Marshall, R. C.: Silviculture of the Trees of Trinidad & Tobago, British West Indies, *Meliaceae*, 39–70, 1939. — Meijer, W.: Botanical Notes on Ceylon Trees, 1., *Soymida febrifuga* (Roxb.) A. Juss. *(Meliaceae)* indigenous in Ceylon, 1971. — Mueller-Eckhardt, H.: Final Report to the Government of the Sudan on wood-based board materials. United Nations Development Programme, FAO, Rome. Report No. 2402, 1967, pp. 1–26. — Nobles, R. W., and Briscoe, C. B.: Height Growth of Mahogany Seedlings in St. Croix, Virgin Islands. U. S. Forest Service Research Note No. ITF 10, Puerto Rico, 1966. — Pawsey, R. G.: Forest diseases in Trinidad & Tobago, with some observations in Jamaica. Comm. For. Rev. 49 (1): 66–77 (1970). — Pennington, T. D., Styles, B. T., and White, F.: Generic Conspectus of the *Meliaceae.* (In preparation). — Richardson, S. D. in Frankel, O, H., and Bennett, E.: Gene Pools in Forestry. — IBP Handbook No. 11, Genetic Resources in Plants — their exploration and conservation: 353–367, 1970. — Stehlé, H.: Les Mahoganys des Antilles françaises et le *Swietenia aubrévilleana* Stehlé et Cusin nov. sp. Mém. Soc. Bot. Franc. 1956–7: 41–51 (1958). — Styles, B. T., and Vosa, C. G.: Chromosome numbers in the *Meliaceae* I. Taxon 20: 489—499 (1971). — Troup, R. S.: The Silviculture of Indian Trees, *Meliaceae.* 1: 178–208, 1921. — Vermoesen, F. M. C.: Notes sur quelques Méliacées. Rev. Zool. Afric. 10, Suppl. Bot.: B14—B57, 1922. — White, F., and Styles, B. T.: Flora Zambesiaca *(Meliaceae)* (2) 1: 285—319, 1963. — Yang, B.-Y.: Study on Techniques and Possibilities for Mahogany Breeding. Bull. Taiwan For. Res. Inst. 113: 1–13 (1965).

Supernumerary Chromosomes in Picea sitchensis (Bong.) Carr.

By R. B. Moir[1]) and D. P. Fox

Department of Genetics
University of Aberdeen
Aberdeen, AB9 1AS,
Scotland

(Received January 1972)

Introduction

In many genera of Gymnospermae the basic chromosome number is 11 or 12 (Darlington and Wylie, 1955). In addition to the relative constancy of the basic chromosome number, polyploidy is rare (Khoshoo, 1958) and supernumerary chromosomes (with the possible exception of Dark, 1932) have never been recorded. This uniformity even extends to the pattern of secondary constrictions within chromosomes of nine species of Pinus (Pederick, 1970).

However, karyotypic stability is more apparent than real in some cases. For example, Miksche (1967) showed that for eleven diploid species within the Pinaceae ($x = 12$) and two species within the Cupressaceae ($x = 11$), there is a range of X2.9 in DNA content between equivalent cells. Indeed, a similar variation exists within the species Picea glauca and Pinus banksiana when seed samples from different geographical locations are compared (Miksche, 1968). This variation in cellular DNA content has a regular geographical basis at least for the western populations of Picea glauca where the DNA content is higher in seed from the most northerly provenances. Since nuclear volume generally increases with DNA content (Sunderland and McLeish, 1961; Sparrow and Evans, 1961; Paroda and Rees, 1971), we might expect that a similar relationship between latitude and DNA content exists for Pinus *silvestris* and Picea sitchensis since in these species nuclear volume increases with latitude (Mergen and Thielges, 1967). Recently, Miksche (1971) has confirmed this relationship for Picea sitchensis, plants derived from more northerly provenances again having higher DNA contents. He considers that his data combined with the observation by Burley (1965) that total length of the A-chromosome karyotype of Picea sitchensis increases with latitude, imply that structural variations have occurred within the chromosomes which result in the geographical variations in RNA content.

In this report we describe the presence of supcrnumerary (B) chromosomes in seed derived from eight provenances of Picea *sitchensis.* This is the first definite report of B-chromosomes in the Gymnospermae and further calls into question the karyotype uniformity of the group.

Materials and Methods

(i) Seed Sources. Seed samples were provided by the Forestry Commission Research Station, Alice Holt Lodge, Wrecclesham, Farnham, Surrey. A summary of the provenances is contained in table 1.

(ii) Cytological Techniques. Seed was germinated on moist filter paper in petri dishes for 10—14 days at 20° C. At this time the radicle was 1–2 cms long. Mitotic spindle inhibition was achieved by immersing the seedlings in a saturated aqueous solution of paradichlorobenzene for eight hours, with aeration. Radicles were subsequently fixed in Carnoy's fluid (6 alcohol : 3 chloroform : 1 acetic acid, v/v/v) overnight at 4° C. A few drops of concentrated ferric chloride solution were then added to the fixative as premordant and the material returned to the refrigerator for a further two days. Squash preparations of individual radicle tips were made after macerating in 2% orcein (G. T. Gurr) dis-

Table 1. — Origins of the eight seed samples of Sitka spruce.

Sample No.	Source	Map reference N	W	Altitude
22	Masset, Q. C. I.	54°	132° 20'	< 500
23	Skidegate, Q. C. I.	53° 16'	132°	< 500
24	Ucluelet, Vancouver Is.	49° 10'	125° 55'	< 500
25	Washington State	General		
26	Washington State	General		
27	Radnor Exp. 20 (Siskiyou, Calif.)	58° 18' (41° 30')	3° 11' (123° 30')	1200 (200)
28	Radnor Exp. 20 (Siuslaw, Oregon)	58° 18' (44° 05')	3° 11' (123° 50')	1200 (150)
29	Radnor Exp. 20 (Olympia Mt. Washington)	58° 18' (47° 50')	3° 11' (123° 40')	1200 (200)

[1]) R. B. M. participated in this work during the course of an honours B.Sc. degree at Aberdeen University.

solved in 1 lactic acid : 1 propionic acid : 2 water (v/v/v). All slides were observed in the temporary condition.

Results and Discussion

(i) Chromosome morphology.

The gross morphology of the A-chromosome set was found to be the same in seed from all provenances. BURLEY (1965) has previously described the karyotype of *Picea sitchensis*, all chromosomes being meta- or submetacentric and the smallest having a length about 60% that of the largest. To this extent our results are in agreement though we have observed secondary constrictions on five pairs of homologous chromosomes compared with one on chromosome 10 (group 3 of this paper) in BURLEY's description. The positions of these constrictions are indicated by arrows in the karyotypes of *figs. 1* and *2*. It will be seen that their position, along with the absolute length of the chromosomes, allows a subdivision of the karyotype into five groups.

The B-chromosome is metacentric *(fig. 2)* and consistently smaller than the smallest A-chromosome. Plants with O, 1 or 2 B-chromosomes were found *(table 2)*. Those seedlings in which B-chromosomes were seen in arrested metaphase cells also had conspicuous, darkly-stained chromocentres in most of their interphase nuclei *(fig. 3)*. It is clear from *table 2* that these chromocentres are derived from the B-chromosomes since the most frequent B-chromosome number in metaphase is also the most frequent chromocentre class in interphase. At least a part of the B-chromosome must therefore be heterochromatic.

(ii) Geographical distribution of the B-chromosome.

Although B-chromosomes were found in seed derived form all eight provenances *(table 2)* one must be wary of drawing any conclusions as to their frequency in these provenances for several reasons:

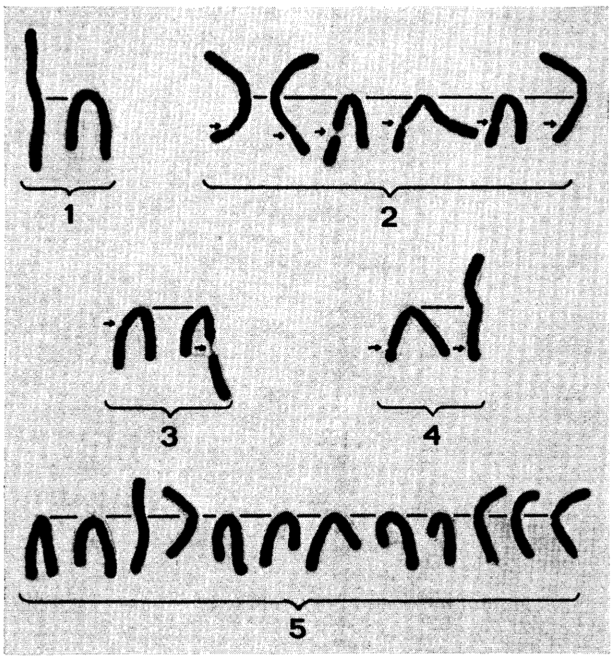

Fig. 1. — Karyotype of an arrested metaphase cell from a seedling without B-chromosomes. The chromosomes with secondary constrictions are subdivided into three groups (2—4) on the basis of their position. Groups 1 and 5 do not have secondary constrictions but may be separated on the basis of absolute length, group 1 being longer than any chromosome of group 5. The positions of the centromeres (primary constrictions) are indicated by the horizontal lines and the positions of secondary constrictions by arrows. (×1000.)

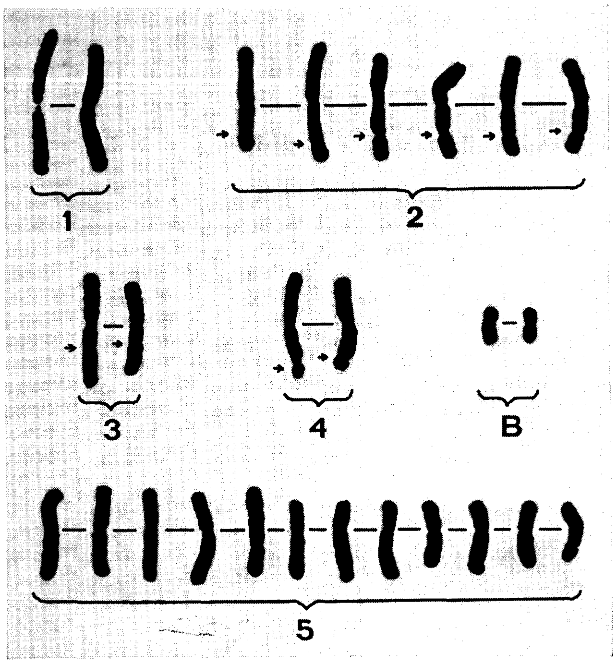

Fig. 2. — Karyotype on an arrested metaphase cell from a seedling with two B-chromosomes. The grouping within the karyotype is the same as for *fig. 1* with the addition of the B-chromosome group (B). (×1000.)

1. The seed are derived from an unknown (though possibly small) number of parent trees and thus may be subject to sampling error.
2. The number of seed studied is small in each case.
3. Provenances 27, 28 and 29 are derived from experimental populations grown in Radnor Forest in Wales. Thus sampling error could have occurred twice, once in collecting seed from the wild and once in collecting seed from the experimental trees.

Finally, exact map references are not available for provenances 25 and 26.

However, the finding of B-chromosomes in all eight provenances, extending over 12° latitude, indicates a widespread distribution in the natural range of the species.

(iii) Significance for cytogenetical studies in Picea sitchensis

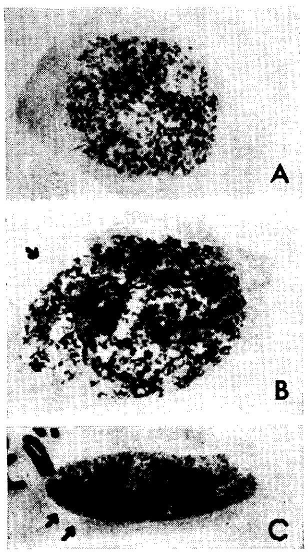

Fig. 3. — Interphase nuclei from plants with various B-chromosome numbers. A — OB, B — 1B, C — 2B. Large chromocentres are indicated by arrows. (A ×500, B ×500, C ×250.)

Table 2. — The relationship between number of heterochromatic chromocentres seen in interphase nuclei and the number of B-chromosomes counted in arrested metaphase. 50 interphase cells were scored for each plant.

Seed source	Plant	No. cells with chromosome no.						Total mitotic cells	No. of cells with chromocentres.		
		24	?24	25	?25	26	?26		O	1	2
22	1	7						7	50		
	2	13						13	50		
	3	2						2	50		
	4	15						15	50		
	5	17						17	50		
	6	1						1	50		
	7	13						13	50		
	8	3	1					4	50		
	9		1	4				5	18	32	
	10	9						9	50		
	11	6			1			7	50		
	12	5	1					6	50		
	13	7						7	50		
	14	2	1		1			4	50		
	15							0	50		
23	1		2	4				6	3	47	
	2	9						9	50		
	3	10			1			11	50		
	4	10						10	50		
	5	6						6	50		
	6	8						8	50		
	7	8						8	50		
	8	3	3					6	50		
	9			5			1	6	10	40	
	10	3						3	50		
	11	1						1	50		
	12	2	3					5	50		
24	1			1				1	4	46	
	2	10		2				12	47	3	
	3	1		5	1			7	8	42	
	4	9	1					10	50		
	5	7	1					8	50		
	6			8	2		1	9	2	48	
	7					7		7	3	6	41
	8	4	2					6	50		
	9			6				6	14	36	
	10	5						5	50		
	11			1	1	8		10	3	4	43
25	1			5				5	7	43	
	2			1		6		7		4	46
	3		1					1	49	1	
	4			3	2			5	7	43	
	5					6		6	6	6	38
	6	15						15	50		
	7	16						16	50		
	8			12	3			15	11	38	1
	9	8	1		1			10	50		
	10	5			1			6	50		
	11				1	8	2	11	4	13	33
26	1	1	1	1				2	49	1	
	2	2						2	50		
	3	12	1					13	50		
	4	5	1					6	50		
	5		2	2	1			5	8	42	
	6	8						8	50		
	7	5						5	50		
	8	5	1					6	50		
	9	5						5	50		
	10	6	2					8	50		
27	1	4	1					5	49	1	
	2	16						16	48	2	
	3	5						5	50		
	4	7	1					8	50		
	5	9			1			10	50		
	6		1	12	1	2		16	1	47	2
	7	5						5	50		
	8	4	3					7	50		

Inevitably several recent investigations of *Picea sitchensis* have not taken account of the B-chromosome polymorphisms. This may require some re-evaluation of data since in other systems B-chromosomes are known to influence many aspects of the exo- and endo-phenotype. Thus in rye the presence of B-chromosomes in the parents increases the variability of the progeny (Moss, 1966). This effect is over and above a direct effect of the B-chromosome on various aspects of growth (Müntzing, 1963). Further, the increasing total length of the karyotype with latitude (Burley, 1965) could also be influenced by the B-chromosome since in rye (Jones and Rees, 1968) their presence reduces the metaphase length of the A-chromosome set (though not its volume). The B-chromosome of rye also has a complex effect upon the dry mass of the interphase nuclei (Jones and Rees, 1968) and may therefore be expected to influence nuclear volume (see Mergen and Thielges, 1967). Finally, the B-chromosome of *Picea sitchensis* probably contains about 4% of haploid DNA content of the A-chromosomes and may therefore contribute to the variation of DNA content recently described by Miksche (1971).

We are not suggesting that the B-chromosome of *Picea sitchensis* can alone account for all these phenomena, nor do we think that inferences drawn from other work are a substitute for experimentation. However, until the effect of the B-chromosome is known, the exact significance of these other variations in the nuclear phenotype cannot be assessed. We are at present pursuing these investigations.

Acknowledgements

We are indebted to Mr. G. Buszewicz of the Forestry Commission for supplying the seed samples.

Summary

1. The karyotype of *Picea sitchensis* seedlings from eight provenances has been investigated.

2. Five secondary constrictions on five pairs of A-chromosomes are described, only one of which has previously been noted.

3. Supernumerary (B) chromosomes are found in some seedlings from all provenances indicating a widespread distribution in the natural range of the species.

4. The B-chromosome is isobrachial and consistently smaller than the smallest member of the A-chromosome set.

5. During interphase, the B-chromosome (or a part of it) remains condensed as a prominent heterochromatic chromocentre.

6. The importance of this finding for cytogenetical studies in *Picea sitchensis* is discussed.

Key words: Sitka spruce, supernumerary chromosomes, heterochromatin, populations.

Zusammenfassung

1. Der Karyotyp von *Picea sitchensis*-Sämlingen von 8 Provenienzen wurde untersucht.

2. Fünf sekundäre Constrictions werden bei 5 A-Chromosomenpaaren beschrieben, von denen bisher nur eines bekannt war.

3. Überzählige (B) Chromosomen werden bei manchen Sämlingen aller Provenienzen gefunden, was auf eine weite Verbreitung der Erscheinung im natürlichen Verbreitungsgebiet der Art hinweist.

4. Das B-Chromosom ist isobrachial und immer kleiner als das kleinste Chromosom des A-Chromosomensatzes.

5. Während der Interphase bleibt das B-Chromosom (oder ein Teil von ihm) als wesentliches heterochromatisches Chromozentrum erhalten.

6. Der Wert dieser Befunde für cytogenetische Untersuchungen bei *Picea sitchensis* (Sitka-Fichte) wird diskutiert.

References

Burley, J.: Karyotype analysis of Sitka spruce, *Picea sitchensis* (Bong.) Carr. Silvae Genet. **14**, 127—132 (1965). — Dark, S. O. S.: Chromosomes of *Taxus, Sequoia, Cryptomeria* and *Thuya*. Ann. Bot., Lond., **46**, 965—977 (1932). — Darlington, C. D., and Wylie, A. P.: Chromosome Atlas of Flowering Plants. George Allen and Unwin Ltd., 1955. — Jones, R. N., and Rees, H.: The influence of B-chromosomes upon the nuclear phenotype in Rye. Chromosoma, Berl., **24**, 158—176 (1968). — Khoshoo, T. N.: Polyploidy in gymnosperms. Evolution, Lancaster, Pa., **13**, 24—39 (1959). — Mergen, F., and Thielges, B. A.: Intraspecific variation in nuclear volume in four

(Continued from Table 2)

Seed source	Plant	No. cells with chromosome no.						Total mitotic cells	No. of cells with chromocentres.		
		24	?24	25	?25	26	?26		O	1	2
28	1	6						6	50		
	2	3	1					4	50		
	3	4						4	50		
	4	4	1					5	50		
	5	4			1			5	50		
	6	6						6	50		
	7		1					1	6	44	
	8	3						3	50		
	9	4	2					6	49	1	
	10	10	3					13	50		
	11	7	3					10	50		
	12			9	2			11	4	46	
29	1	8	1					9	50		
	2	12						12	50		
	3	16			1			17	50		
	4	4	2					6	50		
	5	8						8	50		
	6	6						6	50		
	7	7						7	50		
	8		1					1	50		
	9	10						10	50		
	10	9						9	50		
	11		1	6				8	8	42	

conifers. Evolution, Lawrence, Kans., 21, 720—724 (1967). — MIKSCHE, J. P.: Variation in DNA content of several gymnosperms. Canad. J. Genet. Cytol. 9, 717—722 (1967). — MIKSCHE, J. P.: Quantitative study of intraspecific variation of DNA per cell in *Picea glauca* and *Pinus Banksiana*. Canad. J. Genet. Cytol. 10, 590—600 (1968). — MIKSCHE, J. P.: Intraspecific variation of DNA per cell between *Picea sitchensis* (BONG.) CARR. Provenances. Chromosoma, Berl., 32, 343—352 (1971). — MOSS, J. P.: The adaptive significance of B-chromosomes in rye. Chromosomes today 1, 15—23 (1966). — MÜNTZING, A.: Effects of accessory chromosomes in diploid and tetraploid rye. Hereditas, Lund, 49, 371—426 (1963). — PARODA, R. S., and REES, H.: Nuclear DNA variation in Eu-Sorghums. Chromosoma, Berl., 32, 353—363 (1971). — PEDERICK, L. A.: Chromosome relationships between *Pinus species*. Silvae Genet. 19, 171—180 (1970). — SPARROW, A. H., and EVANS, H. J.: Nuclear factors affecting radiosensitivity. I. The influence of nuclear size and structure, chromosome complement and DNA content. Brookhaven Symp. Biol. 14, 76—100 (1961). — SUNDERLAND, N., and MCLEISH, J.: Nucleic acid content and concentration in root cells of higher plants. Exp. Cell Res. 24, 541—554 (1961).

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, Combretaceae and Myrtaceae

By P. N. MEHRA and P. K. KHOSLA,

Department of Botany,
Panjab University,
Chandigarh-14, India

(Received June 1971)

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 Himalayan region. Flower buds were fixed in CARNOY's fluid. Squashing of anthers was accomplished in 1% acetocarmine. Slides were made permanent in Euparal. Figures are drawn at a uniform magnification of × 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 chromosome 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 2—4 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 timber and forest products. Timbers of several species of *Terminalia* LINN. and *Anogeissus* WALL. ex GUILL. & PEER. are exploited for commercial purposes. The Myrabolan nuts