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The Chromosome study of Giant Sequoia, Sequoiadendron giganteum[1])[2])

By S. E. SCHLARBAUM and T. TSUCHIYA[3])

(Received November / December 1974)

Awesome in size and height at maturity, the giant sequoia tree grows to be the most massive living organism in the world. Sequoiadendron is a monotypic genus belonging to the coniferous family of Taxodiaceae and related to another forest giant, the coastal redwood, Sequoia sempervirens.

Since the discovery of giant sequoia, botanical classification has been a problem. The tree has had no fewer than eight scientific names and is still called by an assortment of common namens. In recent years botanists have classified giant sequoia as Sequoia gigantea (LINDL.) DECNE. (putting giant sequoia in the same genus as Sequoia *sempervirens* (D. DON) ENDL. or Sequoiadendron giganteum (LINDL.) BUCHHOLZ (cf. Harlow & Harper, 1969). Presently, the latter name is accepted as correct, evidence being based primarily upon morphological, internal gametophytic, and embryological differences between giant sequoia and coastal redwood (BUCHHOLZ, 1939 a, b).

The haploid chromosome number, n = 11, was first observed by BUCHHOLZ (1939 a). JENSEN and LEVAN (1941) recorded the diploid chromosome number (2n = 22) and described the centromere position in ten chromosome pairs as median or submedian and subterminal in the remaining pair. In an induced tetraploid plant they occasionally observed a small satellite to be proximally attached to a subterminal chromosome. Detailed karyotype analysis has not been conducted.

In this paper the results of cytological studies in *Sequoiadendron* will be briefly reported with special reference to the finding of an extraordinary chromosome pair in the somatic cells.

Materials and Methods

Root tips of two seedlings were used in the study. The somatic cells in the meristematic region were isolated and analyzed. Many of these cells were at metaphase and counts of the complete chromosome number were frequent.

The root tips were pre-treated with eight oxyquinoline for 36 hours at 4° C and fixed with 3 : 1 mixture of 95% ethanol and glacial acetic acid.

Staining Methods

Method I

Feulgen staining after hydrolyzing the materials for 10 minutes in 1 N HCl at 60° C.

Method II

Stained with acetocarmine after hydrolyzing the materials for 15 minutes in 1 N HCl at 60° C (SAYLOA, 1961).

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

The nomenclature system of LEVAN et al. (1964) was used in the designation of the chromocomes, except for the SAT-chromosomes in which the length of the satellite has not been included in the arm length.

Results and Discussion

The somatic chromosome number is 2n = 22 (Fig. *1—3*), which confirms the results of previous work by BUCHHOLZ (1939) and JENSEN and LEVAN (1941).

JENSEN and LEVAN (1941) mentioned that 10 pairs were median and submedian, and one pair was subterminal without giving actual data.

Chromosome length was measured in the best cell in this study with results shown in Table 1. The two longest pairs are most likely metacentric chromosomes or M-type, eight pairs are near median or m-type, and the remaining pair,

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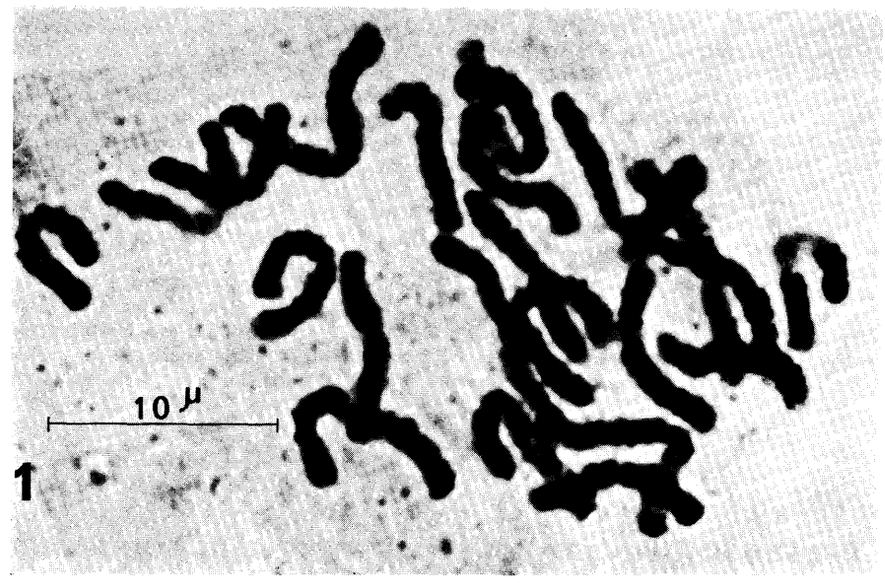

Fig. 1. — Somatic chromosome complement (2n = 22) of giant sequoia *(Sequoiadendron giganteum)* at late prophase of mitosis in root tips. A specific pair of chromosomes which showed "negative heteropycnosis" or lightly stained segment at the proximal region. ×3,000.

the second smallest in the complement, is submedian or sm-type of LEVAN *et al.* (1964). There were no subterminal chromosomes based on the nomenclature of LEVAN *et al.* (1964). The subterminal pair mentioned by JENSEN and LEVAN (1941) could be the sm-type pair of this study, since, the sm-type chromosome in the new system of LEVAN *et al.* (1964) might have been considered as subterminal by JENSEN and LEVAN (1941).

Some SAT-chromosomes were observed in various cells. As clearly seen in the photomicrograph *(Fig. 3)* and *Table 1*,

Table I. Measurements (in microns) of somatic chromosomes at meta-anaphase in the root-tip mitosis of Sequoiadendron giganteum.

Chromosome	Long arm	Short arm	Satellite	Total	L/S	Centromere
1	4.0	3.7		7.3	1.081	m
2	4.0	3.3		7.3	1.212	m
3	3.7	3.7		7.4	1.000	M
4	3.5	3.0		6.5	1.061	m
5	3.3	2.8*	0.7	6.1**	1.179	m
6	3.2	3.0		6.2	1.067	m
7	3.5	2.7		6.2	1.296	m
8	3.0	2.3		5.3	1.304	m
9	3.0	2.7		5.7	1.111	m
10	3.0	2.7		5.7	1.111	m
11	2.3	1.7*	1.0	4.0**	1.353	m
12	2.7	2.3		5.0	1.174	m
13	2.7	2.3		5.0	1.174	m
14	2.7	2.3		5.0	1.174	m
15	2.7	2.3		5.0	1.174	m
16	2.7	2.3		5.0	1.174	m
17	3.0	2.0		5.0	1.500	m
18	3.0	2.0		5.0	1.500	m
19	3.0	1.7		4.7	1.765	sm
20	3.0	1.7		4.7	1.765	sm
21	2.0	1.7		3.7***	1.176	m
22	2.0	1.7		3.7***	1.176	m

* One chromatid of one arm showed satellite.

** Satellite was not included.

*** Non-stained segment at the proximal region in this pair was not included in the measurements because of difficulty in distinguishing centromere and the non-stained segment at the meta-anaphase stage shown in Figures 2 and 3.

chromosome No. 5 and 11 showed obvious secondary constriction and satellite in one of the chromatids. At prophase *(Fig. 1)* at least three satellite chromosomes are recognized. However, it has been difficult to establish the relationship of the SAT-chromosomes in prophase *(Fig. 1)* with those at meta-anaphase *(Fig. 2 and 3)*. More critical analysis is necessary to ascertain the number and type of SAT-chromosomes in this species.

An unexpected result in this study was the discovery of one pair of chromosomes in which the proximal regions stained lightly or showed negative heteropycnotic nature. This nature is especially obvious at late prophase *(Fig. 1)*. At metaphase this region is almost completely discolored and difficult or impossible to distinguish from unstained centromere *(Figs. 2, 3, and 4)*. Instead, small dot-like bodies became visible at the supposedly centromere region in some cases *(Fig. 4c)*. The length of the unstained region is approximately 30% of the total length of the chromosome. The stained segment of two arms seems to be almost the same length. At late metaphase or meta-anaphase, the chromosomes were extremely contracted and it was difficult to recognize this special region easily, except for the fact that the smallest pair of chromosomes showed much wider "centromere" or unstained region compared to the centromere region of other chromosomes in the complement *(Figs. 2, 3 and 4)*.

The nature and the function of this specific pair is not known at present. However, this chromosome pair could be a good cytological marker for the study of phylogenetic relationships between *Sequoiadendron* and other related species, such as a diploid species, *Metasequoia glyptostroboides* (2n = 22) and a hexaploid species, *Sequoia sempervirens* (2n = 6x = 66).

There is no information on the karyotype analysis in *Metasequoia* in which only the diploid chromosome number, 2n = 22, was reported (STEBBINS, 1948). It is, therefore, impossible to make detailed comparisons of karyotypes between *Sequoiadendron giganteum* and *Metasequoia glyptostroboides*.

However, the preliminary results by the present authors showed that at least two pairs of chromosomes in the com-

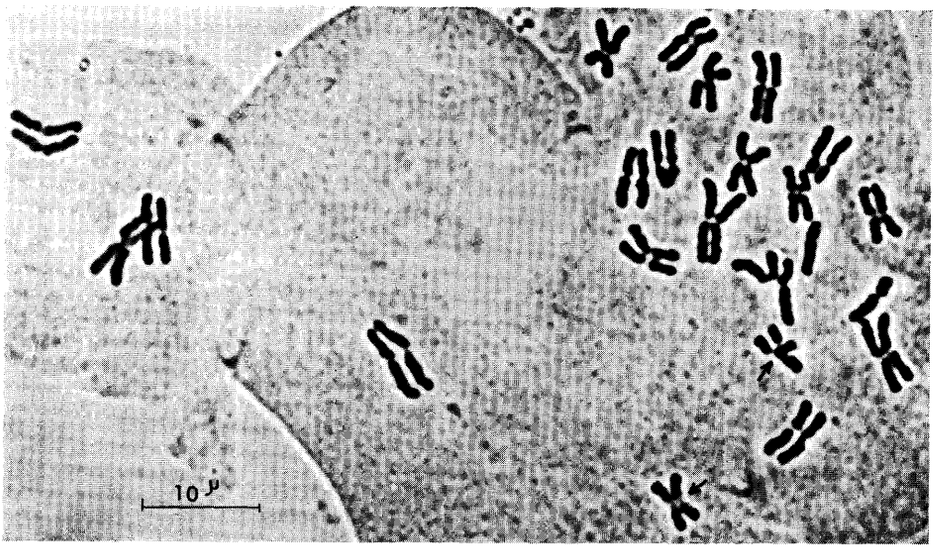

Fig. 2. — Somatic chromosome complement (2n = 22) of giant sequoia at meta-anaphase of a mitotic cell. The specific pair with lightly stained proximal segment was shown by arrow. ×1,500.

Fig. 3. — Somatic chromosome complement (eleven pairs) of giant sequoia. The chromosomes in the cells of Figure 2 were arranged in order of length. The specific pair is the shortest or 11th in the complement. ×3,000.

Fig. 4. — The specific pair with lightly stained proximal segment from six cells. a ~ c, from late prophase cells. d ~ e, from metaphase cells. f, from meta-anaphase cell (Figs. 2 & 3). Note: only one member of the specific pair was shown in e, since the other one was not able to be shown in photomicrograph. ×3,000.

plement of *Metasequoia* had peculiar structure at the centromere and/or proximal region. One of the pairs was definitely the shortest chromosomes corresponding to the specific pair in *Sequoiadendron*. Detailed karyotype analysis is now under way by the present authors.

A detailed karyotype analysis has been conducted by Saylor (1970) on *Sequoia sempervirens*, the hexaploid species with 2n = 6x = 66 chromosomes (Hirayoshi and Nakamura, 1943; Stebbins, 1948). However, it is extremely difficult to identify the chromosome pair similar to the specific pair (No. 11) in *Sequoiadendron* in this study. More extensive karyotype analysis of *Sequoia sempervirens* may be necessary to study the phylogenetic relationships among three species in the sequoia group.

Summary

Chromosomes of the giant sequoia, *Sequoiadendron giganteum*, were studied with the following results:

1. Chromosome number is 2n = 22, which is the same as the previous results reported by Buchholz (1939 a) and Jensen and Levan (1941).
2. Two pairs were most likely metacentric (M-type), eight pairs had near-median centromere (m-type), and the remaining one pair was submetacentric or sm-type of Levan *et al.* (1964).

3. The smallest pair of chromosomes having near-median centromere showed peculiar structure at proximal region. The proximal region (about 30% of the total length of the chromosome) of the chromosome showed light staining or negative heteropycnosis at late prophase. This segment is totally discolored at metaphase and can not be distinguished from the centromere. This chromosome pair may be used as a good cytological marker in the study of chromosomal evolution in the species of family *Taxodiaceae*.

Key words: Sequoiadendron giganteum, Chromosome.

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Pollen Germination and Pollen Tube Growth of Juniperus from Autumn and Winter Collections

By Karim Djavanshir and Gilbert H. Fechner[1])

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Introduction

The occurrence of a low proportion of filled seeds in developed fruits of *Juniperus* has been observed frequently. This low set of sound seeds may be the result of poor pollination due to a lack of synchronization between the dates of pollen release from male trees and those of ovular receptivity on female trees in the vicinity. If this were true, artificial pollinations from forced and stored pollen could enhance sound seed set, provided that pollen viability could be maintained. However, no information is available on the forcing or storage of *Juniperus* pollen, collected during the dormant period, or on the most suitable media for testing pollen germination and pollen-tube development of *Juniperus*.

The germination of pollen and pollen-tube development of conifers have been studied on different artificial media. Several authors (Dengler and Scamoni, 1939; Fechner, 1958) have found that aqueous solutions of less than 20 percent sucrose stimulate the germination of both fresh and stored pollen of *Pinus, Picea,* and *Abies,* compared to that on plain-water media. Fechner and Funsch (1966) found that for pollen stored eleven years the optimum concentration for germination of blue spruce pollen was 15 percent and that it was 5 percent for ponderosa pine pollen. This constituted a change, during storage, in the optimum concentration from 10 percent sucrose for unstored pollen of both species (Fechner, 1958).

Reichle (1960) showed that 0.01 percent, or even 0.001 percent, boric acid in a medium of 0.75 percent agar plus 7.5 percent sucrose stimulated the pollen-tube growth of *Pinus, Picea,* and *Abies,* but at a concentration of 0.1 percent boric acid, pollen germination and pollen-tube growth of most species that he studied were inhibited. *Pinus contorta* Dougl. was not stimulated at any of the three boric acid levels tested by Reichle. Vasil (1964) explained that the stimulatory effect of boron in pollen germination may be due to: a) increased absorption, translocation, and metabolism of sugars because of the formation of sugar-borate complexes, b) increased oxygen uptake, and c) the role of boron in the synthesis of pectic materials for the walls of the actively-growing pollen tubes.

Other substances are known to affect pollen germination and pollen-tube growth. For example, Brewbaker and Kwack (1964) found that calcium increased pollen germination and pollen-tube growth of angiosperms. The authors also pointed out that the effect of calcium is directly a function of the balance between potassium, magnesium, and hydrogen ions. Fins (1972) found that silver solutions in very low concentrations stimulated pollen-tube growth of Engelmann spruce *(Picea engelmannii* Parry); however, she also found that silver nitrate was highly toxic to pollen, limiting both germination and pollen-tube growth at a concentration of 0.1 ppm.

Benner (1972), studying the pollen-tube growth of alsike clover used four different nutrient solutions: those proposed by Kendall and Taylor (1965), Kendall (1967), Brew-

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[2]) For a single collection, December 10, a few branches were forced in a 1.0 percent boric nitrate solution, with no noticeable difference in pollen shedding or subsequent pollen germination or pollen-tube growth, compared to those forced in water.