Technical Papers

Probable Chromosome Number of Fossil Sequoia and Metasequoia Found in Japan[1]

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In 1943, Hirayoshi and Nakamura (*1*) determined the chromosome number of *Sequoia sempervirens*, the coast redwood of California, as 2n = 66, the highest number recorded for any species of conifer. Because of the lack of communication during the war period, their publication was not known outside Japan until after Stebbins (*2*) had independently made the same determination. In the latter publication, the chromosome number of the recently discovered *Metasequoia glyptostroboides* was determined as 2n = 22, in common with most other species of the coniferous families Taxodiaceae and Cupressaceae.

Fossil remains of *Sequoia* and *Metasequoia*, found in abundance in the late Tertiary (Pliocene) deposits of Japan (*3*), resemble closely the living species. These resemblances extend to the details of cell wall structure in the well-preserved leaf epidermis of the remains. Since the differences in size of the epidermal cells, and particularly of the guard cells of the stomata, in related plant species correspond closely with differences in chromosome number, the authors have compared the fossil remains with the living material of these species with respect to the sizes of these cells. The results, reported here, suggest the probable chromosome numbers of the species of *Sequoia* and *Metasequoia* that formerly existed in Japan.

Living material of *S. sempervirens* was studied from the same two sources as those that provided the chromosome counts. One of these was a tree grown by the College of Forestry of Kyoto University, and studied by Hirayoshi and Nakamura (*1*); the other, studied by Stebbins (*2*), was a tree growing on the campus of the University of California, Berkeley. The specimen of living *Metasequoia* was from the same material, collected in China in 1947, which Stebbins used for determining the chromosome number. The fossil remains studied were those of both *Sequoia* and *Metasequoia* found by Miki (*3*) at Tokiguti, Prefecture Gihu, and those of *Metasequoia* collected by Hikita at Noboritate, Awaji, Prefecture Hyogo.

Leaves selected for study were as nearly as possible of the same size. Each leaf was prepared for study first by decolorization for a long time in ammonium hydroxide, followed by neutralization in hydrochloric acid and mounting in balsam to make a permanent preparation. For each specimen, measurements were obtained for the length and width of 100 epidermal cells and 100 stomatal guard cells. Means and standard deviations were calculated for each of these sets of measurements, with the following results.

TABLE 1

SIZES OF STOMATA AND EPIDERMAL CELLS IN LIVING AND FOSSIL *Sequoia* AND *Metasequoia* (FIG. 2)

Material		Stomatal length, μ		Epidermal cells, length		Width, μ	Chromosome no.
		N	M ± σ	N	M ± σ	M ± σ	
Sequoia	Living, California	228	59.8 ± 4.28	100	139.3 ± 23.68	19.7 ± 4.72	2n = 66 !
	Living, Kyoto	300	56.1 ± 3.63	100	128.9 ± 34.04	17.5 ± 4.08	2n = 66 !
	Fossil, Tokiguti	162	54.6 ± 2.37	100	125.8 ± 31.04	18.7 ± 4.24	2n = 66 (prob.)
Metasequoia	Living, China	772	41.8 ± 3.12	100	50.9 ± 18.59	16.4 ± 3.49	2n = 22 !
	Fossil, Noboritate	324	42.9 ± 2.60	100	61.5 ± 18.20	19.9 ± 5.52	2n = 22 (prob.)
	Fossil, Tokiguti	228	31.3 ± 3.26	100	55.2 ± 14.01	14.9 ± 4.38	2n = 22 (prob.)

1. *Stomata*: In both living *Sequoia* and its fossil remains the mean length of the stomatal guard cells was 55–60 μ. In *Metasequoia*, these cells were distinctly smaller, the mean being about 40 μ in living material and in fossils from Noboritate, and about 30 μ in those from Tokiguti (Table 1 and Fig. 1). From these figures, it can be seen that the guard cells of both living and fossil *Sequoia* are significantly larger than those of living and fossil *Metasequoia*, but that the differences between the living species and its fossil counterpart are in both instances very slight and not significant. Although the *Metasequoia* remains found at Tokiguti have smaller stomata than the fossils from Noboritate and the living material, the cones found at the two fossil sites closely resemble each other, so that the senior author has concluded (*3*) that they both belong to the same species, *Metasequoia japonica*. The number of stomata within

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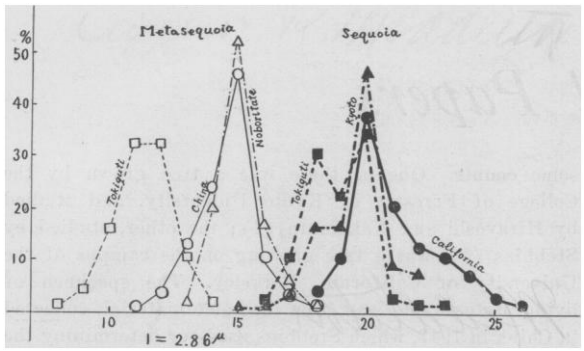

FIG. 1. Distribution of stomatal lengths in living and fossil *Sequoia* and *Metasequoia*.

a circle of 500 μ in diameter was counted in various regions of the leaf, but no significant differences were found between the two genera.

2. *Epidermal cells*: As was observed by Sterling (*5*) in the living *Metasequoia*, and confirmed by the present authors in both living and fossil material, the walls of the epidermal cells in this genus are undulate, a characteristic rarely found in conifers. Since the walls of the epidermal cells are straight in both fossil and living *Sequoia*, this difference is an additional and valuable diagnostic character for separating the two genera. In respect to size, the cells of *Sequoia* are about twice as long as those of *Metasequoia*, although there are no significant differences in width (Table 1).

The close similarity in size between both the guard and epidermal cells of the fossil remains of *Sequoia* and *Metasequoia* with the corresponding cells in living plants of the same genera is strong circumstantial evidence that the fossil *Sequoia* of Japan, like the living *S. sempervirens* of California, had the chromosome number 2n = 66, whereas the fossil *M. japonica* had the chromosome number 2n = 22.

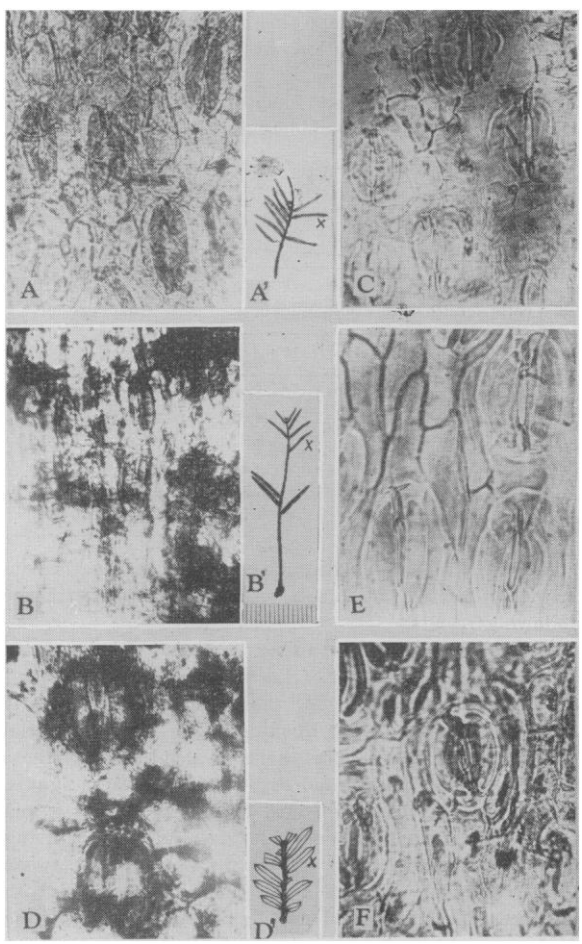

FIG. 2. *A,A'*, *Metasequoia* from Noboritate, Awaji, Pref. Hyogo; *B,B'*, *Metasequoia* from Tokiguti, Pref. Gihu; *C*, epidermis of *Metasequoia* from living species in China; D,D', *Sequoia* from Tokiguti, Pref. Gihu; E,F, epidermis of *Sequoia* from living species; *E*, material from Stebbins, and *F*, from Hirayoshi and Nakamura, *A,B,C,D,E,F*, × 400; *A',B',D'*, × 1; *x* shows leaf measured.

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Destruction of Amino Acids during Filter Paper Chromatography[1,2]

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Berry and Cain (*1*) have stated that one of the most critical operations in the preparation of a filter paper chromatogram involves removal of the solvent. They reported that the intensity of ninhydrin color which resulted from the same amount of an amino acid decreased with an increase of temperature over 80° C or with an increase in the time of heating, and they suggested that the amino acids were oxidized under these conditions. It was concluded that the solvent was best removed by blowing heated air at 85° C over the sheets for 8–10 min. Although many investigators have followed the methods described by Consden, Gordon, and Martin (*2*), which often involve solvent removal in an oven at temperatures up to 110° C, others have allowed the papers to dry at room temperature without giving reasons for doing so; frequently the temperature of drying is not specified in the description of experimental conditions.

Experiments summarized in the present report show that chromatograms wet with phenol should not be heated

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[2] After this paper had been submitted, Lowden and Penny (*Nature*, **165**, 846 [1950]), reported that the recovery of certain amino acids was decreased by heat-drying of paper chromatograms.