in Metaphase I. Seventeen chromosomes have been seen in one pole of Metaphase II.

## Genus—*Verbena*

Two species of this genus have been investigated, both having the same chromosome number i.e. $2n=10$ and also having one pair of chromosomes with secondary constrictions. In general the chromosomes are comparatively longer than in the other genera studied so far. In all five different types of chromosomes have been identified from the idiograms of the two species, and distinctive characters, mainly involving the size and the location of constrictions, have been described below:—

Type *A*—Long chromosome with nearly submedian primary constriction.

Type *B*—Long chromosome with two constrictions, primary and secondary, one nearly median and the other nearly subterminal at the distal end of the short arm.

Type *B′*—This type resembles type B but differs from the latter by the presence of a satellite at the distal end of the short arm.

Type *C*—Long chromosome with median primary constriction.

Type *D*—Medium sized chromosome with nearly submedian to submedian primary constriction.

1. *Verbena erinoides* Lam. (mauve flower) ($2n=10=A_4+B'_2+D_4$)

Ten chromosomes are present in the somatic complement. They form a graded series and the size ranges from $3{\cdot}4\mu$ to $4{\cdot}1\mu$. One pair possesses secondary constrictions.

The somatic complement is formed of two pairs of *A*, one pair of *B′* and two pairs of *D* type. Pairs of Type *A* show a little size difference (Figs. 53 and 53a).

Five clear bivalents have been observed in diakinesis and Metaphase I and five chromosomes in the two poles of Metaphase II.

2. *V. aubletia* Jacq. (red flower) ($2n=10=B_2+C_4+D_4$)

The somatic complement is formed of ten chromosomes. They are graded and the size varies from $3{\cdot}7\mu$ to $4{\cdot}4\mu$. Only one pair bears secondary constrictions.

Analysis of the karyotype reveals that the complement is composed of one pair of B, two pairs of C and two pairs of D (Figs. 54 and 54a).

Genus—*Nyctanthes*

Only one species of this genus has been investigated.

1. *Nyctanthes arbortristis* Linn. ($2n=44=A_2+B_2+C_{14}+D_{26}$)

Fortyfour chromosomes constitute the normal somatic number of this species. They are in general short and their size varies from $0{\cdot}8\mu$ to $2{\cdot}0\mu$. Four chromosomes in the complement possess secondary constrictions.

A critical study of the karyotype reveals that the complement is composed of four types of chromosomes (Figs. 55 and 55a) namely:—

Type *A*—Comparatively long chromsome with two constrictions, primary and secondary, one is nearly median and the other is nearly submedian in position. One pair has been observed.

Type *B*—Comparatively long chromosome with nearly median primary constriction and a satellite at the distal end of one arm. One pair is present.

Type *C*—Comparatively medium sized chromosome with median to nearly median primary constriction. It is represented by seven pairs in the complement, showing size difference amongst them.

Type *D*—Short chromosome with median primary constriction, represented by thirteen pairs in the set.

In addition to the normal complement a somatic nucleus with thirtyfour chromosomes has been worked out (Fig. 56).

Twentytwo clear bivalents have been found in Metaphase I.

## Discussion

*Chromosome number and its significance in interpreting evolution in* Clerodendron

Species of *Clerodendron* studied by previous authors show a range of chromosome numbers between 24 and 108. The haploid numbers so far recorded are 12 and 23. In the present investigation $2n=52$ chromosomes have been seen in *C. infortunatum* and all its varieties as well as *C. minahassae, C. fragrans, C. nutans, C. siphonanthus* and *C. squamatum*. $2n=46$ chromosomes have been found in *C. thomsonae* var. I, *C. inerme* and *C. splendens* whereas $2n=48$ is present in another variety of *C. thomsonae*. *C. ugandense* shows a somatic chromosome number as high as 184. The number $2n=30$, noted in the present work, is the lowest of all species worked out in the present investigation. In addition to the haploid numbers 12 and 23 recorded previously (Patermann, 1938; Bowden, 1940, 1945; Nishiyama and Kondo, 1942) the present investigation provides evidences of the existence of two more haploid numbers i.e. 26 and 15 in species of *Clerodendron*. The number $2n=184$, being a multiple of 23, once more indicates the occurrence of polyploidy in *Clereodendron* and is the highest number so far noted.

The nature of origin of *Clerodendron*, whether mono- or polyphyletic, is yet to be ascertained. It is worth noting that in *C. thomsonae* both 46 and 48 chromosomes have been recorded by previous authors, indicating apparently that one number is derived from the other. In the present work 46 chromosomes have no doubt been found in the somatic cells of *C. thomsonae*, but in another variety of the same species, which differs principally from the former in the colour of the perianth, $2n=48$ chromosomes have been found. Unless properly scrutinised there is the possibility of confusing one variety with the other. It may be pointed out that the second variety was formerly identified as *C. balfourii* Hort. which later on has been found to be a synonym of *C. thomsonae*. It is not unlikely that the numerical difference in chromosomes between these two species may account for their phenotypic difference.

Clear bivalent formation in all the species of *Clerodendron* suggests that whatever may be their mode of origin, homozygosity and a stable condition have at present been reached by all the species. On the basis of evidences so far obtained it appears that the numbers, 46, 48 and 52 are closely interrelated. The existence of $2n=24$ chromosomes in *C. fargesii* reported by Patermann (1938) indicates that 48 chromosomes represent a polyploid level. The close phenotypic similarity between two varieties of *C. thomsonae* showing at the same time 46 and 48 chromosomes respectively, suggests that $2n=46$ chromosomes is therefore derived possibly from $2n=48$ by the loss of one chromosome in the haploid set. It is also likely therefore that $2n=52$ chromosomes, i.e. a multiple of 13 noted in several species of *Clerodendron* including *C. infortunatum*, might have been initially derived from species like *C. fargesii*, etc. with $2n=24$ chromosomes, by the duplication of one chromosome in the basic set. Evolution through several generations has ultimately resulted in structural changes in the duplicated chromosomes so that multivalent formation has been eliminated. On the basis of these evidences it appears that species with 24, 46, 48 and 52 chromosomes and their multiples form a common assemblage.

*C. macrosiphon* with $2n=30$ chromosomes presents a different problem. Here also fifteen clear bivalents have been recorded in meiosis. At present there is no cytological evidence to suggest that $n=15$ has been derived from species with $n=12$ chromosomes. In external morphology however the species has close affinity with other species of *Clerodendron* and bears the character of the genus. Unless data suggesting that $n=12$ and $n=15$ have been derived from the basic set with much lower chromosome number are obtained it is reasonable to consider that in the genus *Clerodendron* at least two lines of evolution are operating, one starting with 12 and the other with 15 chromosomes.

### *Evidences from karyotype analysis and chromatin content in* Clerodendron

That the different species of *Clerodendron*, including *C. macrosiphon*, have been rightly included under the genus *Clerodendron* is borne out by their karyotypes. Chromosomes are grossly divisible into eight distinct types, though modifications have been noticed in different species and varieties. Chromosomes in general are not very long, being medium to small in size, and the karyotype is graded. Size difference between members of the complement is not abrupt. The total chromatin length varies between $28{\cdot}2\mu$ to $46{\cdot}1\mu$. The slight difference in chromatin matter and difference in minute details of karyotype account to some extent for their individual specific and varietal status. It is clear that in this genus structural changes of chromosomes have played a significant role in the origin of different taxa.

*Method of speciation through somatic alteration in* Clerodendron

With regard to the origin of different species and strains of *Clerodendron* a discussion on the behaviour of chromosomes in the somatic tissue is necessary. Several species worked out during the present investigation exhibited variations in the chromosome complements in the somatic tissue occurring in a few cells (5%—12%) along with the normal nuclei. For example, in *C. squamatum* ($2n=52$) fortyeight chromosomes have been found in the variant nuclei. Similarly, in *C. thomsonae* var. II ($2n=48$), fortytwo, fifty and fortysix chromosomes are present in some of the complements. In *C. siphonanthus* normally containing $2n=52$ chromosomes, fortysix chromosomes have been noted in the variant nuclei. It is worthy of note that in several other species of *Clerodendron*, $2n=46$ is the normal number for the species.

No doubt species of *Clerodendron* excepting a few, such as *C. siphonanthus*, are characterised by regular occurrence of sexual reproduction and propagation through seeds. But in addition to sexual means a very common method of propagation in them is vegetative. The numerical alteration of chromosomes as noted in the somatic tissue has not however been found in the pollen mother cells. Evidently, it appears that they do not survive up to the germ cell stage. But their evolutionary significance can be appreciated if vegetative reproduction of the species is taken into consideration. Speciation can be affected if they participate in the formation of new daughter shoots during reproduction. This may be one of the additional means through which evolution in this genus has been facilitated. The occurrence in the variant nucleus of a particular chromosome number (46) amongst others, which is otherwise normal for other species, may point to such a possibility.

*Cytological data and the taxonomical status of other genera of the Verbenaceae*

The twelve genera on which investigations have been carried out in the present work fall under different tribes of Verbenaceae. Of these six genera belong to the tribe Vitieae, two to Verbeneae, and one each to Caryopterideae and Symphoremeae. As most of the genera fall under Vitieae a discussion on these genera will be followed by others.

In Vitieae along with *Clerodendron* the other genera are *Callicarpa, Tectona, Gmelina, Vitex* and *Holmskioldia*. In *Callicarpa macrophylla* 34 chromosomes have been observed whereas previous records in *C. japonica* were 16 and 18 chromosomes in the same species. In view of the lower chromosome number in the allied species it is apparent that *C. macrophylla* represents a polyploid level. The record of 16, 18 and 34 chromosomes in the same genus also suggests that aneuploid variations have contributed to the evolution of this genus.

In *Tectona grandis* the chromosome number observed is $2n=36$ indicating a multiple of either 9 or 18. As no other species of *Tectona* has so far been recorded it is not possible to assert its polyploid nature, though the multiples of 9 chromosomes ($2n=18$)

noted in the allied genus *Callicarpa japonica*, the individuals of which are also large trees like *Tectona grandis*, may point to such a possibility. However meiosis in *T. grandis* is characterised by clear bivalent formation. As in the family Verbenaceae, even in species with very high chromosome numbers, such as *Clerodendron infortunatum* ($2n=52$), regular bivalent formation has been noticed, it is evident that mere bivalent formation may not indicate the diploid level of the species concerned and it may be due to amphidiploidy.

Therefore there is a possibility that *Tectona grandis*, though showing formation of bivalents, may not be a diploid, specially in view of the chromosome numbers noted in the allied genera. On the basis of chromosome number and taking into account that habit of the species as well, one is inclined to believe that *Callicarpa* is very much related to *Tectona* not only in external morphology but in the cytology as well.

In the other genus *Gmelina* which is also characterised by both arborescent and shrubby habit, $2n=38$ chromosome number has been found in *G. hystrix*. In view of the absence of any previous records on any other species of this genus it is not possible to state precisely its diploid and polyploid status. In view of the arborescent nature of the plants, their similarity with *Callicarpa* and *Tectona* cannot be disregarded.

Chromosomes of the species of *Vitex* present interesting data. The previous record in *V. agnus-castus* is $2n=24$ and in *V. trifolia* $2n=32$. In individuals of *V. agnus-castus* studied here the chromosome number has been found to be 32 and in *V. trifolia* it is 34. The occurrence of 24 and 32 chromosomes in different individuals of *V. agnus-castus* suggests that intraspecific polyploidy exists in the species. In *V. trifolia* on the other hand 32 and 34 chromosomes indicate the occurrence of aneuploid variations at an intraspecific level as noted in *Callicarpa japonica* as well. Another species studied here, i.e., *Vitex negundo*, also shows $2n=34$ chromosomes. On the basis of the chromosome number in *Vitex*, i.e., 24, 32, 34 and the occurrence of both 32 and 34 chromosomes in the same species, it may be stated that eight in all probability is the basic number of this genus whence both polyploid and aneuploid variations have later contributed to its evolution. In *V. negundo* ($2n=34$) seventeen clear bivalents have been observed. This may be due to fact that continued evolution involving structural changes has ultimately resulted in the elimination of any evidence of duplicated chromosomes. There can be no doubt that species of this genus represent a natural assemblage.

Leaving aside *Clerodendron* which has already been discussed the other genus in Vitieae of Verbenaceae is *Holmskioldia*. The habit of the species of *Holmskioldia* is very similar to that of *Vitex*. The chromosome number noted is also 32, indicating its affinity with *Vitex* as well. On the basis of chromosome numbers at least the genera *Clerodendron*, *Vitex* and *Holmskioldia* show a close relationship.

Cytological evidence therefore suggests that the tribe Vitieae of Verbenaceae contains at least two assemblages—one including genera like *Vitex*, *Clerodendron*, *Holmskioldia*, and the other including *Callicarpa*, *Tectona* and in all possibility *Gmelina* too. It would be more natural to separate these two groups of genera at least in two separate tribes.

The tribe Verbeneae of this family is represented in this investigation by four genera namely *Verbena*, *Stachytarpheta*, *Petrea*, and *Duranta*. In both species of *Verbena* ten chromosomes have been found, which are quite long. Of the other species of *Verbena* too, a large number has been shown to possess the same chromosome number by previous authors. It may be pointed out in this connection that secondary association has been found in *V. erinoides*, and if this issue is confirmed in other species as well then the basic number of this genus may be even lower than five.

In *Stachytarpheta indica* on the other hand a chromosome number as high as 160 has been found. Previous records show the lowest number as $2n=48$ (Patermann, 1938) in this genus. This number is a clear indication of its polyploid nature. The chromosomes too are very small as compared to that of *Verbena*. No doubt the high degree of polyploidy may to some extent be responsible for the decrease in chromatin matter as found in a number of other species of flowering plants (Delaunay, 1926; Babcock and Cameron, 1934). But even then the wide difference in external morphological characters between *Verbena* and *Stachytarpheta* cannot be overlooked. The spike in the former is condensed whereas in the latter it is elongated, thus giving an entirely different appearance to the two. It may be noted that the two genera of Verbeneae, namely *Lippia* and *Lantana* studied previously, are also characterised by condensed cymes and the chromosome size too is not very small. On the basis of wide difference in external morphological characters as well as the chromosome number it appears that *Stachytarpheta* will be in a more natural position if placed in a separate tribe other than Verbeneae. The basic number in this genus is yet to be determined. Species of *Verbena* with long chromosomes and fairly symmetrical karyotype not only represent a primitive level in Verbeneae, but are possibly primitive to all other genera of Verbenaceae.

Three species of *Duranta* containing $2n=34$ chromosomes can also be conveniently classed under Verbeneae not only because of their chromosomes but also the nature of the inflorescence. Similar is the case with *Petrea volubilis* ($2n=34$). The complements of *Petrea* and *Duranta* are alike not only in chromosome number but in chromosome morphology as well. Their meiosis too shows seventeen clear bivalents. It may be noted in this connection that a previous reports in *D. repens* (*plumieri*) is $2n=36$ (Patermann, 1938) as against $2n=34$ in the present record. It is evident therefore that an aneuploid biotype exists in this species as well.

Two varieties of *Caryopteris mastacanthus* belonging to the tribe Caryopterideae have been shown to possess different chromosome numbers. In one the number has been found to be $2n=40$ and in the other $2n=52$. Such intraspecific variation in chromosome numbers indicates the possibility of existence of biotypes and suggests that aneuploidy is a significant factor in their evolution.

In *Congea tomentosa*, the only species of the tribe Symphoremeae which has been studied here, the chromosome number is $2n=34$. Seventeen clear bivalents have been observed during meiosis. This number however is not uncommon for the family and a correct systematic assignment would await availability of data on related genera.

*Karyotype analysis and its interpretation*

In spite of the fact that different genera of this family are characterised by large differences in chromosome numbers in different genera and species, the karyotype similarity is remarkable. This is a clear indication that the family itself represents a natural assemblage. Chromosomes are mostly medium sized varying to short. The size difference is never marked and the karyotype is graded. Most of the chromosomes are provided with median to submedian primary constrictions and the numbers of satellites and secondary constrictions vary between two and eight. The total amount of chromatin content too does not show much difference. In the case of *Verbena* alone the chromosomes are fairly large, and the chromosome number too in this genus is $n=5$. This genus undoubtedly represents a very primitive state.

Minute details of the karyotypes however distinguish each species from another, and every species has its distinctive karyotype formula. This is an indication that slight structural changes in chromosomes have been associated with the evolution of different genera and species of Verbenaceae.

*Variation of chromosome number in the somatic tissue*

Similar to that of *Clerodendron* a considerable amount of variation of chromosome complements in the somatic tissue has been found along with the normal complements. Though the frequency of their occurrence is very low, not exceeding 12% of the dividing cells, their occurrence itself in most of the species cannot be ignored.

Such somatic variations are common in species reproducing through vegetative means where they play a significant part in the speciation. In the Verbenaceae the significance of such variations is borne out by the fact that a considerable degree of aneuploidy exists not only between species and genera of this family but even at an intraspecific level. The meiosis so far studied has been found to be regular. The origin of large differences in chromosome numbers between genera and species of a natural family like the Verbenaceae cannot be explained if their meiotic behaviour alone is taken into consideration. Such regular meiosis with hardly any variations cannot account for chromosome diversity between different taxa of this family. Evidently the variations in the somatic complement brought forward in the present investigation seem to be playing a significant role in speciation. In spite of sexual reproduction, vegetative reproduction too is profuse in a majority of the species. It is therefore quite likely that such somatic variation participates in the formation of daughter shoots during vegetative reproduction and helps in the origin of new phenotypes and genotypes (Sharma, 1956).

*Cytological evidences of* Nyctanthes arbortristis *in support of its taxonomic position*

Considerable debate has been held on the systematic position of the genus *Nyctanthes*. In view of certain morphological characters Jussieu and Linnaeus supported the inclusion of the genus under Oleaceae, whereas recently it has been placed by Airyshaw (1952) under Nyctanthioideae—a new tribe—between Callicarpeae and Tectoneae (Engler and Prantle).

Cytologically the chromosomes of *N. arbortristis* ($2n=44$) are quite interesting. All of them are very small, and the size variation is not marked, ranging between $0{\cdot}8\mu$

and 2·0$\mu$. There are only four types of chromosomes, and two pairs are provided with secondary constrictions or satellites. This karyotype finds remarkable similarity with *Tectona grandis* ($2n=34$). There only three types of chromosomes are present which are very small, and one pair is provided with secondary constrictions. The number 44 is also not uncommon for the family Verbenaceae where aneuploid variations have played a significant role in speciation. In view of these facts the position of *Nyctanthes* in a separate tribe allied to Tectoneae is justified. Its relationship with *Callicarpa* is yet to be found out.

## SUMMARY

Cytotaxonomic investigation was carried out on thirtyfive taxa of Verbenaceae including twentyeight species under thirteen genera covering the four tribes of the family.

The haploid numbers $n=26$ and 15 are new additions in the genus *Clerodendron* where previous records show $n=12$ and 23. On the basis of cytological data it has been suggested that $2n=52$ chromosomes, as found in *C. siphonanthus*, *C. infortunatum*, *C. fragrans*, etc. might have been initially derived from species with $2n=24$ chromosomes by the duplication of one chromosome in the set. Similarly $2n=46$ chromosomes might have been derived from $2n=48$ by the loss of one chromosome in the haploid set. Regular bivalent formation in all the species suggests that evolution through several generations has ultimately resulted in structural changes in the duplicated chromosomes and thus multivalent formation has been eliminated. It has been suggested that in the genus *Clerodendron* at least two lines of evolution are operating, one starting with 12 and the other with 15 chromosomes as represented by *C. macrosiphon*.

Evidence has been presented to show that structural changes of chromosomes have played a role in the origin of different taxa. Variation in chromosome complements have been noted and their significance in speciation through vegetative means has been pointed out.

Five other genera besides *Clerodendron* have been studied under the tribe Vitieae. Polyploidy in species of *Callicarpa* has been established and different chromosome numbers have been noted in the genus. *Tectona grandis*, resembling the species of *Callicarpa* in habit, shows 36 chromosomes. Its exact polyploid status and the basic set is not possible to assert at present. The same is the case with *Gmelina hystrix*. The occurrence of $2n=24$ and 32 in the same species of *Vitex agnus-castus* suggests that intraspecific polyploidy exists in this species. That aneuploid variations exist in intraspecific level is evident from the occurrence of 32 and 34 chromosomes in *V. trifolia*. It has been suggested that eight in all probability is the basic number of the genus. *Holmskioldia* with its chromosome number and habit shows close affinity with *Vitex*. Cytological evidence therefore suggests that the tribe Vitieae of Verbenaceae contain at least two assemblages—one including *Vitex*, *Clerodendron*, *Holmskioldia* and the other including *Callicarpa*, *Tectona* and in all probability *Gmelina* too.

In the tribe Verbeneae species of *Verbena* having $2n=10$ and long chromosomes with fairly symmetrical karyotype represent not only a primitive level in Verbeneae

but are possibly primitive to all the other genera of Verbenaceae. Cytological data as well as characteristic inflorescence in *Stachytarpheta* differ from *Verbena*, and it has been suggested that the former will be in a more natural position if placed in a separate tribe. The numbers 34 and 36 reported in *D. plumieri* suggest that aneuploid biotypes exist in this species.

Existence of chromosomal biotypes in *Caryopteris mastacanthus* with different chromosome numbers 52 and 40 has been shown. The systematic position of *Congea tomentosa* must await further work on related genera of the tribe Symphoremeae.

*Nyctanthes arbortristis* with $2n=14$ chromosomes, formerly belonging to the Oleaceae, has been placed under Nyctanthioideae in the Verbenaceae by Airyshaw (1952). Considering the similarity with *Tectona grandis* in cytological data, and the chromosome number, which is also present in family Verbenaceae, its position in a separate tribe allied to the Tectoneae has been justified.

## REFERENCES

Airyshaw, H. K. (1952). Note on the taxonomic position of *Nyctanthes* L. and *Dimetra* Kerr. *Bull. Misc. Inf. Kew.*, **1952**, 271.

Babcock, E. B. and Cameron, O. R. (1934). Chromosomes and phylogeny in *Crepis*. II. The relationships of 108 species. *Univ. of Calif. Publ. Agr. Sci.*, **6**, 287-324.

Bowden, W. B. (1940). Diploidy, Polyploidy and winter hardiness relationship in the flowering plants. *Amer. J. Bot.*, **27**, 357.

Bowden, W. B. (1945). A list of chromosome numbers in higher plants. II. Menispermaceae to Verbenaceae. *Amer. J. Bot.*, **32**, 191-201.

Darlington, C. D. and Wylie, A. P. (1955). *Chromosome atlas of flowering plants*. George Allen & Unwin Ltd.: London.

Datta, P. C. (1953). Cytogenetics of *Verbena tenera* Spreng. *Caryologia*, **5**, 359-370.

Delaunay, L. (1926). Phylogenetische Chromosomenbekurzung. *Zeitschr. Zellf. u. mikr. Anat.*, **4**, 338-364.

Natarajan, A. T. and Ahuja, M. R. (1957). Cytotaxonomic studies in the genus *Lantana*. *J. Ind. Bot. Soc*, **36**, 35-45.

*Nishiyama, I. and Kondo, N. (1942). *Seiken Ziho*, **1**, 26.

*Patermann, B. (1938). Vide Darlington & Wylie, 1955.

*Schnack, B. (1947). *Haumania*, **1**, 32.

*Taken from *Chromosome atlas of flowering plants* by Darlington & Wylie, 1955.

Sharma, A. K. (1956). A new concept of a means of speciation in plants. *Caryologia*, **9**, 93-130.

Sharma, A. K. and Mukherjee, D. Cytology of some members of Verbenaceae. (Unpublished).

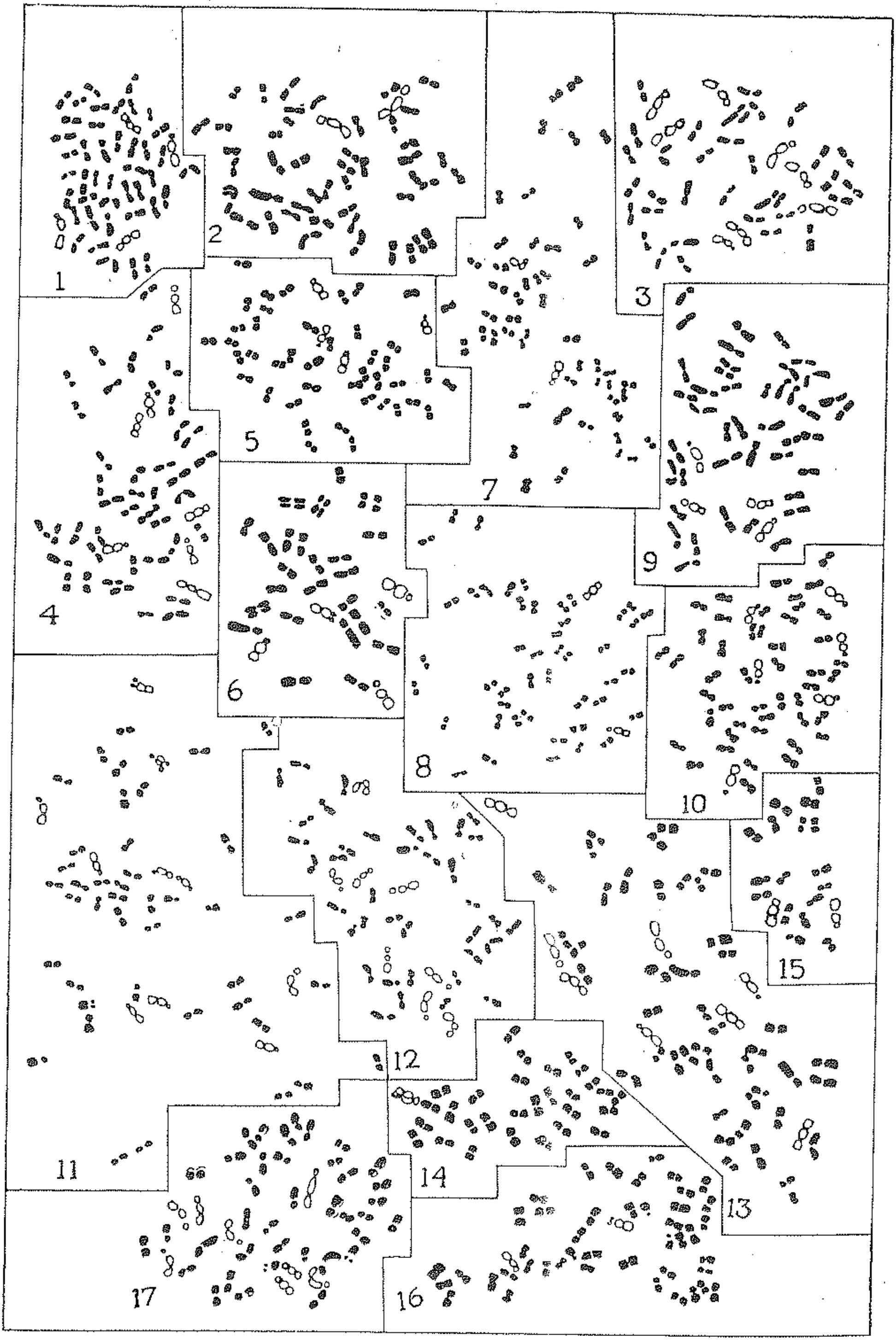
Singh, B. (1951). Chromosome number in some flowering plants. *Curr. Sci.*, **20**, 105.

Sugiura, T. (1936a). A list of chromosome numbers in Angiospermous plants. II. *Proc. Imp. Acad. Jap.*, **12**, 144-146.

Sugiura, T. (1936b). Studies on the Chromosome numbers in higher plants with special reference to cytogenetics. *Cytologia*, **7**, 544-595.

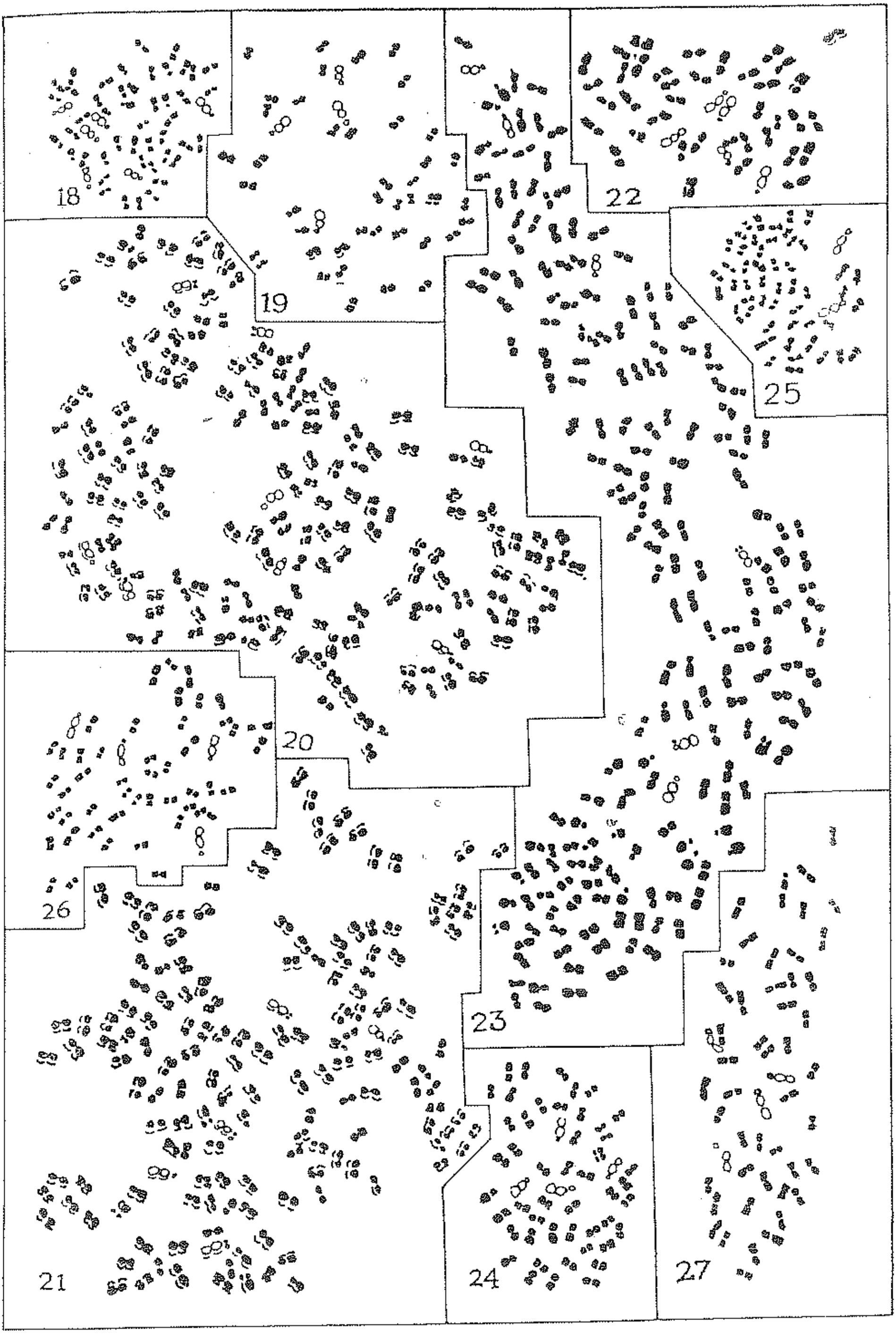
Tjio, J. H. (1948). The somatic chromosomes of some tropical plants. *Hereditas*, **34**, 135.

PLATE 9



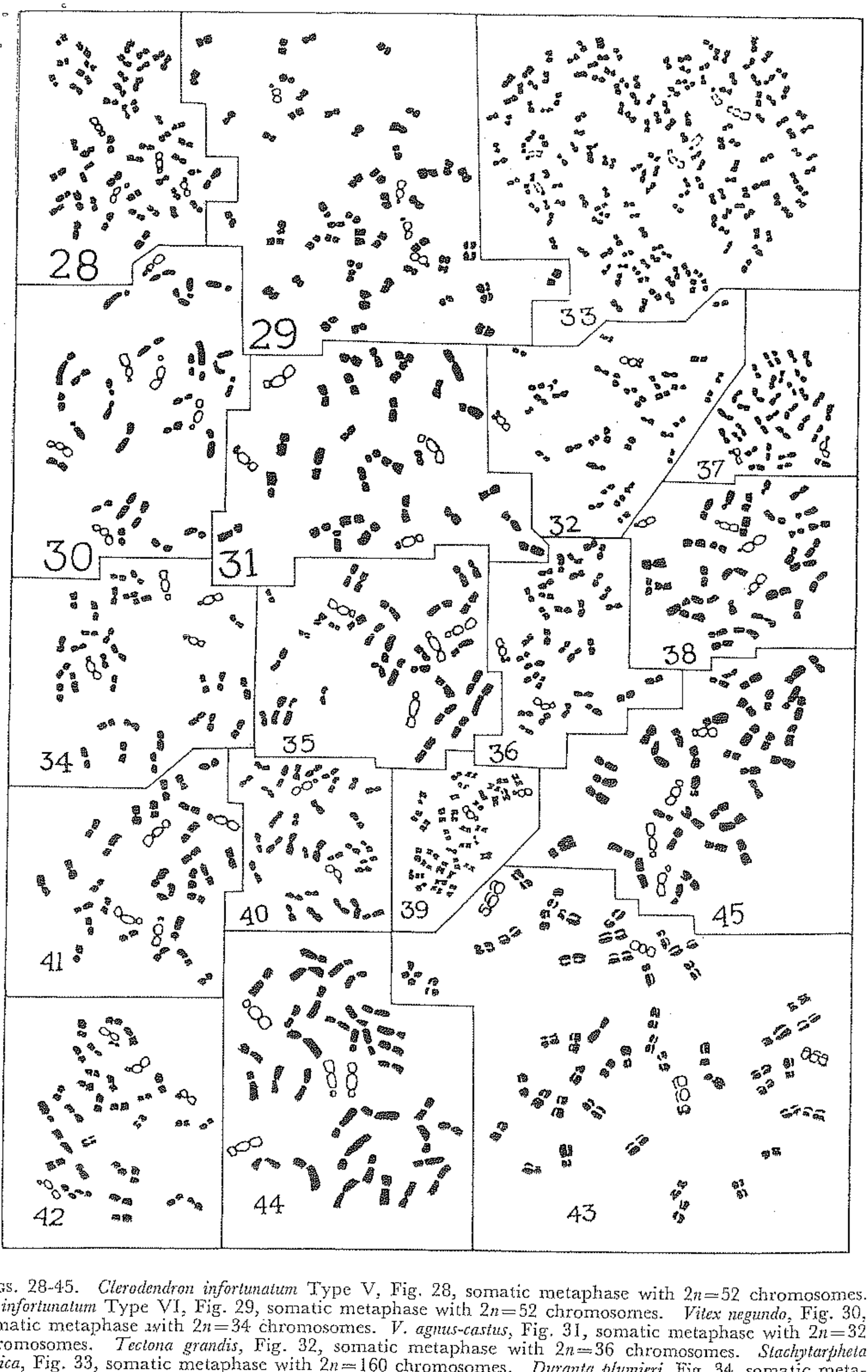

FIGS. 1-17. *Clerodendron thomsonae* var. I, Fig. 1, somatic metaphase with $2n=46$ chromosomes. *C. thomsonae* var. II, Figs. 2-5, somatic metaphase with $2n=48$, 42, 50 & 46 chromosomes, respectively. *C. macrosiphon*, Fig. 6, somatic metaphase with $2n=30$ chromosomes. *C. squamatum*, Figs. 7 & 8, somatic metaphase with $2n=52$ & 48 chromosomes respectively. *C. inerme*, Fig. 9, somatic metaphase with $2n=46$ chromosomes. *C. nutans*, Fig. 10, somatic metaphase with $2n=52$ chromosomes. *C. siphonanthus*, Figs. 11 & 12, somatic metaphase with $2n=52$ & 46 chromosomes respectively. *C. fragrans*, Figs. 13-16, somatic metaphase with $2n=52$, 30, 13 & 46 chromosomes respectively. *C. splendens*, Fig. 17, somatic metaphase with $2n=46$ chromosomes.

PLATE 10




FIGS. 18-27. *Clerodendron minahassae*, Figs. 18 & 19, somatic metaphase with $2n=52$ & 46 chromosomes respectively. *C. ugandense*, Figs. 20-23, somatic metaphase with $2n=184$, 154, 43 & 195 chromosomes respectively. *C. infortunatum* Type I, Fig. 24, somatic metaphase with $2n=52$ chromosomes. *C. infortunatum* Type II, Fig. 25, somatic metaphase with $2n=52$ chromosomes. *C. infortunatum* Type III, Fig. 26, somatic metaphase with $2n=52$ chromosomes. *C. infortunatum* Type IV, Fig. 27, somatic metaphase with $2n=52$ chromosomes.

PLATE 11

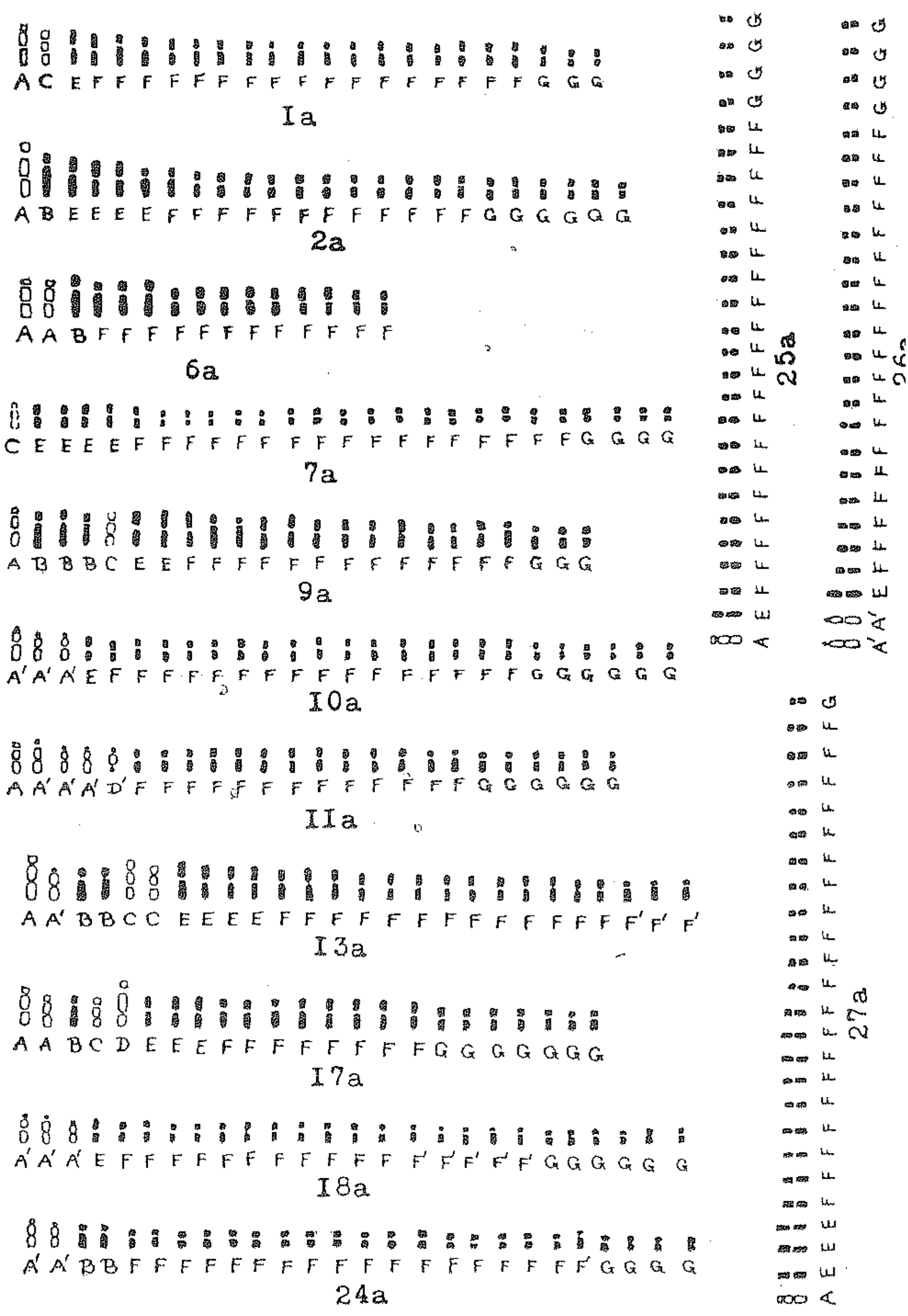



FIGS. 28-45. *Clerodendron infortunatum* Type V, Fig. 28, somatic metaphase with $2n=52$ chromosomes. *C. infortunatum* Type VI, Fig. 29, somatic metaphase with $2n=52$ chromosomes. *Vitex negundo*, Fig. 30, somatic metaphase with $2n=34$ chromosomes. *V. agnus-castus*, Fig. 31, somatic metaphase with $2n=32$ chromosomes. *Tectona grandis*, Fig. 32, somatic metaphase with $2n=36$ chromosomes. *Stachytarpheta indica*, Fig. 33, somatic metaphase with $2n=160$ chromosomes. *Duranta plumieri*, Fig. 34, somatic metaphase with $2n=34$ chromosomes. *D. plumieri* var. alba, Fig. 35, somatic metaphase with $2n=34$ chromosomes. *D. macrophylla*, Fig. 36, somatic metaphase with $2n=34$ chromosomes. *Petrea volubilis*, Figs. 37 & 38, somatic metaphase with $2n=34$ & 35 chromosomes respectively. *Gmelina hystrix*, Figs. 39 & 40, somatic metaphase with $2n=38$ & 35 chromosomes respectively. *Holmskioldia sanguinea*, Figs. 41 & 42, somatic metaphase with $2n=32$ & 34 chromosomes respectively. *Caryopteris mastacanthus* (blue),

PLATE 12

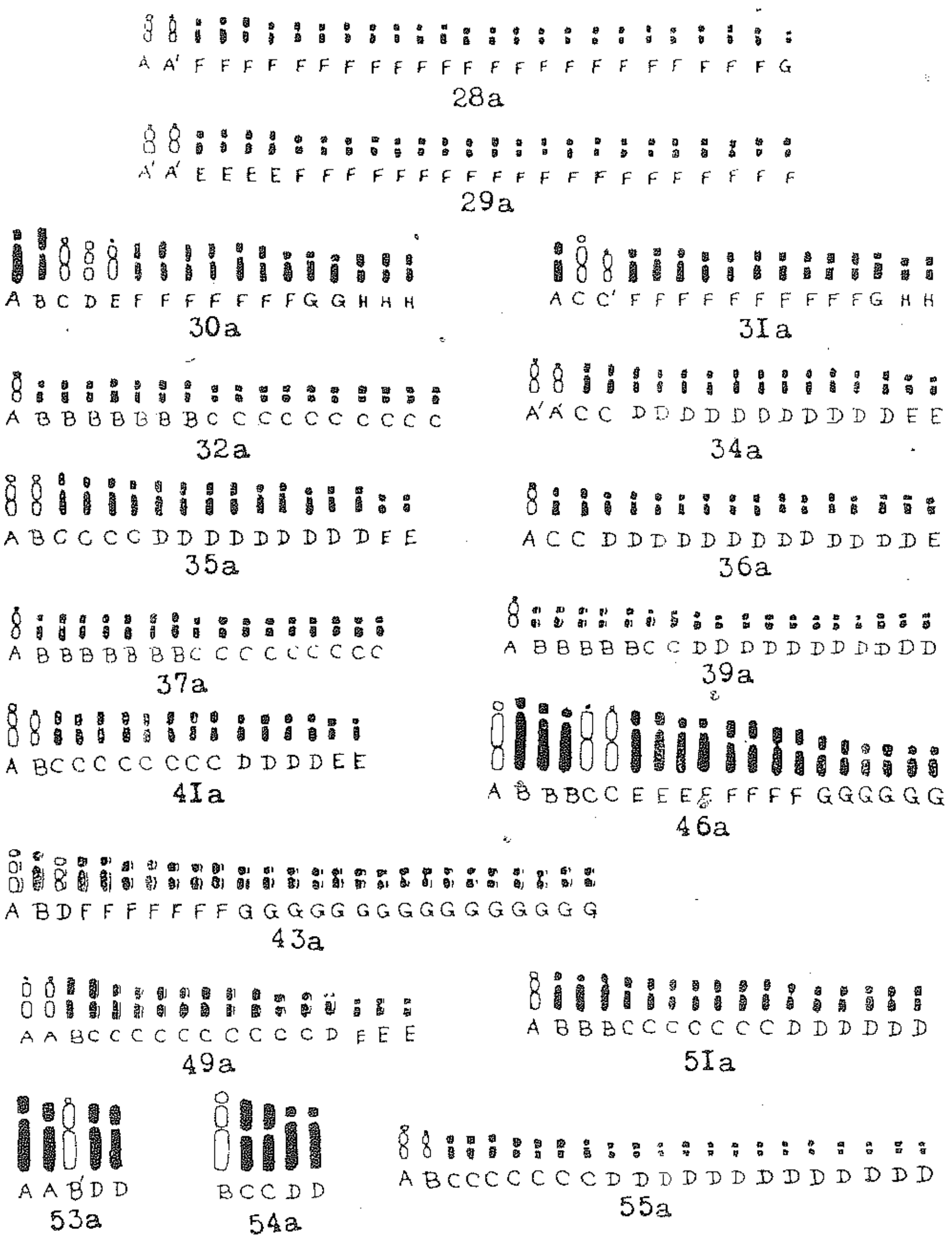

FIGS. 46-56. *Caryopteris mastacanthus* (pink), Figs. 46-48, somatic metaphase with $2n=40$, 39 & 41 chromosomes respectively. *Callicarpa macrophylla*, Figs. 49 & 50, somatic metaphase with $2n=34$ & 32 chromosomes respectively. *Congea tomentosa*, Figs. 51 & 52, somatic metaphase with $2n=34$ & 31 chromosomes respectively. *Verbena erinoides* (mauve), Fig. 53, somatic metaphase with $2n=10$ chromosomes. *V. aubletia* (red), Fig. 54, somatic metaphase with $2n=10$ chromosomes. *Nyctanthes arbortristis*, Figs. 55 & 56 ,somatic metaphase with $2n=44$ & 34 chromosomes respectively.

PLATE 13




Idiograms showing the chromosome morphology of different species and varieties of Verbenace investigated.

PLATE 14




Idiograms contd.