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Cytological Studies of Himalayan Rutaceae

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Introduction

The family Rutacae embraces 1,600 species in 150 genera (BRIZICKY, 1962). It is widely distributed throughout the warmer regions of the world, extending into temperate regions of Europe, Asia and America. The family is best represented in S. Africa and Australia. About 50 species among 20 genera are reported from India, out of which 9 species are classed as commercial timbers (PEARSON & BROWN, 1932). The most important genus is Citrus LINN. which yields the well known citrus fruits of commerce.

SMITH-WHITE (1954, 1959) made a detailed study on the cytology of Australian Rutaceae, Tribe Boronieae. Almost a complete lack of cytological data on Indian species initiated the present investigations on the Himalayan taxa. The aim of the work has been to determine their chromosomal constitution, process of meiosis, pollen fertility and biology of flowering and fruiting.

Material and Methods

Material for meiotic studies was collected from wild sources in the forests of Nainital (lat. 29°. 22' N, long. 79°. 29' E), Darjeeling (lat. 27°. 30', long. 88°. 18' E) and Khasia and Jaintia hills (lat. 25°. 40' N, long. 91°. 55' E). Flower buds were fixed in CARNOY's fluid. Squashing of anthers

Table 1

Name	Locality	Flowering & fruiting period	Chromosome number	Fig. No.	Previous reports
+*Evodia fraxinifolia* Hook. f.	Darjeeling: Senchal forest, 2,500 m; Shillong: Barapani, 800 m.	3—5; 6—9	n = 39	2	2n = 72: Bowden, 1945
**E. meliaefolia* Benth.	Darjeeling: Sukna, 150 m; Assam; Digboi, 150 m.	4—6; 9—10	n = 18	14	
Aegle marmelos Correa	Darjeeling: Manjitar, 300 m;	4—5; 7 onwards	n = 18	15	2n = 36: Janaki-Ammal vide Darlington & Wylie, 1955.
	Nainital; Ranibag, 600 m.		n = 9	3	n = 9 or 2n = 18: Banerji & Pal, 1957; Raghavan, 1957; Nanda, 1962.
Murraya paniculata (L.) Jack.	Khasia & Jaintia hills: Tharia, 300 m; Nainital: Nalina, 1,200 m.	3—6; 7 onwards	n = 9	16	n = 9 or 2n = 18: Toxopeus, 1933; Pathak *et al.*, 1949; Raghavan, 1957.
M. koenigii Spreng.	Khasia & Jaintia hills; Dawki, 300 m.	3—5; 7—8	n = 9	4	2n = 18: Raghavan, 1957; Janaki-Ammal vide Darlington & Wylie, 1955.
**Zanthoxylum limonella* Alston	Darjeeling: Sukna, 150 m; Khasia & Jaintia hills: Dawki, 300 m.	4—5; 9 onwards	n = 34	5	
**Z. armatum* DC.	Darjeeling: Tonglu, 300 m; Nainital: Sariatal, 1,600 m.	5—6; 7 onwards	n = 33	17	
**Z. acanthopodium* DC.	Darjeeling: Jalapahar, 2,300 m.	7—9; 10 onwards	n = 32	6	
**Z. ovalifolium* Wight.	Khasia & Jaintia hills: Pongtong reserve, 1,200 m.	3—4	n = 18	18	
	Darjeeling: Mongpu, 900 m;	4—6	n = 34	7	
	Khasia & Jaintia hills: Sorrarim, 1,500 m.	3—4	2n = ca. 136	8, 9	
**Z. oxyphyllum* Edgew.	Darjeeling: Jalapahar, 2,300 m; Nainital: Lariakanta, 2,400 m.	3—6; 7 onwards	n = 36	10	
**Z. nitidum* DC.	Darjeeling: Rongtong, 600 m.	4—5; 6—7	n = 34	11	
**Z. scandens* Bl.	Shillong: Shillong peak, 1,800 m.	5—6; 8 onwards	n = 34	12	
**Z. tomentellum* Hook. f.	Sikkim		n = 36		
**Clausena pentaphylla* DC.	Nepal		n = 18		
**C. heptaphylla* Wight. & Arn.	Khasia & Jaintia hills	4—5; cold season	n = 18		
**Acronychia penduculata* (L.) Miq.	Darjeeling: Rambi, 500 m; Shillong: Botanical garden, 1,500 m.	4 onwards; winter	n = 18	19	
+*Toddalia asiatica* Schult.	Shillong: Barapani, 800 m.	1—10; 8—1	n = 18	13	
***Micromelum integerrimum* Wight. & Arn.	Khasia & Jaintia hills: Shella, 300 m.	12—2; 3—4	n = 9	20	
**Paramignya monophylla* Wight.	Khasia & Jaintia hills	4; 8	n = 18		
**Skimmia laureola* Hook. f.	Sikkim: Yoksum, 2,500 m.	4; rainy season	n = 15	21	

**Genus worked out for the first time.
*Species worked out for the first time.
+New cytotype.

was accomplished in 1% acetocarmine. Slides were made permanent in Euparal. Stomatal studies (in case of *Zanthoxylum ovalifolium)* were made from the centre of the dried leaves treated in 5% KOH at 60° C for about 2 hrs. The peels were stained in 1% safranin and mounted in 50% glycerine. Pollen fertility was estimated by their capacity to stain uniformly with aceto-carmine. All figures are at a uniform magnification of ×1360. Voucher specimens have been deposited in the Herbarium Panjab University, Chandigarh-14 (India). Numerical strength of the genera is adopted from WILLIS (1966).

Results and Discussion

Cytological data on 23 taxa belonging to 20 species in 10 genera are given in *Table 1* along with specific localities of collection and flowering and fruiting periods. The course of meiosis was normal in all cases except in an intraspecific race of *Zanthoxylum ovalifolium* which showed multivalent configurations. Detailed observations are given only for species of cytological interest.

Zanthoxylum LINN. includes 200 species which are largely pantropical in distribution. Ten species occur in India. The genus is represented by *Z. limonella (Fig. 5)* in the plains, but the other species are restricted to montane and sub-montane regions. All the 8 species, namely, *Z. ovalifolium*, n = 18, 34; 2n = ca. 136 *(Fig. 7—9)*; *Z. acanthopodium*, n = 32 *(Fig. 6)*; *Z. armatum*, n = 33 *(Fig. 17)*; *Z. nitidum*. n = 34 *(Fig. 11)*; *Z. scandens*, n = 34 *(Fig. 12)*; *Z. limonella*, n = 34; *Z. oxyphyllum*, n = 36 *(Fig. 10)*; and *Z. tomentellum* n = 36 are cytologically investigated for the first time. Since 18 is the lowest haploid chromosomal number (falling in series with x = 9, which is a common base number of the family), it can be assumed as the base number of the genus. *Z. oxyphyllum* and *Z. tomentellum* would then be tetraploids, whereas the others are aneuploids at the tetraploid level.

The current work on *Z. ovalifolium* has shown the occurrence of three cytotypes. The population from Darjeeling Hills (Mongpo, 1,500 m) revealed n = 34 at M-I. Meiosis was normal with 100% fertile pollen. Two taxa were discovered from Khasia and Jaintia Hills in Assam. The one from Pongtong reserve forest (1,200 m) showed n = 18 *(Fig. 18)* with regular meiosis, while that from Sorrarim (1,500 m) exhibited highly abnormal meiosis with 2n = ca. 136. Chromosomes were sticky forming multivalents at M-I. The following 3 configurations were noticed:

$$1_{XII} + 3_{IV} + 1_{III} + 54_{II} + 1_{I},$$
$$1_{XII} + 1_{VI} + 4_{IV} + 50_{II} + 2_{I},$$
$$2_{IV} + 64_{II}$$

Pollen sterility was about 20—25%.

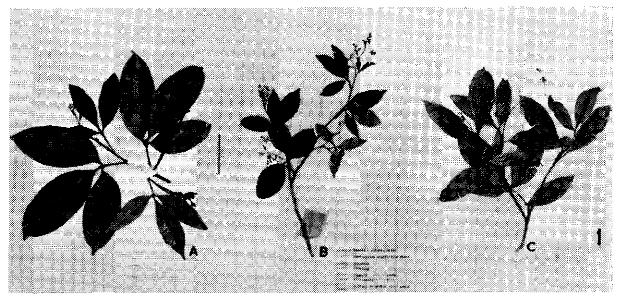
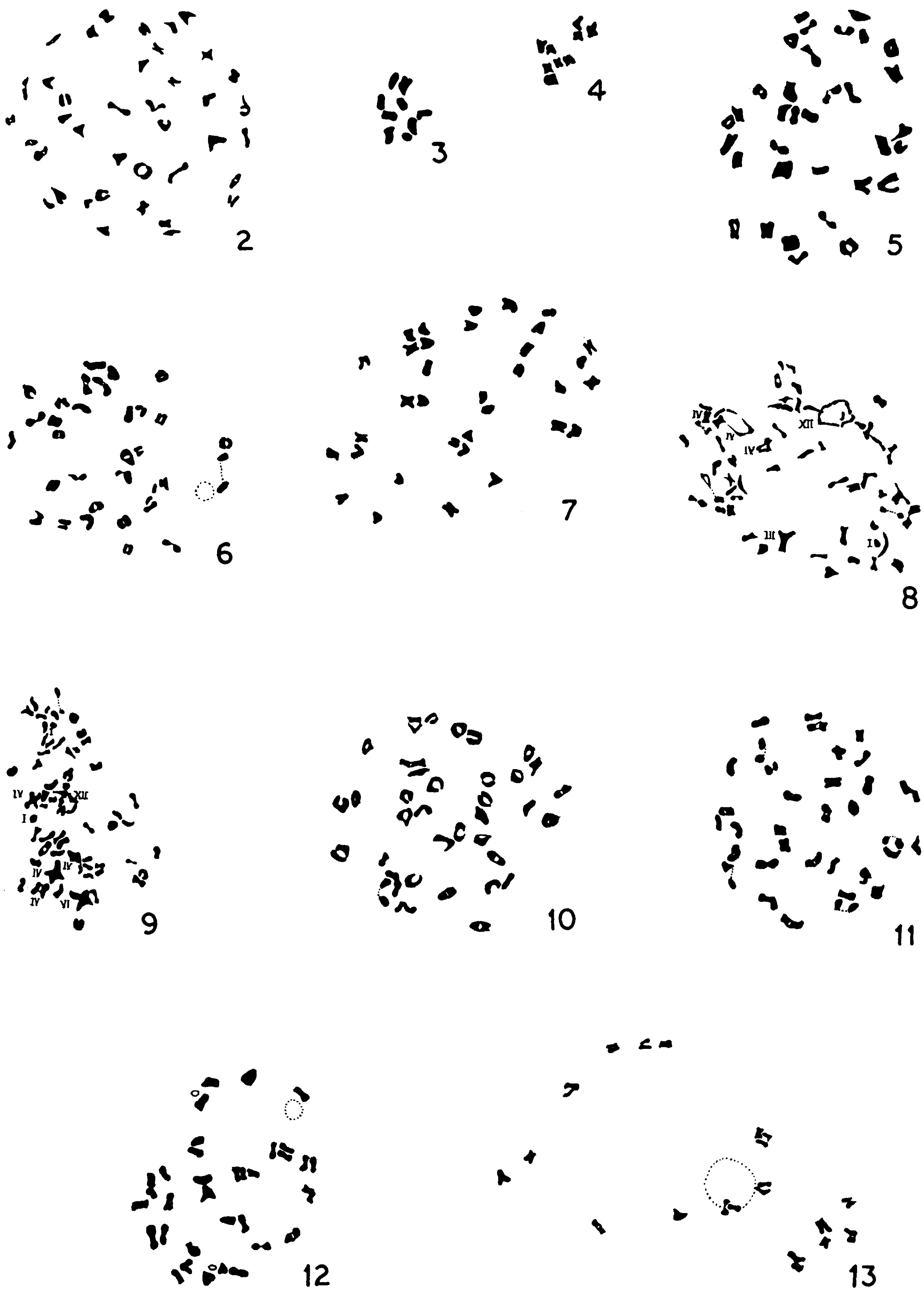


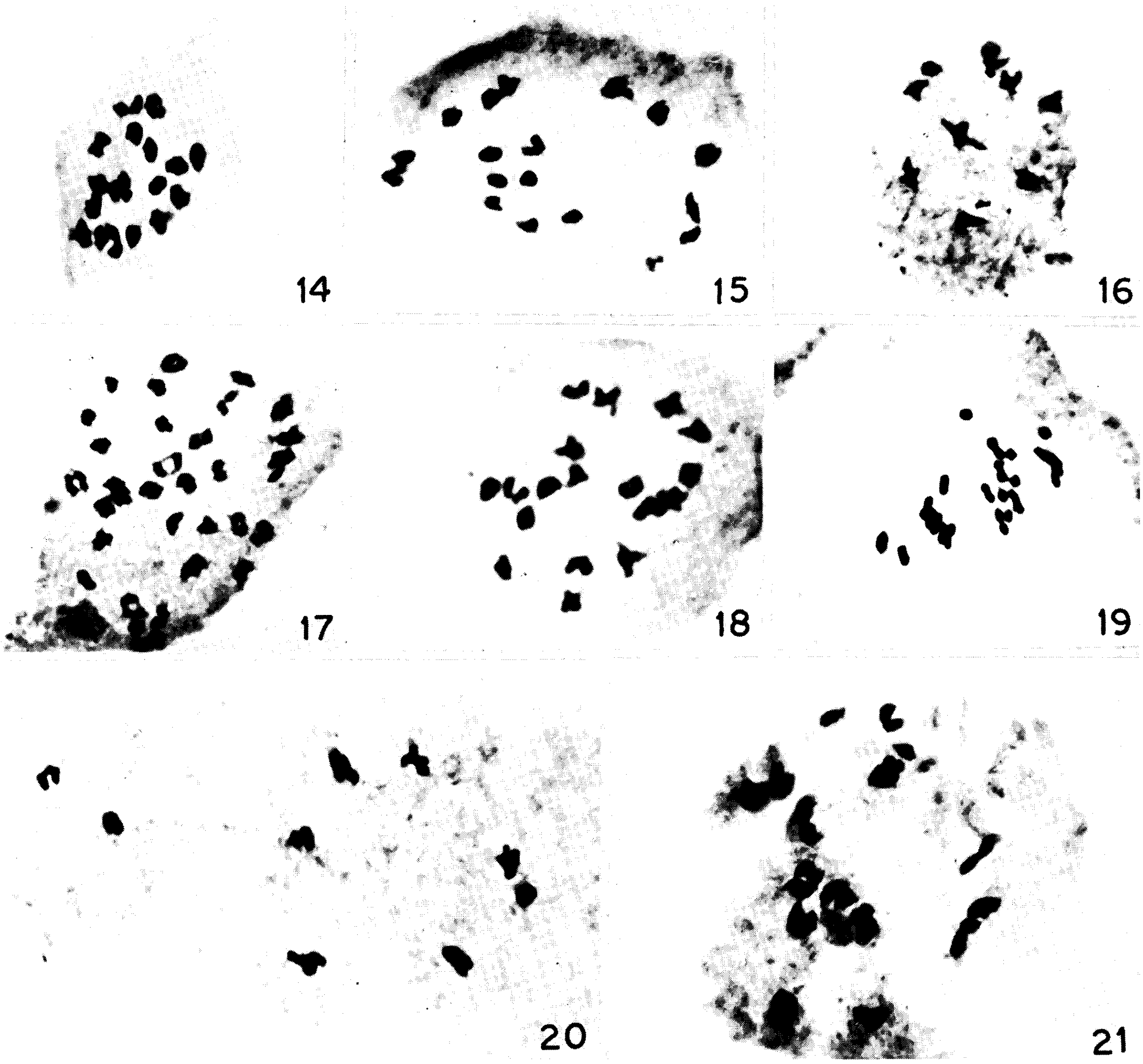
Fig. 1. — Cytotypes of *Zanthoxylum ovalifolium*, showing morphological variation. A (2n = ca.136), B (n = 18), C (n = 34). A and B from Khasia and Jaintia Hills, C from Darjeeling.

Table 2

Characters	Darjeeling type n = 34	Khasia & Jaintia hill types Pontong reserve n = 18	 Sorrarim 2n = ca. 136
Habitat	Terai and lower hills upto 1,500 m	Subtropical forests upto 1,200 m	Subtropical or subtemperate forests upto 1,800 m.
Habit	Bushy shrub, unarmed	Small tree upto 4—6 m in height, armed with a few conical prickles	Small tree upto 8—12 m in height, profusely armed with prickles with broad base
Leaflet size.	6.0—9.3 cm × 2.3—3.7 cm.	4.4—6.5 cm × 1.9—3.6 cm	8.0—15.3 cm × 2.5—4.4 cm
Shape	Ovate to ovatelanceolate, apex not so acute, sub-coriaceous, entire, veins 9—19 on each side of the midrib, venation obscure.	Ovate to lanceolate, apex acute, coriaceous, margin entire to sub-entire, venation prominent, tertiary nerves netted.	Lanceolate, with acute long pointed acuminate apex, coriaceous, margin broadly dentate, veins 14—25 on each side of midrib, venation prominent, tertiary nerves netted.
Colour	Dark brown	Olive green	Olive green
Stomatal size	30.2—32.8 μ	26.2—29.9 μ	33.9—35.4 μ
Flowers	0.3—0.6 cm in diameter, white	0.2—0.4 cm in diameter, greenish	0.4—0.6 cm in diameter, greenish
Flowering season	April—June	March-April	March—April
Meiosis	Normal	Normal	Abnormal
Pollen size	19.3—21.4 μ	18.4—22.8 μ	22.9—24.7 μ
Fertility	100%	100%	75—80%



Figs. 2—13. — *Fig. 2.* — *Evodia fraxinifolia*, metaphase I, n = 39. —*Fig. 3.* — *Aegle marmelos*, metaphase I, n = 9. — *Fig. 4.* — *Murraya koenigii*, metaphase I, n = 9. — *Fig. 5.* — *Zanthoxylum limonella*, metaphase I, n = 34. — *Fig. 6.* — *Z. acanthopodium*, diakinesis, n = 32. — *Figs. 7—9.* — *Z. ovalifolium*. — *Fig. 7.* — Metaphase I, n = 34. — *Fig. 8.* — Metaphase I, 2n = ca. 136. $1_{XII} + 3_{IV} + 1_{III} + 54_{II} + I_{I}$. — *Fig. 9.* — Metaphase I, 2n = ca. 136, $1_{XII} + 1_{VI} + 4_{IV} + 50_{II} + 2_{I}$. — *Fig. 10.* — *Z. oxyphyllum*, metaphase I, n = 36. — *Fig. 11.* — *Z. nitidum*, metaphase I, n = 34. — *Fig. 12.* — *Z. scandens*, diakinesis, n = 34. — *Fig. 13.* — *Toddalia asiatica*, diakinesis, n = 18.



Figs. 14—21. — Fig. 14. — Evodia meliaefolia, metaphase I, n = 18. — *Fig. 15. — Aegle marmelos*, metaphase I, n = 18. — *Fig. 16. — Murraya paniculata*, diakinesis, n = 9. — *Fig. 17. — Zanthoxylum armatum*, diakinesis, n = 33. — *Fig. 18. — Z. ovalifolium*, metaphase I, n = 18. — *Fig. 19. — Acronychia penduculata*, metaphase I, n = 18. — *Fig. 20. — Micromelum integerrimum*, metaphase I, n = 9. — *Fig. 21. — Skimmia laureola*, diakinesis, n = 15.

The distinguishing characters of the 3 races are summarized in *Table 2*. The population from Darjeeling hills is widely different morphologically from the taxa thriving in the Khasia and Jaintia hills *(Fig. 1)*. The former is unarmed and fits in with the description given by Hooker (1872) of *Z. ovalifolium* var. *ovalifolium*, while the latter two taxa which possess straight prickles are included in *Z. ovalifolium* var. *sepiarium*. These latter are alike in all respects except for the gigas nature of the cytotype with 2n = ca. 136 which also showed slight increase in the stomatal and pollen size which may be attributed to the increase in chromosome number. Earlier Wight (cf. Hooker, l. c.) had treated the two varieties as distinct species designating them as *Z. ovalifolium* and *Z. sepiarium*. These were merged by Hooker (l. c.) as mere varieties of *Z. ovalifolium*. Considering the cytological data now available, the morphological features, and the pattern of distribution, the two varieties, in the opinion of the authors, should be given distinct specific status as suggested by Wight (l. c.). However, the taxa from the two different localities of Khasia and Jaintia hills should be treated as intraspecific races since the increase in chromosome number is connected only with the quantitative augmentation of morphological features.

Aegle Corr. includes 4 species, 2 in the Indo-Malayan region, 1 in Japan and 1 in tropical West Africa. The Indian representative, *A. marmelos*, is well known, both for its timber and medicinal importance of its fruit. Two cytotypes have been observed. A large fruited type from the Eastern Himalayas showed 18 bivalents at M-I (tetraploid) *(Fig. 18)*, while a small fruited type from the Western Himalayas exhibited n = 9 (diploid). Comparative morphological studies showed that there are no appreciable differences in the two cytotypes, excepting for fruit size.

Evodia Forst. embraces 45 species of trees and shrubs distributed from Madagascar through India to Malay Archipelago. Two of its species, *E. fraxinifolia* n = 39 and *E. meliaefolia* n = 18 *(Fig. 14)* have been presently investigated. The report for the former species is at variance with BOWDEN (1945) who gave n = 36 for it. The species has been presently investigated from 3 distant localities in Darjeeling and Khasia and Jaintia hills, always exhibiting the same chromosome number. DARLINGTON & WYLIE (1955) have documented x = 9 as the base number of the genus. Since minimum gametic number recorded till today for *Evodia* is 18, the possibility of this as being its base number is more probable. As such *E. fraxinifolia* is a hypertetraploid.

Murraya KOEN. ex LINN. comprises 5 species distributed in the Indo-Malayan region. The haploid number for both the Indian representatives, viz. *M. paniculata (Fig. 16)* and *M. koenigii* is 9. The counts for these are in agreement with the previous reports *(Table 1)*.

Clausena BURM. comprises 14 species chiefly confined to tropical Asia. A few are met with in Africa and Australia. *C. pentaphylla* and *C. heptaphylla* with n = 18 are new chromosomal reports. DARLINGTON and WYLIE (l. c.) gave x = 9 as the base number of the genus and on this basis both the species are tetraploid.

Acronychia FORST. embraces 20 species which are native of tropical Asia and Australia. The lone Indian member which is distributed in Sikkim Himalayas and Eastern India, possesses n = 18. It is a new report for the species.

Toddalia JUSS. includes 8 species distributed in tropical and subtropical Asia, Africa and Australia. Two species occur in India. *T. asiatica* with n = 18 *(Fig. 13)* is a new chromosomal report. MINFRAY (1963) reported 2n = 72 for the species. The species, thus, exists in nature at diploid as well as tetraploid levels.

Micromelum BL. with 4 species is distributed in tropical Asia. The Indian species *M. pubescens* is met with in the Central and Eastern Himalayas. It shows n = 9 chromosomes. It is a new report for the genus and suggests x = 9 as its base number.

Paramignya WIGHT. includes 4 species distributed in the Indian sub-continent. *P. monophylla,* a common shrub of Sikkim Himalayas, with n = 18 is a new report for the genus.

Skimmia THUNB. with 4 species is indigenous to Himalayas and Japan. *S. laureola* with n = 15 *(Fig. 21)* is a new report for the species and is at variance with a previous report of n = 16 for *S. formanii* (DESAI, 1960) in the genus.

Conclusions

Meiotic behaviour

All the species investigated, except an intraspecific race of *Zanthoxylum ovalifolium,* showed normal meiosis. All the species possess small chromosomes which, therefore, will have low chiasma frequency and as such chromosomal aberrations of small magnitude, if present, would be hard to detect. The meiotic irregularities in *Z. ovalifolium* with 2n = ca. 136 may be traced to homologies between the chromosomes. It is suspected to be an autopolyploid in which diploidisation has not yet been achieved.

Base number

A perusal of the literature coupled with the present findings show that x = 9 is the original base number of the family and characterises all the tribes except Boronieae, where secondary numbers are more frequent forming more or less a continuous series from 7—19 (10,15 excepted). Accordingly, all the other numbers have probably originated through modifications in the primitive chromosome complement of x = 9 either by a stepwise increase or decrease in the base number or by polyploidy. SMITH-WHITE (1954, 1959) correlated the chromosomal diversity in the tribe Boronieae to the delimitation of various genera. According to him polyploidy has also played a significant role in the evolution of groups of generic status and also of species within the genera.

Polyploidy

Out of a total of 23 taxa cytologically investigated 10 are diploid, 5 tetraploid, 1 hypertetraploid, 6 hypotetraploid and 1 hypooctaploid. Thus, 56.5% of the total number of investigated taxa are at the various polyploid levels. This is much higher than the percentage of polyploidy in angiosperms in general which is 30—35% (STEBBINS, 1950; TISCHLER, 1950; DE WOLF, 1957). A chromosomal survey of the genera presently investigated, also taking into account the previously investigated species belonging to them (see *Table 3),* shows even higher percentage of polyploidy, i. e., 60.2%.

The currently established data of high percentage of polyploidy in Rutaceae, a tropical woody family, are not in line with the views expressed earlier by STEBBINS (1938), based on the data on temperate species. MEHRA & BAWA (1970) and MEHRA (1972) have recently contradicted the view of the rarity of polyploidy in woody species. In all probability, as also suggested by MORTON (1966), the differences in the innate potentialities of different genera to form polyploids is the determining factor rather than simply the woody habit.

Table 3.

Genus	No. of taxa	
	Diploid	Polyploid
Zanthoxylum	2(1)	15(9)
Aegle	1(1)	1(1)
Evodia	1(1)	4(1)
Murraya	2(2)	—
Clausena	1(—)	2(2)
Acronychia	2(1)	—
Toddalia	1(1)	1(—)
Micromelum	1(1)	—
Paramignya	1(1)	—
Skimmia	2(1)	—
Total	14(10)	23(13)

Note: Numbers outside the parenthesis refer to the total of previously and currently investigated taxa.
Numbers inside the parenthesis refer to only the currently investigated taxa.

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Summary

Cytological studies of 23 taxa belonging to 20 species in 10 genera from the Himalayas have been carried out. The chromosome counts for *Micromelum pubescens* and *Paramignya monophylla* are the first cytological reports for the genera to which they belong. New chromosomal numbers have been documented in *Evodia fraxinifolia* and *Toddalia asiatica.* The chromosomal counts for rest of the species except three have been made for the first time. Intraspecific polyploidy has been noticed in *Aegle marmelos* and *Zanthoxylum ovalifolium.* The elevation of the variety

sepiarium of the latter to distinct specific rank as suggested by WIGHT is supported on the basis of cytomorphological analysis and pattern of distribution. A high percentage of polyploidy has been noticed in the species presently investigated.

Key words: Rutaceae Himalayas, cytology.

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Cytology of Some Woody Species of Rosaceae from Himalayas

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Introduction

Rosaceae is a large family covering about 100 genera with 2,000 species (WILLIS, 1973), which are cosmopolitan but abundant in the temperate regions. Over 200 species belonging to about 25 genera are met with in India. The members exhibit varied habit being trees, shrubs or herbs. The family is best known for its ornamentals and edible fruits. *Pyrus, Parinarium, Parastemon* and *Pygeum* embrace species which yield timber of considerable importance. Some genera provide woods of local value. Some species of *Cotoneaster,* above the tree line, on account of their compact growth and strong root system, hold up masses of snow, thus aiding in the prevention of erosion.

Materials and Methods

The material for the present study was collected from the wild populations in the Himalayas. For meiotic studies, flower buds were fixed in CARNOY's fluid. The chromosomes were stained in acetocarmine. All figures are at a uniform magnification of $\times 1360$. The voucher specimens have been deposited in the Herbarium, Panjab University Botany Department, Chandigarh.

Results and Discussion

Table 1 summarises the data in respect of the investigated species. The genera and species are arranged according to their forestry importance. Flowering and fruiting months are designated by numbers, e. g. 4 denotes April, 6 denotes June etc. All species except *Cotoneaster bacillaris* displayed normal meiosis.

Prunus is represented in India by 18 species. The genus, in its natural range, is restricted to the Himalayas and Assam except for one species in the Andaman. *P. cerasoides* ($n = 8$) and *P. cornuta* ($n = 16$) *(Fig. 2)* are the only two commercially recognized timber species of Rosaceae in India. The latter is a moderate sized graceful tree met with at higher elevations between 2,000—3,000 m, especially in moist localities, and is commonly associated with *Abies pindrow, Quercus semecarpifolia* and *Q. dilatata (Fig. 1). P. nepalensis* ($n = 16$) is worked out for the first time *(Fig. 5).*

There are 23 species of *Pyrus* in India. *P. pashia* and *P. sikkimensis* showed chromosome number $n = 17$ *(Fig. 6).* The former species confirms identical report by RAO (1967) while the latter is cytologically reported for the first time.

The genus *Photinia* is distributed in S.E. Asia and N. America. *P. integrifolia* with $n = 17$ tallies with the same previous report by ARORA (1961).

Out of seven species of *Eriobotrya* in India, *E. dubia* and *E. pitiolata (Fig. 7 and 8)* are chromosomally reported for the first time. Both these species are diploid with $n = 17$.

Cotoneaster bacillaris is a morphologically variable taxon. The chromosome number of this species is not known before. PMC's showed abnormal meiosis with $2n = 34$ *(Fig. 3).* The common chromosomal configuration observed at M-I was $2_{III} + 12_{II} + 4_{I}$, indicating translocations. More than 50% of the pollen were shrivelled. The present finding of $n = 17$ for *C. frigida* substantiates the earlier report by ZEILINGA (1964).

Only three species of *Crataegus* occur in India. *C. crenulata,* an elegant shrub, is planted as an ornamental and