sens fraglich erscheint, die Polyploidisierung als nutzbringende Methode in der Nadelbaumzüchtung zu verwenden. Eine endgültige Beurteilung ist jedoch noch nicht möglich.

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Comparative Karyotype Analysis of Douglas-Fir

Pseudotsuga menziesii (Mirb.) Franco

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Introduction

Recent reports on the variation of nuclear volume and DNA content between genera and among species within genera in higher plants (13, 14, 16, 18, 24, 29, 31) may appear to contradict the DNA constancy theory put forward by BOVIN *et* al. (1948) and MIRSKY and RIS (1949).

Nuclear volume has been reported to vary with latitude; plants from northern latitudes tend to have a larger nuclear volume than plants of the same species from southern regions (8, 33, 34). The existence of intraspecific clinal variation in DNA content from south to north (13, 25, 26) has been reported. STEBBINS (1964, 1966) indicated that the variation in nuclear characteristics, such as nuclear volume and DNA content, is not random, but has an adaptive significance.

The present study was undertaken on a similar but limited geographical basis to the investigation made by EL-LAKANY and SZIKLAI (1971) on intraspecific variation in DNA content, and compares this variation with karyotypic descriptions based on morphological characteristics of the chromosomes, DE-VESCOVI (1974).

Materials and Methods

Cone samples were collected from their natural habitat in 1966 and 1968 by the International Union of Forest Research Organization (IUFRO), and the seeds were extracted, cleaned and stored as described by YAO (1971).

One hundred and fifty open pollinated seeds from each of the four provenances (Covelo #1, Gasquet #2, Forks #3 and Spokane #4) (*Fig.* 1) were germinated on the Jacobsen Germinator. Some root-tips of all germinants, approximately 5 mm. long, were treated in monobromonaphthalene for three hours at room temperature, fixed in Farmer's solution, hydrolyzed in 1N HCl at 60° C for 10 minutes, stained in leuco-basic fuchsin for 60–90 minutes and then squased in 45% acetic acid. Each of the four locations provided approximately 120 metaphase plates with well spread chromosomes, of which three cells with clearly defined details were measured and analysed (9).

Numerous photomicrographs were taken from each cell to obtain prints of the chromosomes in sharp focus. These prints were then used to measure the chromosomes. Once all the chromosomes were measured on the prints, the short arm length (p), the long arm length (q), the total length (T), and the width (w), were calculated.

Arm ratio was based on long arm length (q) over short arm length (p). Centromere index (C.I.) was calculated using the formula (35) $\text{C.I.} = \frac{100p}{(p+q)}$ Relative chromosome length was calculated in relation to the langest individual chromosome. The maximum value was 1, assigned to the longest chromosome.

Morphological index (M.I.) was used as in a previous work by GIANELLI and HOWLETT (1967). $\text{M.I.} = (p/q)\ (p+q)$.

The average width and the total length of the chromosomes were used for calculating the volume and it was assumed (4, 6, 22, 29, 36) that the cross section of the chromosome is a circle.

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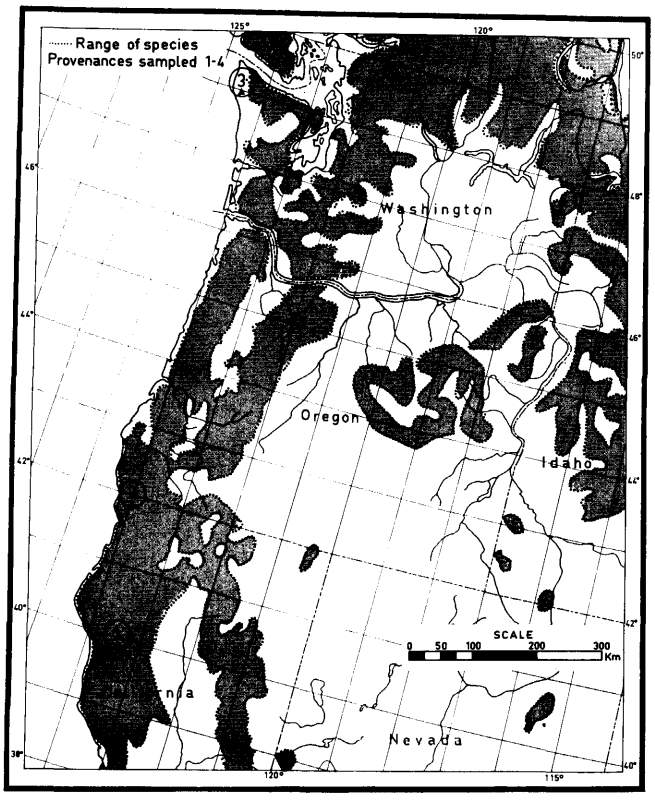

Fig. 1. — Location of Douglas-fir provenances sampled.

No. 1 Covelo, Calif. $\Psi = 39^0\,55'$ $\lambda = 123^0\,18'$,
No. 2 Gasquet, Calif. $\Psi = 41^0\,51'$ $\lambda = 123^0\,59'$,
No. 3 Forks, Calif. $\Psi = 47^0\,59'$ $\lambda = 124^0\,24'$,
No. 4 Spokane, Wash. $\Psi = 47^0\,28'$ $\lambda = 117^0\,12'$.

The chromosomes were classified into metacentric, submetacentric and subtelo-centric morphological categories as recommended by Levan *et al.* (1964).

Results

The haploid chromosome number of Douglas-fir, as observed in root-tip meristematic cells, consistenty appeared to be x = 13 in all provenances. The same haploid chromosome number was reported by other authors using mitotic material (10, 12, 32, 39) and meiotic tissues (1, 5, 21, 42). Using classification of Levan *et al.* (1964) it was possible to identify five metacentric, six submetacentric, and two subtelocentric chromosomes.

The morphometric and index values related to the 13 chromosomes of the four provenances are presented in *Table 1* and *2*.

The short arm length, long arm and total chromosome lengths did not show any different trend among the four provenances; on the other hand, the width and volume measurements indicated a clinal trend among the three coastal provenances, Covelo #1, Gasquet #2, and Forks #3. The Interior provenance, Spokane #4, when compared to the Forks #3 from approximately the same latitude, exhibited smaller values, 3.10 vs. 3.82 for width and 270.99 vs. 413.53 for volume respectively.

Discussion

Douglas-fir presents a large intraspecific variation pattern, which is related to the wide range of the species (20). Previous studies concentrated mainly on morphological, physiological and recently on nuclear characteristics of the species.

Clear morphological differences between Coast and Interior provenances, have been described for seed characteristics (1, 11, 30); cone scale and bract characteristics (41); needle thickness, cone diameter and cone specific gravity (40); and cone and seed characteristics (38).

Physiological studies have been related to germinative energy and capacity (2, 3, 41).

Variation in nuclear volume and relative DNA content from 11 Coast and 10 Interior provenances were investigated by El-Lakany and Sziklai (1971) and they extended the same work to 31 provenances in 1973. For both characteristics, differences were apparent for Coastal and Interior provenances. The relative DNA content was larger in the Coast source than in the corresponding Interior one. Furthermore a clinal increase in nuclear volume and DNA content was established with latitude for coastal sources. A significant clinal increase from south to north in chromosome width has also been demonstrated (9). Thus the present karyotype study reveals trends in chromosome width and volume that correspond with previous results. The southerly provenance, Covelo #1, had the smallest average width of 3.00 units (0.34 μm). This value increased to 3.36 units (0.38 μm) for Gasquet #2 and to 3.82 units (0.43 μm) for the most northerly provenance, Forks #3, on the Coast.

A similar northerly increasing clinal trend was noted for the volume of chromosomes for provenances #1, #2, #3 from the Coastal region with 266.24 units, 275.28 units and 413.53 units respectively. This correlated strongly with the relative DNA content *(Fig. 2)*.

Furthermore, there were also significant differences between the Interior and the Coastal provenances located at the same latitude with regard to the width and volume of the chromosome complement. The values were significantly lower for the Interior than for the Coastal provenances; e.g. for width 3.10 units (0.35 μm) as compared with 3.82 units (0.43 μm) and for volume, 270.99 units compared with 413.53 units *(Table 1)*. The volume of the haploid complement of Coastal provenance #3 was 53% larger than the corresponding Interior one, Spokane #4, at the same latitude *(Figs. 3—4)*.

The evidence from the range of chromosome volume found within the species suggests that the intraspecific variation is due to events which have affected all chromosomes to some extent, as Fox (1960) stated — "it seems improbable that these changes could be accounted for solely by duplications and deletions in the sense commonly used by cytologists"; they could also be the result of changes in the number of lateral redundancy (37). The lateral multistrand was reported for *Vicia faba* species (23). The results obtained in this study support the lateral multiplicity theory and also shows a clinal trend as the volume of the chromosomes of the four provenances which were investigated. Further studies using more samples from a wider distribution range, are needed to confirm these results and substantiate Stebbins' statement, that these differences may not be random but have an adaptive significance.

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Table 1. — Morphometric values of the chromosomes from Covelo (#1), Gasquet (#2), Forks (#3) and Spokane (#4) provenances. in micrometer units = .1136″

	LENGTH														
	SHORT ARM					LONG ARM					TOTAL				
Chrom. Pair #	#1	#2	#3	#4	Av.	#1	#2	#3	#4	Av.	#1	#2	#3	#4	Av.
1	29.10	23.47	26.93	24.43	25.95	37.70	26.25	32.62	32.23	32.20	66.80	49.75	59.55	56.67	58.19
2	24.65	20.88	22.45	21.43	22.35	30.77	24.07	30.08	30.67	28.90	55.42	44.95	52.53	52.10	51.25
3	21.10	18.77	21.33	21.30	20.63	26.12	23.52	24.22	25.90	24.94	47.22	42.28	45.55	47.20	45.56
4	20.48	19.17	19.88	19.07	19.65	23.40	21.00	23.70	23.65	22.94	43.88	40.17	43.58	42.72	42.59
5	18.72	17.70	18.07	17.60	18.02	23.00	20.26	22.35	22.13	22.03	41.72	38.32	40.42	39.73	40.05
6	12.38	9.27	12.98	10.80	11.36	25.92	23.40	23.30	24.98	24.40	38.30	32.67	36.28	35.78	35.76
7	10.82	9.23	10.95	10.10	10.28	23.70	20.20	22.50	22.48	22.22	34.52	29.43	33.45	32.58	32.50
8	10.83	8.88	10.60	10.33	10.16	22.18	18.73	21.07	20.25	20.56	33.02	27.62	31.67	30.58	30.72
9	10.03	8.38	8.65	9.05	9.03	21.52	17.87	21.47	20.38	20.31	31.55	26.25	30.12	29.43	29.34
10	9.25	7.42	9.18	8.10	8.49	20.33	10.03	19.62	19.43	19.10	29.58	24.45	28.80	27.53	27.59
11	9.20	6.57	8.67	7.95	8.10	18.55	16.17	17.80	18.35	17.72	27.75	22.73	26.47	26.30	25.81
12	4.97	3.52	5.60	3.93	4.51	18.47	16.45	17.57	18.82	17.83	23.23	19.97	23.17	22.75	22.28
13	3.52	2.82	3.17	3.50	3.25	16.23	12.13	16.83	14.63	14.96	19.75	14.95	20.00	18.13	18.21

WIDTH					VOLUME				
#1	#2	#3	#4	Av.	#1	#2	#3	#4	Av.
3.09	3.33	3.52	3.00	3.24	496.25	423.11	578.66	402.73	475.19
2.90	3.15	3.62	2.86	3.13	364.69	339.05	543.51	351.97	399.81
2.91	3.37	3.90	3.25	3.36	314.28	374.36	541.36	390.54	405.14
2.92	3.21	3.88	3.50	3.38	293.90	323.88	518.71	413.11	387.40
2.90	3.15	3.86	2.88	3.20	275.73	296.12	474.96	258.95	326.44
2.99	3.58	3.87	3.05	3.37	269.54	326.77	434.92	264.74	323.99
3.01	3.43	3.69	3.11	3.31	245.99	267.52	359.95	248.95	280.60
2.94	3.43	3.83	2.96	3.29	225.09	251.30	370.27	211.30	264.49
2.96	3.33	3.76	3.07	3.28	217.11	225.56	334.58	221.12	249.59
3.09	3.40	3.76	3.09	3.34	221.03	220.51	325.07	211.35	244.49
3.24	3.51	4.04	3.27	3.52	230.35	217.62	343.08	220.66	252.93
2.91	3.32	4.11	3.25	3.40	155.16	177.42	315.76	190.14	209.62
3.09	3.43	3.80	3.04	3.34	151.98	135.42	235.13	137.29	164.96

Table 2. — Index values of the chromosomes from Covelo (#1), Gasquet (#2), Forks (#3) and Spokane (#4) provenances.

Chrom. Pair #	Index values																					
	ARM RATIO					CENTROMERE INDEX					RELATIVE LENGTH					MORPHOLOGICAL INDEX						
	#1	#2	#3	#4	Av.	#1	#2	#3	#4	Av.	#1	#2	#3	#4	Av.	#1	#2	#3	#4	Av.		
1	1.32	1.13	1.22	1.33	1.25	43.37	47.07	45.25	43.10	44.70	0.96	0.96	0.96	0.98	0.97	51.91	44.47	49.21	43.21	47.20		
2	1.28	1.17	1.34	1.45	1.31	44.29	46.56	42.84	40.95	43.66	0.80	0.87	0.85	0.90	0.86	44.59	39.09	39.23	36.56	39.87		
3	1.25	1.27	1.15	1.22	1.22	44.62	44.46	46.68	45.03	45.20	0.69	0.82	0.74	0.82	0.77	38.15	33.79	40.16	38.82	37.73		
4	1.15	1.10	1.22	1.28	1.19	46.72	47.72	45.38	44.61	46.11	0.64	0.78	0.70	0.74	0.72	38.50	36.66	36.64	34.49	36.57		
5	1.24	1.17	1.28	1.28	1.24	44.83	46.52	44.43	44.29	45.02	0.61	0.74	0.65	0.69	0.67	34.01	33.06	32.71	31.62	32.85		
6	2.13	2.55	1.87	2.33	2.22	32.41	28.36	36.38	30.19	31.84	0.56	0.63	0.58	0.62	0.60	18.35	12.94	21.05	15.49	16.96		
7	2.23	2.21	2.08	2.27	2.20	31.33	31.39	32.71	30.90	31.58	0.50	0.57	0.54	0.57	0.55	15.76	13.46	16.32	14.66	15.05		
8	2.09	2.16	1.99	1.98	2.06	32.72	31.94	33.71	33.74	33.03	0.48	0.54	0.51	0.53	0.52	16.17	13.13	15.98	15.61	15.22		
9	2.20	2.18	2.51	2.30	2.30	31.75	31.87	28.97	30.59	30.80	0.46	0.52	0.49	0.51	0.50	14.83	12.32	12.21	13.11	13.12		
10	2.23	2.33	2.16	2.42	2.29	31.31	30.44	31.85	29.43	30.76	0.43	0.48	0.47	0.48	0.47	13.46	10.65	12.34	11.50	11.95		
11	2.03	2.59	2.09	2.33	2.26	33.27	29.13	32.76	30.25	31.35	0.41	0.44	0.43	0.46	0.44	13.82	9.28	13.27	11.41	11.95		
12	3.88	4.83	3.48	5.01	4.30	21.41	17.96	24.56	17.24	20.29	0.34	0.40	0.38	0.40	0.38	6.33	4.31	7.50	4.76	5.73		
13	4.70	4.45	5.34	4.30	4.70	17.75	19.31	15.82	19.52	18.10	0.29	0.30	0.33	0.32	0.31	4.28	3.49	3.76	4.38	3.98		

Summary

A comparative karyotype study of Douglas-fir germinants from four different provenances, three Coastal provenances through the 8° 04' of latitude in California and Washington, Coast #1, #2, #3 and Interior #4 was conducted using root-tip meristematic tissues.

1. A haploid chromosome number of 13 was observed in all provenances.
2. The homologous chromosome pairs were identified using the arm ratio, centromere index, relative length and especially the morphological index.
3. These values also provided the basic information for determining that Douglas-fir has five metacentric, six submetacentric and two subtelocentric chromosomes.
4. A clinal variation of chromosome width and volume was noted among the three Coastal provenances with increasing values toward the north.

Fig. 2. — Correlation between relative DNA content and chromosome volume of the three Coastal provenances.

Fig. 3. — Chromosomes from the Coastal (Forks #3) provenance. (x 8000 $\Psi = 47° 59'$)

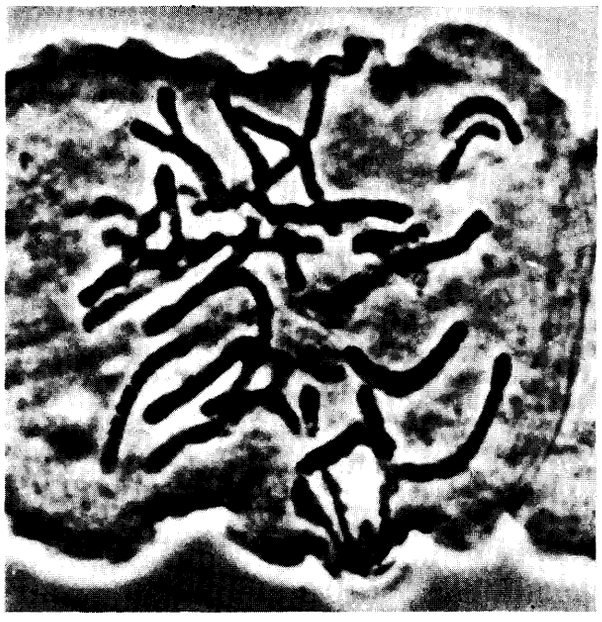

Fig. 4. — Chromosomes from the Interior (Spokane #4) provenance. (x 8000 $\Psi = 47^0\ 28'$)

5. Chromosome width and volume of the Interior provenance were significantly smaller than the width and volume of the corresponding Coastal one.
6. These results substantiated the previous findings of clinal variation in nuclear volume and DNA content in Douglas-fir and emphasized the importance of two and three dimensional chromosomal studies.
7. More studies are needed to be initiated on a broad geographical basis, including samples from the entire range of this species and other species, to describe and understand the significance of this variation.

Key words: *Pseudotsuga menziesii* (Mirb.) Franco, Provenances, Karyotype study, Chromosome number, Chromosome width, Chromosome volume.

Abstract

A comparative karyotype study was made of chromosomes in root-tip meristematic cells of Douglas-fir, *Pseudotsuga menziesii* (Mirb.) Franco germinants obtained from three Coastal (#1, 2, 3) and one Interior (#4) sources distributed through 8^0 04' latitude. The somatic chromosome number in all provenances was $2n = 26$ or $x = 13$. morphological index was found to be the most useful criterion for separating the five meta-, six submeta- and two subtelo-centric chromosomes of the basic set.

The haploid chromosome complement of one Coastal provenance (#3) had 1.5 times more volume than the corresponding Interior one (#4), substantiating previous results as far as variation in nuclear volume and DNA contents are concerned.

A clinal increase from south to north in chromosome width (3.00, 3.36 and 3.82 units) and volume (266.24, 275.28 and 413.53 units) was noted among the chromosomes of the three (#1, 2 and 3) Coastal provenances.

The intraspecific variation in chromosomal volume, correlated with the relative DNA content reported previously for the four provenances, does not contradict the DNA constancy concept, but rather gives more weight to that interpretation of this concept which attributes plasticity to DNA, a stable biological molecule.

The concept of lateral multiplicity was mentioned as a possible factor in the increased chromosomes width and volume, and therefore emphasizes the importance of two dimensional chromosomal studies.

Zusammenfassung

Keimpflanzen der Douglasie *Pseudotsuga menziesii* (Mirb.) Franco aus vier verschiedenen Provenienzen, davon drei aus der Küstenregion Kaliforniens und Washingtons über 8^0 04' nördlicher Breite hinweg und eine aus dem Landesinnern wurden karyologisch untersucht. Hierbei wurde bei allen vier Provenienzen ein haploider Chromosomensatz von $n = 13$ gefunden. Von den 13 Chromosomen erwiesen sich 5 als metazentrisch, 6 als submetazentrisch und 2 als subtelozentrisch. Die drei sehr weit auseinander liegenden Küstenprovenienzen zeigten in nördlicher Richtung zunehmende Werte für die Chromosomenbreite und das Chromosomenvolumen. Beide Werte waren bei der Provenienz aus dem Landesinnern im Vergleich zu der korrespondierenden Küstenprovenienz signifikant kleiner.

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Karyological Studies and Chromosomal Evolution in Meliaceae

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Introduction

This paper embodying cytological research on 30 species of Meliaceae is a continuation of earlier studies of Styles and Vosa (1971). This project was initiated by Dr. B. T. Styles at the Commonwealth Forestry Institute, Oxford, in order to compile chromosome data on a world-wide basis for an arborescent group comprising some of the best tropical timbers (see Styles and Vosa, *loc. cit.*). Other important workers on the cytology of Meliaceae include S. and G. Mangenot (1957, 1958 and 1962), Minfray (1963 a, b), Mehra and Khosla (1969), Mehra and Sareen (1969) and Mehra *et al.* (1972). The latter three contributors have concentrated mainly on Himalayan Meliaceae.

An overall picture of chromosome data on Meliaceae is portrayed in order to understand the process of speciation in the family. This is one of the most important criteria on which to base any programme of forest tree improvement through induced ploidy changes or mutation breeding.

Material and Methods

Methods involved in these studies were the same as those cited by Styles and Vosa *(loc. cit.)*. Root-tip squashes for examination of mitosis were made from freshly germinated seeds in petri dishes and also from seedlings raised in a tropical greenhouse at the University Field Station, Wytham, Oxford. The root-tips were pre-treated with 0.05 per cent colchicine for four hours and fixed in 1 : 3 acetic-alcohol. The material was stained by the Feulgen method and the preparations made permanent. Meiotic counts have been made in three instances where flowering occurred in the greenhouse. Sources of material along with voucher records are given in *Table 1*.

In order to study the karyotype several slides were made of each taxon, the number of which varied with the availability of material. On average 2—5 plants were examined per species. Only those slides that showed well-spread chromosomes with straight or almost straight arms were used for drawing or for making measurements. Usually the best metaphase plate was selected for drawing and the chromosomes were measured with the aid of a stage micrometer. The averages of at least five drawings were calculated to the nearest 0.05μ to show the karyotypic differences in different species.

Attempts have been made to study the morphology of chromosomes but this is often difficult because of their small size. In the text the relative size of chromosomes in the complement is denoted by L = large, M = medium and S = small; the centromeric position is represented by m = median, sm = sub-median, st = sub-terminal, and sc = secondary cosntriction. The average size of the chromosomes is computed by totalling the individual lengths and then dividing the sum by the total number of chromosomes. Measurements in each case are taken from metaphases of five cells. Figures are at a uniform magnification of 1500 X. The circumscription of taxa and nomenclature follows that proposed in A Generic Monograph of the Meliaceae, Pennington and Styles (1975).

Observations

Table 1 summarizes cytological data on 33 taxa. This includes the source of the material and voucher record, chromosome number, total chromatin length, average chromosome size, habit of the plant and any previous chromosome counts.

Subfamily Swietenioideae

Tribe Swietenieae:

Chukrasia A. Juss. (1—2 species; India to S. China and W. Malesia).

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