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## Note added in proof

Since submission of this paper three other publications have come to our notice reporting the presence of supernumerary chromosomes in Gymnosperm species: — M. V. Kruklis: Additional chromosomes in Gymnosperms (Picea obovata). Dokl. Akad. Nauk. 196, 1213 (1971). — J. H. Hunziker: Chromosome studies in Cupressus and Libocedrus. Revista de Investigaciones Agricolas 15, 169 (1961). — L. C. Saylor and H. A. Simons: Karyology of Sequoia sempervirens: karyotype and accessory chromosomes. Cytologia 35, 294 (1970). — The supernumery chromosome reported from Picea obovata is particularly interesting since it resembles the supernumerary of Picea sitchensis in both size and shape.

# Cytogenetical Studies of East Himalayan Hamamelidaceae, Combrestaceae and Myrtaceae

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#### Introduction

The improvement of hardwoods through breeding of selected types, hybridization and induction of polyploidy in trees of timber importance has great potential. It has made rapid strides in some of the advanced countries of the world. However, any such Programme on Indian hardwoods has lagged behind because of the existence of big cytological lacuna with regard to chromosome number, size and morphology, their behaviour at meiosis, frequency of polyploidy in nature and biology of flowering and fruiting. This was the main consideration which initiated the present studies on several members of the above mentioned three families

# **Material and Methods**

Material for meiotic studies was collected from wild sources in the forests of Darjeeling (latitude 27° 30′ N; longitude 91° 55′ E). But also included are 3 exotic species of Eucalyptus which are widely cultivated in the Himala-yan region. Flower buds were fixed in Carnov's fluid. Squashing of anthers was accomplished in 1% aceto-carmine. Slides were made permanent in Euparal. Figures are drawn at a uniform magnification of X 1360. Voucher specimens have been deposited in the Herbarium, Panjab University, Department of Botany, Chandigarh-14, India.

# **Results and Discussion**

Cytological observations on 24 species belonging to 7 genera and 3 families are given in Table 1, which also includes data on specific localities of collection and flowering and fruiting season. The chromosome counts for 2 genera and 16 species are first cytological reports. New chromo-

some number has been added in Terminalia belerica Roxb. The course of meiosis was normal in all taxa except for one individual of the former species which shows multivalent configurations. Detailed observations are given only for species of morphological or cytological interest.

# Hamamelidaceae

The family comprises about 23 genera and 80 species (Willis, 1966). Its representatives are found in Asia from Persia and Himalayas to Malaya, China and Japan, in North America and in South Africa, China being the centre of concentration. The family is of forestry importance as several arborescent species provide useful timber. Two representatives of the family, viz., Symingtonia populnea Van Steen. and Corylopsis himalayana Griff. were cytologically worked.

S. populnea, the lone species of the genus, is common in Darjeeling hills, thriving best on northern slopes. It is a lofty tree 25—30 m in height and 24 m in girth with a straight clean bole and is one of the best for afforestation. This representative of the monotypic genus, with n=32 (Fig. 1), is cytologically investigated for the first time. The high chromosome number is perhaps indicative of its polyploid origin. The chromosomes are small. Pollen is 100% fertile.

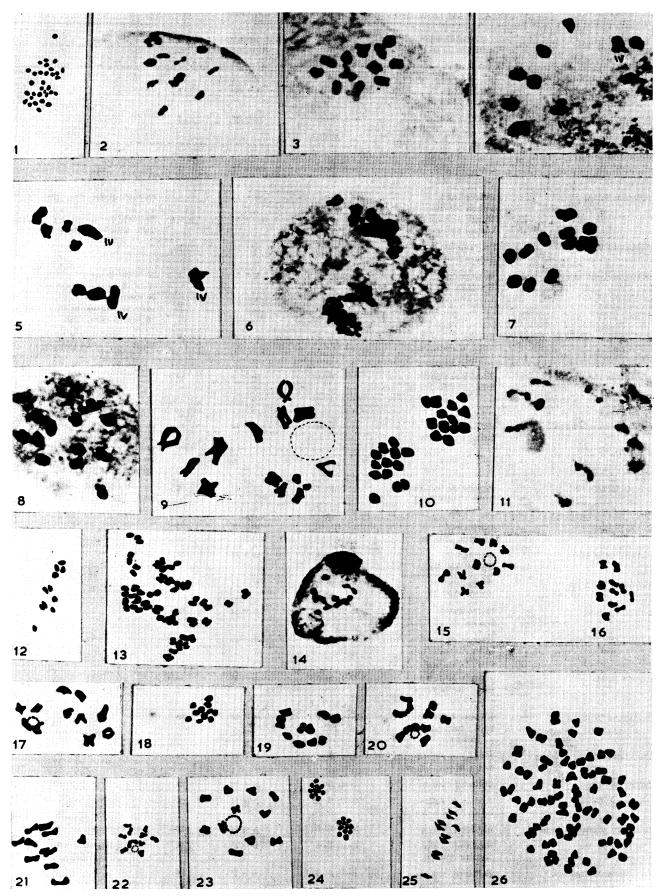
# Combretaceae

Willis (l.c.) attributes to this family 19 genera and 600 species of woody climbers, shrubs or trees, pantropical in distribution. Eight genera and about 27 species occur in India. The members of this family are well known for their tirnber and forest products. Timbers of several species of Terminalia Linn. and Anogeissus Wall. ex Guill. & Peer. are exploited for commercial purposes. The Myrabolan nuts

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Table 1. — Summary of cytological observations, alongwith specific locality, flowering and fruiting season and previous reports. For flowering and fruiting, numbers 1—12 represent months January to December.

Name	Locality with altitude in meters	Flowering and fruiting season	Chromosome number	Fig. No.	Previous reports
HAMAMELIDACEAE					
Symingtonia populnea Van Steen.	Darjeeling: Lebong 1500 m	8—9; 1—10	n = 32	1	
Corylopsis himalayana Griff.	Shillong 1500 m	5—6; 7—9	n = 12	2	
COMBRETACEAE					
Terminalia myriocarpa Heurek et Muell.	Darjeeling: Manjitar 300 m	23; 58	n = 12	3	
T. belerica Roxв.	Darjeeling: Sukna 150 m Upper Assam: Digboi 150 m	4—6; 6—9	n = 12		2n = 26: Subir Sen, 1955.
		4—6;6—9	2n = 24	4, 5, 6	2n = 48: Janaki-Ammal & Sobti 1962; Mehra & Gill, 1968)
T. crenulata Rотн.	Darjeeling: Sukna 150 m	3—5; 6—8	n = 12	7	
T. chebula Retz.	Darjeeling: Manjitar 300 m	5—6; 8—10	n = 12	8	2n = 26: Subir Sen, 1955. n = 12, 24: Janaki-Ammal & Sobti, 1962. n = 12: Mehra & Gill, 1968. n = 7: Nanda, 1962.
Combretum decandrum Roxв.	Khasia & Jaintia hills: Garampani 800 m	3—5; 7—9	n = 13	9	
C. extensum Roxb.	Khasia & Jaintia hills: Shella 300 m	46; 811	n = 13	10	
MYRTACEAE					
Eugenia jambolana Lam.	Shillong: Barapani 800 m	3—4; 5—6	n = 11	11	2n = 44: Tho, 1948. n = 22, 33: Bhaduri & Islam, 1949. 2n = 33, 55: Roy & Jha, 1962.
					n = 11, 33: Mehra & Gill, 1968
E. operculata Roxв.	Khasia & Jaintia hills: Garampani 800 m	4—5; 9—10	n = 11	12	n=11: Nanda, 1962; Mehra & Gill, 1968.
E. kurzii Duthie	Darjeeling: Lopchu, 1500 m	9—10; 1—2	n = 33	13	
E. ramosissima WALL.	Darjeeling: Sukna 150 m	2—3; 6—8	n = 11	14	
E. formosa Wall.	Darjeeling: Sukna 150 m	3—4; 8—9	n = 11	15	
E. grandis Wight	Darjeeling: Sukna 150 m	1—2; 5—6	n = 11	16	
E. mangifolia WALL.	Khasia & Jaintia hills: Garampani 800 m	3—4; 5—6	n = 11	17	
E. oblata Rохв.	Khasia & Jaintia hills: Garampani 800 m	34; 67	n = 11	18	
E. tetragona Wight	Khasia & Jaintia hills: Barapani 800 m	2—4; 5—8	n = 11	19	
E. claviflora Roxв.	Khasia & Jaintia hills: Shella 300 m	3—4; 5—6	n = 11	20	
E. fruticosa Roxв.	Shillong 1500 m	3-4; 5-6	n = 11	21	
E. khasiana Duthie	Khasia & Jaintia hills: Tharia 300 m	6—7; 9—10	n = 11	22	
Eucalyptus globulus Labill.	Shillong 1500 m	2-3; 5-8	n = 11	23	n = 11: McAulay et al., 1936.
E. tereticornis Sm.	Shillong: Lake Garden 1500 m	3—4; 7—8	n = 11	24	n = 11: Ruggeri, 1960.
E. siderophloia Вентн.	Shillong: 1500 m	4—5; 6—7	n = 11	25	
Decaspermum fruticosum Forst.	Khasia & Jaintia hills: Cherrashella road 700 m	4—5; 7—8	n = 44	26	



Figs. 1—26. — Fig. 1. Symingtonia populnea. 32 chromosomes at A-II (one pole drawn). — Fig. 2. Corylopsis himalayana. M-I shows 12 bivalents. — Fig. 3. Terminalia myriocarpa. 12 large sized bivalents at M-I. — Figs. 4—6. Terminalia belerica. — Fig. 4. M-I with  $2_{\text{IV}} + 8_{\text{II}}$ . — Fig. 5. M-I with  $3_{\text{IV}} + 6_{\text{II}}$ . — Fig. 6. Laggards at late A-I. — Fig. 7. T. crenulata. M-I shows 12 large sized bivalents — Fig. 8. T. chebula.

of which India is the principal exporter is obtained from dried fruits of *T. belerica* Roxb. and *T. chebula* Retz. Six species in 2 genera, viz., *Terminalia* and *Combretum* Linn. have been presently investigated.

Terminalia is a pantropical genus of about 250 species (Willis, l. c.). In India, the genus is represented by 9 species of commercial woods. All the four species of the genus currently investigated possess haploid complement of 12 large sized chromosomes. T. myriocarpa Heurek et Muell. is a first class quick growing timber species of Eastern Himalayas. It grows luxuriantly in Namchik forests (150-200 m) around NEFA in gregarious patches of almost pure formation. Exceptional trees with 11-12 m girth have been noticed. The species attains a height of 35-40 m with a clean straight bole upto 15 m high. At M-I, 12 large bivalents are counted (Fig. 3). Pollen are 100% fertile. Meiotic course is normal in all the other species of the genus, except for an individual tree of T. belerica from Digboi, upper Assam in which abnormality is marked by 1-3 quadrivalents leading to laggards at late A-I (Figs. 4, 5, 6) and pollen sterility of 15-20%. Such aberration is obviously due to structural changes.

T. belerica with n=12 is a new chromosomal report and reveals the occurrence of diploids. Tetraploidy has been reported earlier in this species (Janaki-Ammal and Sobti, 1962; Mehra and Gill, 1968). Several varieties exist in T. chebula but they all belong to 2 cytological races, diploid and tetraploid. According to Janaki-Ammal and Sobti (l. c.) there is correlation between ploidy, fruit size and tannin contents. All the remaining cytologically known species of the genus Terminalia are diploid on x=12. The chromosome counts of Subir Sen (1955) 2n=26 for T. chebula, T. catapa Linn., T. arjuna Bedd. and T. belerica and Nanda (1962) n=7 for T. chebula and 2n=14 for T. tomentosa Wt. and Arn. are not in accord with the present findings. As such it is argued that the genus is monobasic on x=12, and these reports are erroneous.

Combretum is a large genus of trees and shrubs, generally climbing. Two species, viz., C. decandrum Roxb. (Fig. 9) and C. extensum Roxb. (Fig. 10) with n=13 are established for the first time. Other counts reported for the genus are n=28 for C. coccineum Lam. (Nanda, l. c.) and 2n=32 for C. lecardii Engl. et Diels (Miège, 1962).

# Myrtaceae

It includes 100 genera and 3,000 species, almost entirely tropical, with two centres of distribution, *Myrtoideae* in tropical America and *Leptospermoideae* in Australia (Willis, l. c.). The family is represented in India by at least 4 genera and 60 species. It is an important family from the forestry point of view. The fast growth, great climatic adaptation and multifarious uses of the wood of different species of *Eucalyptus L'Herit* have led to their introduction in many parts of the world. Sixteen species confined to 3

genera, viz., Eugenia Linn., Eucalyptus and Decaspermum Forst. were presently investigated.

Eugenia\*) Linn. includes about 1,000 species confined to tropical and subtropical America, Asia, Africa and Australia. Amongst the 12 investigated species, E. jambolana Lam. and E. operculata Roxb. are of considerable timber importance. The chromosome counts of n = 11 (Figs. 11, 12), for both of these species are in agreement with earlier similar reports (Table 1). The other diploid species of the genus worked out for the first time are E. ramosissima (Fig. 14), E. formosa (Fig. 15), E. grandis (Fig. 16), E. mangifolia (Fig. 17), E. oblata (Fig. 18), E. tetragona (Fig. 19), E. claviflora (Fig. 20), E. fruticosa (Fig. 21) and E. khasiana (Fig. 22). On the other hand E. kurzii has turned out to be hexaploid with n = 33 (Fig. 13).

A perusal of the literature reveals (Fig. 27) that some of the cultivated species of Eugenia are found in different cytological races. For example, in E. jambolana 2x, 3x, 4x, 5x and 6x races are now known (Table 1). Likewise, in E. jambos 2x and 3x races, and in E. javanica a whole series 3x, 4x, 5x, 6x, 8x and 10x taxa have been discovered (Bhaduri and Islam, 1949, Roy and Jha, 1962). A detailed study is needed in these species to correlate the cytological data with the mechanical strength and quality of the wood.

Eucalyptus is an Australian genus consisting of about 230 Australian species (Willis, l. c.) Eucalyptii are the most important trees of the Australian continent and some of the timbers (E. marginata Sm. and E. diversicolor von MUELL.) are exported for various purposes. They are stated to be among the largest trees of the world. Three species of the genus E. globulus Labill. (Fig. 23), E. tereticornis Sm. (Fig. 24) and E. siderophloia Benth. (Fig. 25) have been presently investigated and all found to possess n = 11. The cytological report for the former two species are in line with the previous studies of McAulay et al. (1936) and Ruggeri (1960), respectively, while the third has been investigated for the first time. So far a total on 52 species in the genus have been investigated of which E. redunea Sch., E. corynocalyx Muell. and E. flocktoniae Maid. have 2n=24, the rest being based on x=11. According to HERBERT (1929), the genus Eucalyptus appeared in the Cretaceous period in Australia. Thereafter it has undergone many climatic, geological and geographical changes and has become stable in the diploid form with a basic set of x = 11. Secondarily derived is the base number x = 12met in the above mentioned three species. Hybrids and hybrid swarms among Eucalyptus have been described frequently (Maiden, 1914; Blakely, 1955; McAulay, 1937; Brett, 1937), indicating extensive cross fertility among species and emphasising close taxonomic relationship. Very likely, speciation in the genus has been at ecospecific level

<sup>\*)</sup> The genus Eugenia is split in 3 genera, viz., Syzygium GAERIN., Jambosa DC. and Eugenia Mich ex Linn. However, because of the confusion in the namenclature of the Indian species, the old arrangement including all these into the traditional genus Eugenia has been adhered to.

<sup>12</sup> large sized bivalents at M-I. — Fig. 9. Combretum decandrum. 13 bivalents and a nucleolus at diakinesis, one bivalent shows early disjunction. — Fig. 10. C. extensum. 13 chromosomes at each pole. — Fig. 11. Eugenia jambolana. 11 loosely associated bivalents at diakinesis. — Fig. 12. E. operculata. M-I shows 11 bivalents. — Fig. 13. E. kurzii. Mixed A-I shows 66 chromosomes. — Fig. 14. E. ramosisima. M-I with 11 bivalents. — Fig. 15. E. formosa. Diakinesis shows 11 bivalents and a nucleolus. — Fig 16. E. grandis. M-I with 11 bivalents with 11 bivalents and a nucleolus, two bivalents are attached to the nucleolus. — Fig. 18. E. oblata. M-I with 11 bivalents. — Fig. 19. E. tetragona. M-I shows 11 bivalents. — Fig. 20. E. claviflora. Diakinesis with 11 bivalents and a nucleolus. — Fig. 21. E. fruticosa. M-I with 11 bivalents. — Fig. 22. E. khasiana. Diakinesis shows 11 bivalents and a nucleolus. — Fig. 23. Eucalyptus globulus. Diakinesis with 11 bivalents and a nucleolus, one bivalent is attached to the nucleolus. — Fig. 24. E. tereticornis. A-I with 11 chromosomes at each pole. — Fig. 25. E. siderophloia. M-I with 11 bivalents. — Fig. 26. Decaspermum fruticosum. Mixed A-I shows 88 chromosomes.

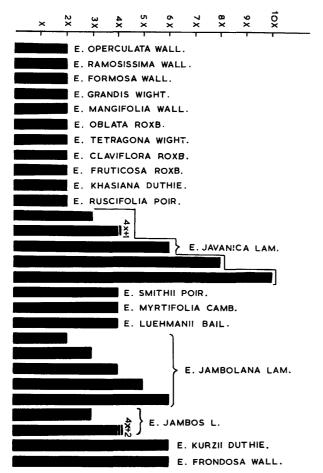


Fig. 27. — Graphic representation to show level of ploidy in cytologically known species of Eugenia.

involving either gene mutation or rapatterning of chromosomes.

Decaspermum with its 4 species is distributed in tropical Asia and Australia, and the Pacific islands. The lone Indian species is D. fruticosum Forst., distributed in the Khasia and Jaintia hills as a shrub or a small tree. At mixed A-I 88 chromosomes are discernible (Fig. 26). It is a new report for the genus falling in the series x=11, thus occurring in nature at the octaploid level.

A perusal of the literature along with the present findings show that in the family Myrtaceae 160 species in 28 genera are known cytologically with an array of base numbers x = 6, 8, 9, 11, 12. Of these, x = 11 is the most common. Smith-White (1942), on the basis of general occurrence of secondary pairing in Eucalyptus and Tristania R. Br. suggested x = 7 as the base number of the family. Airchison (1947) challenged the validity of this view on the grounds of uniformity of chromosome number within the family and its antiquity. Later, Smith-White (1948) suggested x=6 as the base number of the family. This conclusion he drew from the analysis of secondary association together with some supporting evidence of prophase behaviour in Eucalyptus paniculata. We believe that this hypothesis is further strengthened by the existence of x = 6 in Darwinia Rudge and Actinodium Sch. (cf. Darlington and Wylie, 1955) which are members of a primitive tribe in the family. It seems reasonable to postulate that the 11-chromosome genome has evolved by doubling and loss of a chromosome and has proved more successful in the evolutionary development of the family. SMITH-WHITE (1950) opined that gene mutation, small chromosomal alterations and segregation following hybridization were responsible for all speciation in the Australian *Myrtaceae*.

## Acknowledgements

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#### Summary

Cytological studies of 24 species comprising 7 genera belonging to 3 families viz., Hamamelidaceae (2), Combretaceae (6) and Myrtaceae (16) from the East Himalayas have been carried out. Small size of chromosomes is characteristic of all the species except Combretaceae which are endowed with large sized chromosomes. 6 has been suggested to be the original base number of the family Myrtaceae. Two instances of polyploidy have been noted i. e.,  $Eugenia\ kurzii\ (n=33)$  and  $Decaspermum\ fruticosum\ (n=44)$ . Euploidy is altogether absent in Eucalyptus. The monotypic genus Symingtonia has a high base number, x=32.

### Zusammenfassung

2 Arten der Hamamelidaceae, 6 der Combretaceae und 16 der Myrtaceae aus dem Ost-Himalaja wurden cytologisch untersucht. Mit Ausnahme der Arten der Combretaceae hatten alle anderen kleine Chromosomen. Als ursprüngliche Chromosomen-Grundzahl wird 6 für die Familie der Myrtaceae angenommen. Zwei Beispiele von Polyploidie stellte man fest: Eugenia kurzii mit n=33 und Decaspermum fruticosum mit n=44. Die Gattung Symingtonia hatte die hohe Chromosomen-Grundzahl von x=32.

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