1. Failure to form a successful graft or bud union in a high percentage of cases.

2. Appearance of shoot dieback and general ill health of grafted tree.

3. Premature death of the tree in the nursery.

It is evident from *Table 2* that ability to survive at 30 days and 60 days are significantly different indicating that under varied environmental conditions mortality was accentuated, confirming incompatibility. This confirms the view of CHANG (1937) that symptoms of incompatibility appear most quickly and in the severest manner when the tree is grown under adverse environmental conditions. On the other hand under favourable conditions, the symptoms may take some time to appear, although incompatibility actually exists in the tree. The major cause seems to be the discontinuity between wood and bark shown in *Figure 2a* and *2b*. CHANG (1937) says "that the distinctive groups of incompatibility as shown by bark and wood discontinuity seem to be directly correlated with a high degree of incompatibility. Possibly this crevice at the union hinders or lessens the upward and downward transport of water, soil solution and manufactured food, thus causing a weaker growth of shoot and root". *Figure 1a* and *1b* show successful and unsuccessful bud grafts respectively. The unsuccessful graft is associated with shoot dieback and premature death of tree.

On the basis of these observations it can be suggested that incompatibility may exist in teak clones, when they are grafted.

A systematic study must be done to determine the extent of incompatibility using different combinations, viz scion on stock, stock on scion and stock on stock.

Literature Cited

AHLGREN, C. E.: Some factors influencing survival, growth and flowering of intraspecific and interspecific pine grafts. J. Forest **60**: 785—789 (1962). — ALPHEN DE VEER, E. J. VAN: Teak cultivation in Java. Proc. IVth World Forestry Congress, Dehra Dun **3**: 322—338 (1954). — ARGLES, G. K.: A review of the literature on stock-scion incompatibility in fruit trees with particular reference to Pine and Stone - fruits. Tech. Communications, No. 9, Imp. Bar. Fruit Prod. East Malling (1937). — CAMERON, A. L.: Genetic improvement of teak in New Guinea. Australian Forestry **30**: 76—87 (1966). — CHALMERS, W. S.: The breeding of Pine *(Pinus caribaea)* and Teak *(Tectona grandis)* in Trinidad. Some early observations. Caribbean Forester **23**: 100—111 (1962). — CHANG, W. T.: Studies in incompatibility between stock and scion with special reference to certain deciduous fruit trees, Jour. Pom and Hort. Sci. **15**: 267—325 (1937). — CHOUDHARI, N. R.: Experience with raising of teak seed orchard in Maharasthra state. Proc. Seminar-cum-workshop on genetic improvement of Forest tree seeds in India, February 7 to 12, 1970. Forest Research Institute and Colleges, Dehra Dun. 56—65 (1970). — FOWLER, D. R.: Low grafting and deep planting may prevent mortality due to incompatibility in pine. Forest Sci. **13**: 314—315 (1967). — HARTMAN, H. T. and KESTER, D. E.: Plant propagation principles and practices (Second edition). Prentice Hall of India Private Limited, New Delhi, L. F. 357—370 (1972). — HELLINGA, G.: On forest tree improvement in Indonesia. Proc. I.U.F.R.O. meetings **22**: 101 (1956). — KEIDING, H. and BOON-KIRD, S.: Vegetative Propagation of teak. Unasylva **14**: 193—194 (1960). — MAHAPATRA, P.: Experiment of grafting on teak in Orissa. Proc. Seminar-cum-workshop on genetic improvement of Forest tree seeds in India. February 7 to 12, 1970. Forest Research Institute and Colleges, Dehra Dun. 30—33 (1970). — RAWAT, M. S. and KEDHARNATH, S.: Field grafting and budding in Teak *(Tectona grandis* L. f). Indian Forester **94** (3): 259—262 (1968). — SILEN, R. R. and COPES, D. L.: Douglas-fir seed orchard problems a progress report. J. Forest **70**: 145—147 (1972).

A chromosome study of coast redwood, Sequoia sempervirens (D. Don) Endl.[1])

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Summary

A detailed karyotype analysis was made on the somatic chromosome complement of *Sequoia sempervirens*. The species has 2n = 6x = 66 chromosomes which concurs with previous reports. Chromosomes with unusual or specific structures are present in the complement. Two pairs of SAT-chromosomes have unusually long secondary constrictions and are believed to be associated with nucleolar organization regions. Previous studies show that *Sequoia* has six nucleoli, four large and two small, which indicates that there are two other active NOR present, likely associated with microsatellites which may not be visible due to pretreatment effects. Amphiplasty may have been responsible for the morphological differences among the nucleolar organizing chromosomes. Tentatively, *Sequoia* appears to be a segmental allopolyploid, $A_1A_1A_1A_1A_2A_2$, though the possibility that *Sequoia* is an autoallopolyploid, AAAABB, cannot be discounted.

Key words: Sequoia sempervirens, redwood, *Taxodiaceae*, chromosome, karyotype analysis. hexaploid, segmental allopolyploid, autoallopolyploid, amphiplasty.

Zusammenfassung

Am Wurzelspitzenmeristem von *Sequoia sempervirens* wurde eine detaillierte Analyse des Karyotyps durchgeführt. Von den aus früheren Untersuchungen bekannten 2n = 6x = 66 Chromosomen weisen einige spezifische Strukturen auf. 2 SAT-Chromosomen haben ungewöhnlich lange sekundäre Einschnürungen, die für Nucleolus-Organisator-Regionen (NOR) gehalten werden. Da in früheren Untersuchungen 6 Nucleoli nachgewiesen werden konnten, 4 große und 2 kleine, werden 2 weitere NOR-aktive Chromosomenpaare angenommen, deren Mikrosatelliten aber durch die hier angewandte Vorbehandlung des Materials

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nicht mehr sichtbar sind. Auf Grund der morphologischen Unterschiede der NOR-Chromosomen wird für *Sequoia sempervirens* Amphiplastie ($A_1A_1A_1A_1A_2A_2$) angenommen, obgleich auch die Möglichkeit der Autoallopolyploidie (AAAABB) nicht auszuschließen ist.

Introduction

Coast redwood, *Sequoia sempervirens* (D. Don) Endl., is a monotypic genus in the coniferous family *Taxodiaceae*. The species is known worldwide for attaining great height and forming majestic groves on the North American Pacific coast. Though of restricted distribution today, *Sequoia* was once a prominent component of forests in the late Cretaceous and early Tertiary epochs (Florin, 1963). Interestingly, *Sequoia* is unique within Coniferales, being of a hexaploid nature (Hirayoshi and Nakamura, 1943; Stebbins, 1948).

Sequoia was thought to be a polyploid species as early as 1904 (Lawson, 1904). Attempts to obtain a definitive chromosome count of the species were made for a number of years without success (Goodspeed and Crane, 1920; Dark, 1932; Sax and Sax, 1933; Buchholz, 1939; Jensen and Levan, 1941). Eventually, Hirayoshi and Nakamura (1943) counted 2n = 66 chromosomes in the pollen mother cells of *Sequoia*. This count was later independently confirmed by Stebbins 1948) as the war period had disrupted communications. Stiff (1952), Fozdar and Libby (1968), and Saylor and Simons (1970) also counted 2n = 66 chromosomes in somatic cells of *Sequoia*.

Hirayoshi and Nakamura (1943) recorded various metaphase configurations in 4 cells involving hexavalents, quadrivalents, bivalents, and occasionally univalents but believed that *Sequoia* was not of an autopolyploid nature. Stebbins (1948) analyzed 2 microsporocytes and found the metaphase I configurations to comprise of hexavalents, quadrivalents, and bivalents. The observation of multivalents led Stebbins to hypothesize *Sequoia* to be an autoallopolyploid (AAAABB) or a segmental allopolyploid with a genomic formula of $A_1A_1A_1A_1A_2A_2$ or $A_1A_1A_2A_2A_3A_3$.

Saylor and Simons (1970) studied the somatic chromosomes from root tip meristematic cells and made a detailed karyotype analysis. They found that when the haploid set of chromosomes was arranged in descending order according to overall length, there was a preponderance of groupings of three chromosomes of similar lengths. Additionally, these authors recorded three sets of chromosome pairs with satellites (SAT-chromosomes) in the whole complement giving credence to the grouping of chromosomes in trios. Within the groups of three chromosomes, one chromosome was often different in kinetochore position and/or length than the remaining two chromosomes. Saylor and Simons (1970) concluded that *Sequoia* was an autoallopolyploid (AAAABB) based on the observation of four large and two smaller, inconsistently appearing, nucleoli in the somatic cells.

The present authors have been engaged in studies of coniferous trees in search of chromosomes with unusual structures that may indicate cytotaxonomic and phylogenetic relationships (Schlarbaum and Tsuchiya, 1975, 1984; Schlarbaum *et al.*, 1983, 1984). In a preliminary report on the chromosomes and relationships of *Metasequoia glyptostroboides* Hu et Cheng and *Sequoia*, Schlarbaum *et al.* (1984) indicated the presence of marker chromosomes with unusual secondary constrictions in the complement of *Sequoia*. The present report is the result of detailed analysis of the *Sequoia* karyotype and a discussion of the polyploid nature as indicated by the marker chromosomes.

Materials and Methods

Sequoia seed of an unspecified origin was obtained from a commercial company and germinated. Chromosome studies were made on five seedlings. Actively dividing cells in the meristematic region of the root tips were isolated and analyzed. The root tips were immersed in 0.002 M solution of 8-hydroxyquinoline for 36 hours at 4° C (cf. Saylor, 1961) or 0.2 percent colchicine for 6—8 hours at room temperature (cf. Hair, 1968). The materials were then fixed in either a 3:1 mixture of ethanol and glacial acetic acid or Carnoy's fixative for approximately one week. The root tips were then hydrolyzed for 10—15 minutes in 1 N HCL at 60° C and stained in Feulgen or acetocarmine (Schlarbaum and Tsuchiya, 1976).

The slides were prepared by using the squash technique and made permanent by applying several drops of a 10:1 mixture of 45 percent acetic acid and glycerol to the edge of the cover slip (Tsuchiya, 1971).

The nomenclature system for chromosome types of Levan *et al.* (1964) was modified for designation of individual chromosomes, except for chromosomes with unusual structures or satellites in which the length of the unusual structure or satellite has not been included in arm length (Schlarbaum and Tsuchiya, 1984).

The karyotype analysis was made on the chromosome complement of a representative cell in which the chromosomes were well spread and morphological features could be readily observed. Procedures for the karyotype analysis of the polyploid *Sequoia sempervirens* was similar to methods used with diploid conifer species in previous studies (Schlarbaum and Tsuchiya, 1975a, b, 1976, 1984; Schlarbaum *et al.*, 1983). However, the arrangement of chromosomes in the karyotype and corresponding tables were different due to the hexaploid nature of the species.

The chromosomes were initially divided by size into 11 groups of six (designated with Roman numerals), which were further subdivided into subgroups of four and two chromosomes based upon size and kinetochore position. The subdivision of each 11 groups into 4:2 chromosomes was *a posteriori* based on the assumption that *Sequoia* is a segmental allopolyploid with a genomic formula of $A_1A_1A_1A_1A_2A_2$ or an autoallopolyploid with a corresponding genome formula of AAAABB (cf. Stebbins, 1947, 1948). Therefore, in each set of six chromosomes, the A_1 or A genome is represented by a group of four chromosomes and the A_2 or B genome is represented by a chromosome pair.

Genome diagrams were constructed following Tjio and Hagberg (1951) and were used for comparisons of genomes defined by the present study with those defined by Saylor and Simons (1970, cf. *Table 1).* Representative chromosome lengths in the genomes were calculated by averaging the lengths, respectively, of four A_1 or A chromosomes and the two A_2 or B chromosomes in each chromosome set. The percent of the total chromosome length of each genome (relative length) and chromosome index (short arm: long arm ratio) were then calculated from these values for each chromosome in the respective genomes. The relative length (axis of abscissa) and chromosome index (axis of ordinates) values were used for plotting points in the diagrammatic comparisons. A diagrammatic comparison was also made between the A_1 or A and the A_2 or B genomes.

Results

The chromosome number of *Sequoia* was found to be 2n = 66 *(Fig. 1)* which confirms the initial reports of Hi-

RAYOSHI and NAKAMURA (1943) and STEBBINS (1948) and other studies (STIFF, 1952; FOZDAR and LIBBY, 1968; SAYLOR and SIMONS, 1970). The hexaploid chromosome number was reaffirmed in a number of cells, sixteen of which had well spread chromosomes making possible detailed observations of chromosome morphology. There was no notable differences in chromosome morphology among the five seedlings.

Table 1. — Measurements in microns of somatic chromosomes as arranged in *Figure 2* in the root tip mitosis of *Sequoia sempervirens.*

Chromosome		Long Arm (L)	Short Arm (S)	Satellite region	Total	L/S	Kinetochore position	Genome
I	1	5.5	5.1	-	10.6	1.078	m	A_1
	2	5.4	4.1	-	10.5	1.059	m	A_1
	3	5.2	5.2	-	10.4	1.000	M	A_1
	4	5.2	5.1	-	10.3	1.020	m	A_1
	5	5.3	4.9	-	10.2	1.082	m	A_2
	6	5.3	4.9	-	10.2	1.082	m	A_2
II	7	5.3	4.6	-	9.9	1.152	m	A_1
	8	5.1	4.7	-	9.8	1.085	m	A_1
	9	5.1	4.7	-	9.8	1.085	m	A_1
	10	5.1	4.6	-	9.7	1.109	m	A_1
	11	5.1	4.6	-	9.7	1.109	m	A_2
	12	5.1	4.3	-	9.4	1.186	m	A_2
III	13	4.7	4.6	-	9.3	1.022	m	A_1
	14	4.7	4.3	-	9.0	1.093	m	A_1
	15	4.7	4.3	-	9.0	1.093	m	A_1
	16	4.5	4.4	-	8.9	1.023	m	A_1
	17	4.9	4.1	-	9.0	1.195	m	A_2
	18	4.4	4.3	-	8.7	1.023	m	A_2
IV	19	6.8	3.5*	2.0	10.3†	1.940†	sm	A_1
	20	5.5	3.3*	3.0	8.8†	1.667†	msm	A_1
	21	5.5	3.2*	2.3	8.7†	1.719†	sm	A_1
	22	5.3	3.2*	1.8	8.5†	1.656†	msm	A_1
	23	5.5	3.2	-	8.7	1.719	sm	A_2
	24	5.4	3.2	-	8.6	1.688	msm	A_2
V	25	4.5	4.3	-	8.8	1.047	m	A_1
	26	4.3	3.9	-	8.2	1.103	m	A_1
	27	4.0	4.0	-	8.0	1.000	M	A_1
	28	4.0	3.8	-	7.8	1.053	m	A_1
	29	3.9	3.8	-	7.7	1.026	m	A_2
	30	4.3	3.4	-	7.7	1.265	m	A_2
VI	31	3.9	3.4	-	7.3	1.147	m	A_1
	32	3.8	3.4	-	7.3	1.085	m	A_1
	33	3.7	3.6	-	7.3	1.028	m	A_1
	34	3.7	3.6	-	7.3	1.028	m	A_1
	35	4.3	3.2	-	7.5	1.344	msm	A_2
	36	4.4	3.0	-	7.4	1.467	msm	A_2
VII	37	4.0	3.1	-	7.1	1.290	m	A_1
	38	4.5	2.6	-	7.1	1.731	sm	A_1
	39	4.5	2.6	-	7.1	1.731	sm	A_1
	40	4.0	3.0	-	7.0	1.333	msm	A_1
	41	3.6	3.6	-	7.2	1.000	M	A_2
	42	3.7	3.4	-	7.1	1.088	m	A_2
VIII	43	3.6	3.3	-	6.9	1.091	m	A_1
	44	3.5	3.4	-	6.9	1.029	m	A_1
	45	3.4	3.4	-	6.8	1.000	M	A_1
	46	3.4	3.4	-	6.8	1.000	M	A_1
	47	3.5	3.3	-	6.8	1.061	m	A_2
	48	3.8	2.8	-	6.6	1.357	msm	A_2
IX	49	4.0	2.7	-	6.7	1.481	msm	A_1
	50	4.0	2.6	-	6.6	1.538	msm	A_1
	51	3.8	2.8	-	6.6	1.357	msm	A_1
	52	3.7	2.6	-	6.3	1.423	msm	A_1
	53	4.3	2.3	-	6.6	1.870	sm	A_2
	54	4.0	2.4	-	6.4	1.667	msm	A_2
X	55	3.3	2.8	-	6.1	1.179	m	A_1
	56	3.2	2.8	-	6.0	1.143	m	A_1
	57	3.1	2.8	-	5.9	1.107	m	A_1
	58	3.0	2.6	-	5.6	1.154	m	A_1
	59	3.4	3.1	-	6.5	1.097	m	A_2
	60	3.3	3.1	-	6.4	1.065	m	A_2
XI	61	2.9	2.6	-	5.5	1.115	m	A_1
	62	2.8	2.6	-	5.5	1.077	m	A_1
	63	2.8	2.4	-	5.2	1.167	m	A_1
	64	2.8	2.4	-	5.2	1.167	m	A_1
	65	2.6	2.6	-	5.2	1.000	M	A_2
	66	2.6	2.6	-	5.2	1.000	M	A_2

* Satellite attached to short arm.
† Satellite region not included in calculation.

The majority of the chromosomes in the complement have median kinetochores *(Table 1).* Eight chromosome sets of the A_1 or A genome are m-type, chromosome groups VII and IX are msm-type, and chromosome group IV is submedian with a long secondary constriction attached to the short arm *(Fig. 2; Table 1).* The secondary constriction *(Fig. 3a, b)* is similar to those found in the marker chromosomes of *Cunninghamia* spp. (SCHLARBAUM and TSUCHIYA, 1984). The region is lightly stained and contains visible chromomeres and has a satellite body connected to the distal portion of the region *(Fig. 3a, b).* The secondary constriction is inconsistent in appearance among different cells and variation was observed between homologous chromosomes of the same cell *(Fig. 3b).*

Seven chromosome pairs of the A_2 or B genome have median kinetochores and one pair is M-type *(Fig. 2; Table 1).* Chromosome pairs VI and IX have median-submedian kinetochores. Chromosome pair IV is submedian and has no visible satellite region unlike the corresponding chromosomes of the A_1 genome.

The chromosome groups of the A_1 or A genome gradually decreased in size with a relative difference of 5.4 microns between the largest and the smallest chromosomes *(Table 1).* The chromosome pairs of the A_2 or B genome follow the same pattern with a relative size difference of 5.0 microns between the largest and smallest chromosomes *(Table 1).*

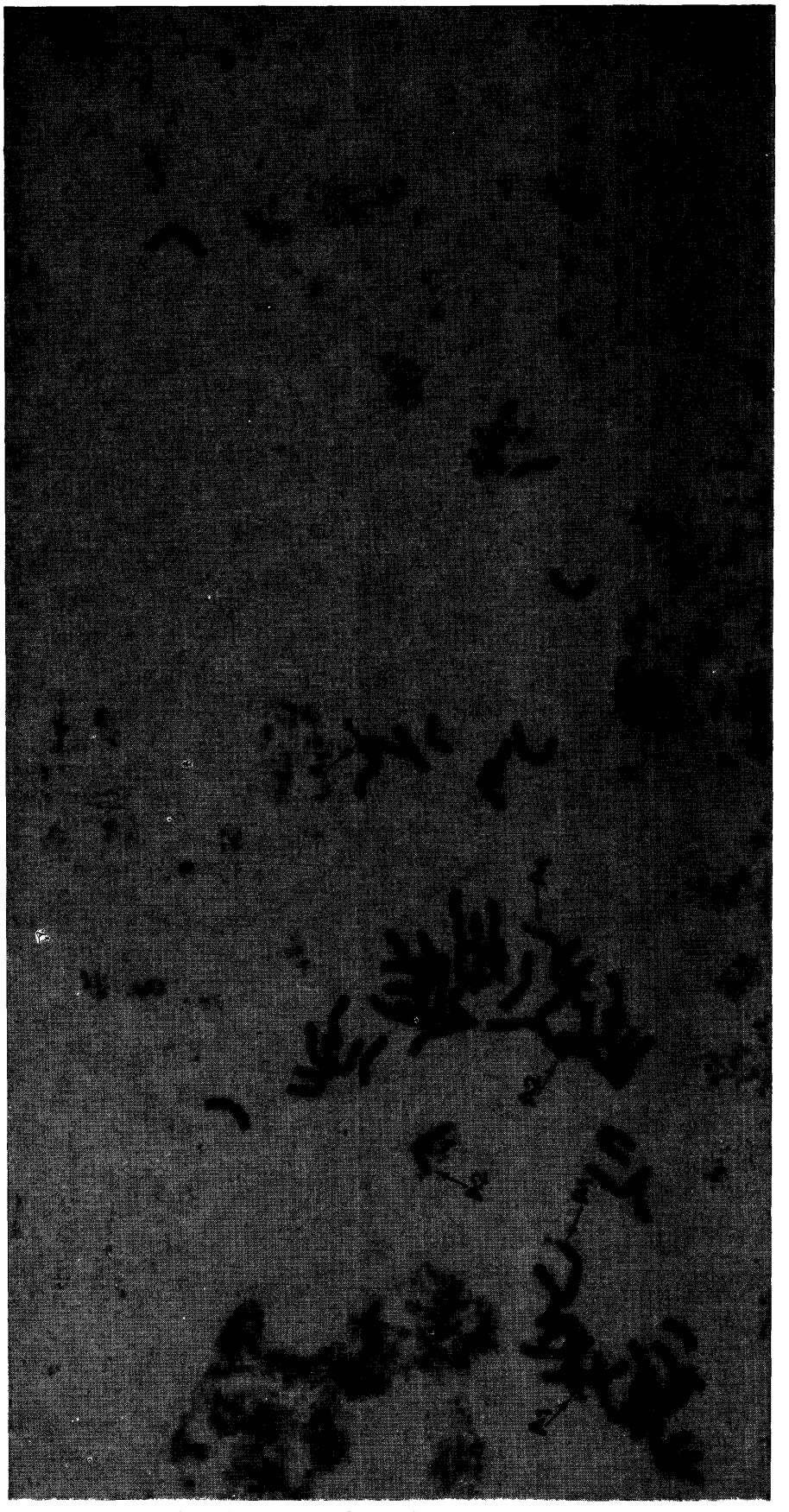

Figure 1. — Somatic metaphase cell of *Sequoia sempervirens* (2n = 6x = 66). A_1 arrows indicate SAT-chromosomes with long secondary constriction. A_2 arrows indicate corresponding chromosomes without visible satellites.

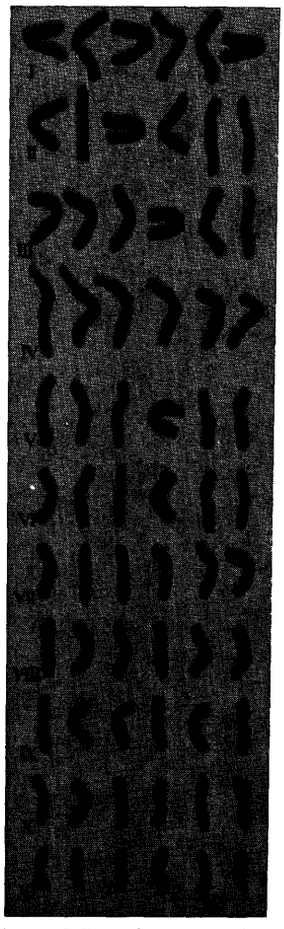

Figure 2. — Karyotype of *Sequoia sempervirens;* taken from *Figure 1.* Chromosomes are arranged according to *Table 1.*

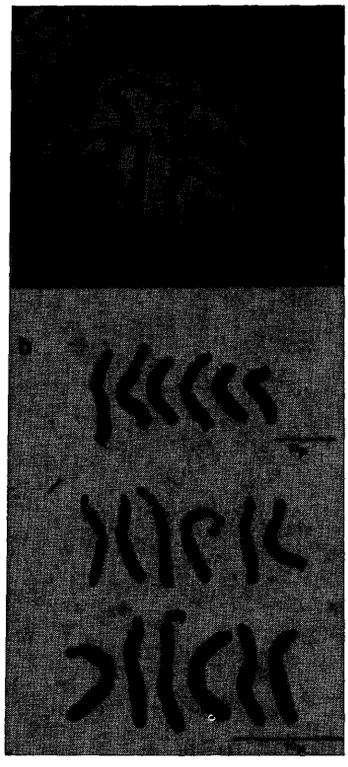

Figure 3. — a) Isolated SAT-chromosome of *Sequoia sempervirens* showing the long secondary constriction. b) Isolated marker chromosome pairs from cells of *Sequoia sempervirens.*

Discussion

Comparisons of the results of the present study with those of Saylor and Simons (1970) are presented by the diagrams in *Figure 4a, b.* Comparison of the A_1 or A genomes shows close agreement of the paired points *(Fig. 4a).* The A_2 in comparison with B shows the same general point distribution pattern but some discrepancies between the paired points *(Fig. 4b).* It may be that a real difference exists between the results of Saylor and Simons (1970) and the present study, but nonbiological factors such as slide preparation technique or different methods of arranging

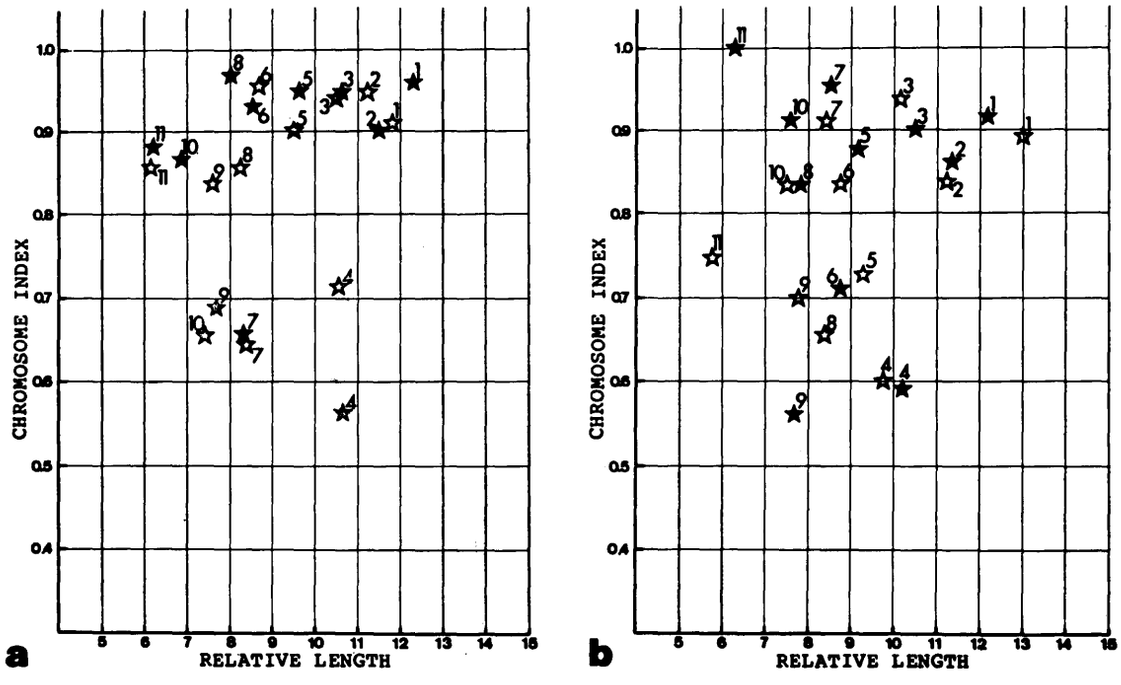

Figure 4. — Diagrammatic comparisons of *Sequoia sempervirens* genomes of the present study results (solid stars) with the results of Saylor and Simons (1970) (hollow stars). a) Comparisons of the A_1 or A genomes. b) Comparisons of the A_2 or B genomes.

chromosomes may have contributed to the apparent differences between the two works. For example, in the present study a karyotype was developed by cutting and arranging chromosomes from a photomicrograph of a cell whereas SAYLOR and SIMONS (1970) displayed only an idiogram.

SAYLOR and SIMONS (1970) concluded that three SAT-chromosome pairs existed in the chromosome complement of *Sequoia* based on the analysis of fifteen cells. They observed that the number of satellited chromosomes ranged from 0—6 per cell and all six satellited chromosomes were observed in three cells. However, none of the photographs in the paper shows all six SAT-chromosomes. As previously mentioned, SAYLOR and SIMONS (1970) had found six nucleoli per somatic cell, four large and two smaller, which may have influenced their opinion of the number of SAT-chromosomes in *Sequoia.*

In the present study only two SAT-chromosome pairs were visible in the sixteen cells examined. Although the morphology of the SAT-chromosomes and secondary constrictions was variable, this variation was inconsistent among the chromosomes. Nothing was observed to suggest that the satellites and the associated secondary constrictions of individual chromosomes were consistently different as in the SAT-chromosome observed in *Secale* by BOSE (1956, 1957, 1958). In no instance were more than four SAT-chromosomes visible in the complements. However, if only four SAT-chromosomes are present in the complement, then only four nucleoli should be visible in somatic cells instead of the six nucleoli reported by SAYLOR and SIMONS (1970). This is based on the assumption that the secondary constriction of each SAT-chromosome is associated with a nucleolar organizer region. Apparently, two other chromosomes are involved in nucleolar organization and are not exhibiting secondary constrictions in the slide preparation. This phenomenon is not uncommon in other plant species such as sugar beet (NAKAMURA and TSUCHIYA, 1982) or barley (TSUCHIYA, 1964). It is likely that microsatellites are present in a chromosome pair but are not visible in the slide preparation due to the pretreatment procedure used to shorten the chromosomes. Although the report of six SAT-chromosomes in *Sequoia* by SAYLOR and SIMONS (1970) is technically correct, it is felt that the additional two satellites observed are not the postulated microsatellites of the present study. SAYLOR and SIMONS (1970) make no distinction among satellite morphology in the idiogram. The two additional satellites observed by SAYLOR and SIMONS were likely artifacts affecting the morphology of several standard chromosomes in the complement or due to allocycly as shown in *Cryptomeria japonica* D. DON by SCHLARBAUM and TSUCHIYA (1981). Artifacts such as chromosome twisting and breakage and allocycly were observed to occur in *Sequoia* chromosomes by the present authors.

The chromosome number of *Sequoia sempervirens* 2n = 66, reveals the hexaploid nature of the species. STEBBINS (1947) classified polyploids into three categories: autopolyploid, allopolyploid, and segmental allopolyploid. STEBBINS (1948) considered *Sequoia* an autoallopolyploid (AAAABB) or a segmental allopolyploid ($A_1A_1A_1A_1A_2A_2$ or $A_1A_1A_2A_2A_3A_3$), which is independently supported by the chromosome configurations shown by HIRAYOSHI and NAKAMURA (1943). The karyotype analyses of SAYLOR and SIMONS (1970) and of the present study closely concur with Stebbins' hypothesis, indicating that the A or A_1 genome is repeated four times, AAAA or $A_1A_1A_1A_1$. This classifies *Sequoia* as an autoallopolyploid (AAAABB) or a segmental allopolyploid ($A_1A_1A_1A_1A_2A_2$). The determination of the genomic formula of the third chromosome complement, BB or A_2A_2 is more problematical. It appears from the previous discussion of SAT-chromosome types that one pair of chromosomes, with postulated microsatellites and responsible for nucleolar organization, is different in morphology than the other SAT-chromosomes and forms smaller nucleoli. The critical question is: whether the morphology of proposed microsatellites and associated nucleolar organizer regions (NOR) remained the same after development into the polyploid or whether the morphology was changed by amphiplasty. Synthesis of an artifical hexaploid *Sequoia* to determine the effects of amphiplasty on the combining genomes is impossible due to the relictual nature of *Sequoia.* The existence of a chromosome pair with microsatellites and associated NOR has not been observed in any of the studied genera of *Taxodiaceae* in the present or previous studies (SCHLARBAUM, 1980; SCHLARBAUM and TSUCHIYA, 1975, 1976, 1981, 1984; SCHLARBAUM *et al.* 1983, 1984), though no detailed meiotic study has been conducted in any species. A strong argument, however, can be made for alteration of the morphology of the SAT-chromosome pair and general karyotype of the BB or A_2A_2 genome by the effects of amphiplasty.

Amphiplasty or nucleolar dominance was first reported by NAVASHIN (1927, 1928, 1934) to occur in the karyotypes of hybrids of interspecific crosses in *Crepis* spp. Navashin found that the nucleolar SAT-chromosomes of one genome frequently had dominance over the SAT-chromosomes in the other genome in the hybrid condition. Amphiplasty often causes the absence of secondary constrictions, partial or complete inactivation of the nucleolus organizer regions, and morphological changes in the chromosomes of the dominated genome (cf. RIEGER *et al.*, 1979). A recent review by RIEGER *et al.* (1979) shows that amphiplasty has occurred in karyotypes of many plant and animal species.

From the earlier discussion, it was shown that *Sequoia* likely has a genome formula of $A_1A_1A_1A_1A_2A_2$ or AAAABB. Considering a genomic formula of $A_1A_1A_1A_1A_2A_2$ the following hypothesis can be made: if A_2A_2 originally contained nucleolar SAT-chromosomes similar to the SAT-chromosome pair in the $A_1A_1A_1A_1$ genomes and A_1 has nucleolar dominance over A_2 in the hybrid, the A_2A_2 SAT-chromosomes could likely be microsatellited in morphology and as a consequence, effect a corresponding reduction or suppression of nucleoli. Considering a genomic formula of AAAABB the following hypothesis can be made: if the B originally contained a chromosome with the NOR associated with an unusual structure, such as a long kinetochore region, and the SAT-chromosomes in the A genome had nucleolar dominance over nucleolar chromosomes in the B genome of the hybrid, the resulting morphology of the nucleolar chromosomes in the B genome could be similar to a conventional chromosome. The corresponding BB nucleoli would likely be affected also, and become smaller or absent.

Evidence from the karyotype analysis of the present study favors the interpretation that *Sequoia* is a segmental allopolyploid ($A_1A_1A_1A_1A_2A_2$). As shown by the diagrammatic comparison between the A_1 or A and A_2 or B genomes *(Fig. 5),* the chromosomes are similar, yet the karyotype of the A_2 or B genome is somewhat different than the A_1 or A genome which might be expected if amphiplasty has occurred. Moreover, the morphology of the large submedian

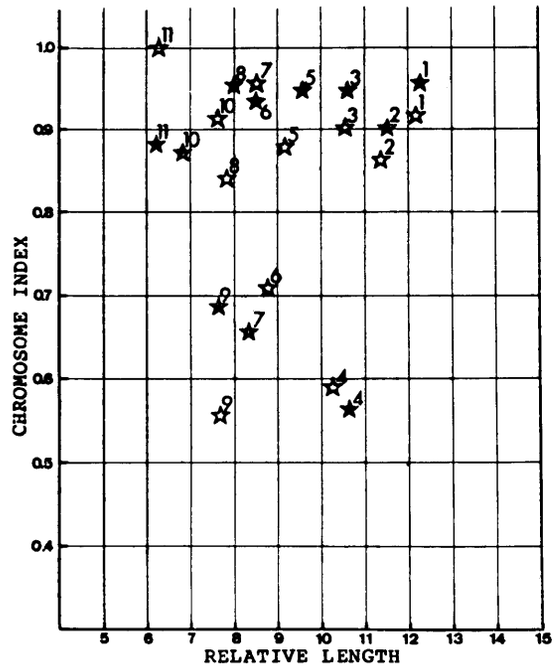

Figure 5. — Diagrammatic comparison between the A_1 or A (solid stars) and A_2 or B (hollow stars) genomes of *Sequoia sempervirens.*

chromosome pair of the A_2 or B genome corresponds to the SAT-chromosomes in the A_1 or A genome. This is what would be expected if the A_2 or B genome were affected by nucleolar dominance of the A_1 or A genomes. However, the possibility that *Sequoia* is an autoallopolyploid (AAAABB) must not be completely discounted. Detailed studies at mitotic late prophase and meiosis are needed in *Sequoia* to determine which chromosomes are involved in the nucleolar organization and what regions of the chromosomes possess the nucleolar organizing region.

The karyological relationships among *Sequoia, Metasequoia,* and *Taxodium* have been briefly discussed in previous publications (Schlarbaum *et al.,* 1983, 1984). The types and numbers of marker chromosomes found in *Metasequoia* and *Taxodium distichum* (L.) Rich. are different than those present in *Sequoia* making it unlikely that these species contributed to the polyploidy of *Sequoia.* Comparison between the marker chromosomes in *Sequoia* with the negative heteropycnotic pair found in *Sequoiadendron giganteum* (Lindl.) Buchholz (Schlarbaum and Tsuchiya, 1975) also indicates that genomic contribution by *Sequoiadendron* to *Sequoia* is not probable. Further discussion of cytotaxonomic and evolutionary relationships of *Sequoia* with other species in the *Taxodiaceae* will be presented in a future publication.

Literature Cited

Bose, S.: Aberrations in the nucleolar chromosomes of inbred rye. Hereditas **42:** 263—296 (1956). — Bose, S.: Aberrations in the nucleolar chromosome of inbred rye. II Size variation in inbred lines and population plants. Hereditas **43:** 621—643 (1957). — Bose, S.: Aberrations in the nucleolar chromosome of inbred rye. III Size variation in inbred lines in relation to vigor. Hereditas **44:** 257—279 (1958). — Buchholz, J. T.: The embryogeny of *Sequoia sempervirens* with a comparison of the Sequoias. Am. J. Bot. **26:** 248—257 (1939). — Dark, S. O. S.: Chromosomes of *Taxus, Sequoia, Cryptomeria,* and *Thuja.* Ann. Bot. **46:** 965—977 (1932). — Florin, R.: The distribution of conifer and taxad genera in time and space. Acta Horti. Berg. **20:** 121—312 (1963). — Fozdar, B. S. and W. J. Libby: Chromosomes of *Sequoia sempervirens:* 8-hydroxyquinoline-castor oil pretreatment for improving preparation. Stain Tech. **43:** 97—100 (1968). — Goodspeed, T. H. and M. P. Crane: Chromosome numbers in the Sequoias. Bot. Gaz. **64:** 348—349 (1920). — Hair, J. B.: The chromosomes of the *Cupressaceae 1. Tetraclineae* and *Actinostrobeae (Callitroideae).* N. Z. J. Bot. **6:** 277—284 (1968). — Hirayoshi, I. and Y. Nakamura: Chromosome number of *Sequoia sempervirens.* Bot. Zool. **2:** 73—75 (1943). (In Japanese with English summary). — Jensen, J. and A. Levan: Colchicine-induced tetraploidy in *Sequoia gigantea.* Hereditas **27:** 220—224 (1941). — Lawson, A. A.: The gametophyte, archegonia, fertilization, and embryo of *Sequoia sempervirens.* Ann. Bot. **18:** 1—28 (1904). — Levan, A., K. Fredga and A. A. Sandberg: Nomenclature for centromeric position on chromosomes. Hereditas **52:** 210—220 (1964). — Nakamura, C. and T. Tsuchiya: Cytogenetics of alien addition trisomics in sugar beet. I. Meiotic chromosome behavior in nematode-resistant trisomics. Biol. Zbl. **101:** 227—240 (1982). — Navashin, M.: Changes in the number and form of chromosomes as a result of hybridization. Z. Zellforsch. Mikroskop. Anat. **6:** 195—233 (1927). — Navashin, M.: "Amphiplastie" — eine neue karyologische Erscheinung. Proc. Int. Cong. Genet. **5:** 1148—1152 (1928). — Navashin, M.: Chromosome alterations caused by hybridization and their bearing upon certain general genetic problems. Cytologia **5:** 169—203 (1934). — Rieger, R., H. Nicoloff and M. Anastassova-Kristeva: "Nucleolar dominance" in interspecific hybrids and translocation lines — a review. Biol. Zbl. **98:** 385—398 (1967). — Sax, K. and H. J. Sax: Chromosome number and morphology in the conifers. J. Arnold Arbor. **14:** 356—375 (1933). — Saylor, L. C.: A karyotypic analysis of selected species of *Pinus.* Silvae Genet. **21:** 155—163 (1961). — Saylor, L. C. and H. A. Simons: Karyology of *Sequoia sempervirens:* Karyotype and accessory chromosomes. Cytologia **35:** 294—303 (1970). — Schlarbaum, S. E.:Cytotaxonomic relationships within *Taxodiaceae.* Ph. D. dissertation. Colorado State University. 217 pp. (1980). — Schlarbaum, S. E. and T. Tsuchiya: The chromosome study of giant sequoia, *Sequoiadendron giganteum.* Silvae Genet. **24:** 23—26 (1975). — Schlarbaum, S. E. and T. Tsuchiya: Chromosome study of Japanese umbrella pine. J. Hered. **67:** 65—67 (1976). — Schlarbaum, S. E. and T. Tsuchiya: Differential reactivity to staining in tree chromosomes. J. Hered. **72:** 62—63 (1981). — Schlarbaum, S. E. and T. Tsuchiya: The chromosome studies of *Cunninghamia konishii* Hay., *Cunninghamia lanceolata* (Lamb.) Hook., and *Taiwania cryptomerioides* Hay. Plant Syst. Evol. **143:** (1984) (In press). — Schlarbaum, S. E., T. Tsuchiya and L. C. Johnson: The chromosomes and relationships of *Metasequoia* and *Sequoia:* an update, J. Arnold Arbor. 65: 00—00 (1984) (In press). — Schlarbaum, S. E., L. C. Johnson and T. Tsuchiya: The chromosome studies of *Metasequoia glyptostroboides,* dawn redwood and *Taxodium distichum,* bald cypress. Bot. Gaz. **144:** 559—565 (1983). — Stebbins, G. L.: Types of polyploids: their classification and significance. Adv. Genet. **1:** 403—429 (1947). — Stebbins, G. L.: The chromosomes and relationships of *Metasequoia* and *Sequoia.* Science **108:** 95—98. — Stiff, M. L.: The geographical distribution and cytology of Coniferales. Ph. D. thesis. University of Virginia. 172 pp. (1952). — Tjio, J. H. and A. Hagberg: Cytological studies on some x-ray mutants of barley. Anales de la Estachion Experimental de Aula Dei **2** (2): 149—167 (1951). — Tsuchiya, T.: Chromosome aberrations and their uses in genetics and breeding in barley. Barley Genetics. I. Proc. 1st Intern. Barley Genet. Symp. (eds. Broekhuizen, S.; G. Dantuma; H. Lamberts; W. Lange), pp. 116—150 (1964). — Tsuchiya, T.: An improved acetocarmine squash method, with special reference to the modified Rattenbury's method of making a preparation permanent. Barley Genet. Newslett. **1:** 71—72 (1971).