The Chromosomes and Relationships of *Metasequoia* and *Sequoia*

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THE DISCOVERY BY CERTAIN CHINESE foresters, and the recognition by Profs. Cheng and Hu, of living trees belonging to a genus described a few years earlier from fossil material was related in an earlier issue of this journal by Merrill (*7*). To this genus, *Metasequoia,* belong the great majority of the fossils which paleobotanists had previously judged to be the same species as, or a close relative of, the California coast redwood, *Sequoia sempervirens* (Chaney, oral communication). The systematic description of the living *Metasequoia* is either unpublished or, if published, has not yet been available to the present writer, although he has seen a copy of the illustration which Profs. Hu and Cheng are including with their description. Nevertheless, the extraordinary interest of this plant and the fact that both living and preserved material of it are now available make possible and desirable at least a preliminary evaluation of its relationships. The discussion and opinions presented here are based on examination of a series of herbarium specimens collected both in 1946 and by the 1947 expedition sponsored by the Arnold Arboretum and described by Dr. Merrill; of seedlings grown by N. T. Mirov, of the California Forest and Range Experiment Station, from seeds collected by the 1947 expedition; and of young trees as well as of microsporangiate and ovulate strobili in the stage of pollen shedding and pollination, collected by Ralph W. Chaney in March 1948.

A chromosome count of *Metasequoia* was obtained from acetocarmine smears of a vegetative shoot taken from one of the young trees brought back by Dr. Chaney and fixed in a 4:3:1 mixture of chloroform, absolute alcohol, and glacial acetic acid after a pretreatment of one hour in an 0.2% aqueous solution of colchicine. The chromosome number is 2n = 22, and the chromosomes are similar in both size and morphology to those illustrated by Jensen and Levan (*6*) for *Sequoiadendron giganteum* (Fig. 1). Other counts reported for the family Taxodiaceae are $n = 11$ in *Cryptomeria japonica,* 2n = "about 22" in *Taiwania cryptomerioides* and *Taxodium distichum* (*8*), and 2n = 20 in *Sciadopitys verticillata* (*10*). The writer has verified the number 22 in shoot smears of a tree of *Taxodium distichum* growing on the University of California campus and has found the chromosomes to be essentially similar to those of *Sequoiadendron* and *Metasequoia.*

For *S. sempervirens* the counts recorded by various workers are n = 16, 2n = "about 50," and n = 22 (*2, 6*).

FIG. 1. Somatic chromosomes of *Metasequoia* from an acetocarmine preparation of a shoot smear (× 1,900).

Seven years ago the writer attempted to study meiosis from acetocarmine preparations of microsporogenesis made from trees of *S. sempervirens* growing on the University of California campus, but in nearly all of the sporocytes the chromosomes were so closely packed together that they could not be separated well enough for counting without breaking individual chromosomes. Nevertheless, one or two cells could, with a little interpretation, be analyzed approximately in their entirety, and the chromosome complement of such a cell at first metaphase is illustrated in Fig. 2.

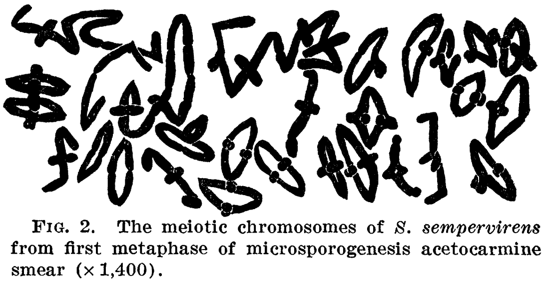

FIG. 2. The meiotic chromosomes of *S. sempervirens* from first metaphase of microsporogenesis acetocarmine smear (× 1,400).

It shows 2 chains of 6, 2 quadrivalents, and 22 bivalents, adding up to 2n = 66 chromosomes. In another cell the complement was approximately 3 chains or rings of 6, 3 quadrivalents, and 18 bivalents. In two different chromosome groups at first anaphase, a haploid number of about 33 was counted. Finally, somatic smears of both leafy shoots and ovules, some of which were made during the current year, yielded a few counts of approximately 66 and several of more than 50. Some of these made from the same tree, growing on the campus of Stanford University, from which Dr. Buchholz obtained the material which was the basis of his count of $n = 22$ ($2n = 44$). Furthermore, in all of these preparations of somatic tissues, 6 nucleoli were clearly seen in many telophase or resting nuclei. The writer is reasonably certain, therefore, that the coast redwood is a hexaploid with a somatic number of about 66 chromosomes. No other natural polyploids are known in the Taxodiaceae, and only a few have been recorded for the entire order Coniferales (*6*).

The cytological evidence, therefore, tells us little about the interrelationships between the genera of Taxodiaceae, except that *S. sempervirens* must have been derived from one or more other species not very different from it genetically and morphologically by means of polyploidy and perhaps hybridization. This intriguing problem will be discussed below, after a discussion of the morphological characteristics of the genera which appear to be most closely related to *Sequoia* and *Metasequoia.*

Merrill (*7*) has said of *Metasequoia* that "its botanical alliance is scarcely with *Sequoia,* as one might infer from its generic name. In its vegetative characters it suggests *Glyptostrobus* and *Taxodium,* but it may prove to be not closely allied to either of these two genera. . . ." This indicates that the four genera which should be considered as most closely related to *Metasequoia* are *Glyptostrobus,* the Asiatic "water pine"; *Taxodium,* the bald cypress of the eastern United States and Mexico; *S. sempervirens,* the redwood of the California coast; and *Sequoiadendron giganteum,* the "big tree" of the Californian Sierra Nevada. Two other genera of Taxodiaceae, *Taiwania* and *Athrotaxis,* may be as closely related as some of those included in this study, but adequate material of them was not available.

The most conspicuous morphological characteristic of *Metasequoia* is the opposite or decussate arrangement of all its parts: leaves, branches, bud scales, microsporophylls, and megasporophylls. In this it departs from all other genera of Taxodiaceae and resembles the Cupressaceae, although it does not resemble any genus of the latter family in other characteristics. The next most striking vegetative characteristic, the deciduous nature of the foliage and branchlets, is shared with *Taxodium* and *Glyptostrobus.* However, there is some evidence that this resemblance is due to parallel evolution rather than to true relationship. The cataphylls or winter bud scales of *Metasequoia* are much larger and more numerous than those of *Glyptostrobus* and *Taxodium,* and their epidermal cells are very different in appearance. In this connection the fact must be noted that *Sequoia sempervirens,* although evergreen, forms winter buds covered by cataphyll-like scales of about the same size and shape as those of *Metasequoia,* although in the California redwood these structures are thicker, usually green, and bear stomata on their outer (abaxial) surface. The branchlets of *Sequoia* disarticulate at the position of these scales, just as they do in *Metasequoia.* In *Sequoiadendron* the leaves are all similar, and no structures resembling bud scales are found.

In the morphology of its leaves, *Metasequoia* differs from all four of the other genera in that only flat, needle-like leaves are found. The leaves of *Sequoiadendron* are all scale-like or acicular; those of *Sequoia* and *Taxodium* are either needle-like or scale-like; while *Glyptostrobus* possesses scale-like, acicular, and needle-like leaves, with all three types often occurring on the same tree (*5*). A cursory microscopic examination of the leaf epidermis of *Metasequoia* indicates that the orientation and cellular structure of the stomatal apparatus resemble those of *Sequoia, Sequoiadendron,* and *Glyptostrobus,* as described by Florin (*4*) and observed also by the present writer. *Taxodium* differs in the irregular, usually transverse orientation of its stomata. There are, however, certain anomalous features of the leaf epidermis of *Metasequoia* which require further study.

Metasequoia resembles *Taxodium* in the arrangement of its pollen-bearing cones racemosely on specialized branches. When shedding pollen, the individual cones are short-stiped, as in *Sequoia, Taxodium,* and *Glyptostrobus,* while in *Sequoiadendron* they are sessile (*3*). The decussate arrangement of the microsporophylls or cone scales is unique among the Taxodiaceae. They bear on the summit of a conspicuous stalk an ovate sterile tip and three microsporangia, being in all of these respects very similar to the pollen-bearing scales of *Sequoia* and *Sequoiadendron.* Those of *Glyptostrobus* and *Taxodium* have much shorter stalks, and more numerous (6–9) smaller microsporangia (*5*).

The ovulate cones are also racemosely arranged along the branches, emerging in spring from lateral buds covered with numerous conspicuous cataphylls and toward maturity becoming elevated on elongate naked or scaly peduncles. In contrast, the ovulate cones of all other genera of Taxodiaceae are formed on the ends of twigs formed in the previous season or seasons. The decussately arranged sporophylls, when young, are similar to those of *S. sempervirens,* bearing about 8 ovules in a single row. In *Sequoiadendron* the ovules are arranged in two rows (*2*), while in both *Glyptostrobus* and *Taxodium* the much narrower sporophylls bear only two ovules. In *Metasequoia,* as in *Sequoia* and *Sequoiadendron* (as well as *Athrotaxis, Taiwania,* and *Cunninghamia*), the growth of the cone scale during maturation produces an inversion of the position of the seeds, so that at maturity they are reflexed, with their micropylar or apical ends pointing toward the axis of the cone. In *Glyptostrobus* and *Taxodium,* on the other hand, they remain erect until maturity. The seeds of *Metasequoia* resemble closely those of *Sequoiadendron,* having two conspicuous, pale wings on either side of a narrow body. The seeds of *Sequoia* have somewhat narrower, darker-colored wings, but are otherwise similar. Those of *Glyptostrobus* have no lateral wings, but a wing-like structure at their base, while the seeds of *Taxodium* are angular, thick, and wingless. The scales of the mature cones are essentially similar in *Metasequoia, Sequoia,* and *Sequoiadendron,* except that in the latter genus they are much larger. Those of *Taxodium* are somewhat similar, but *Glyptostrobus* has much narrower, oblanceolate cone scales. Judging from the considerable number of seedlings raised by Dr. Mirov, the number of cotyledons in the embryo of *Metasequoia* is consistently two, which is the usual number in *S. sempervirens. Sequoiadendron* ordinarily has four cotyledons, while the usual number in both *Glyptostrobus* and *Taxodium* is six.

These comparisons can be summed up as follows: In the decussate arrangement of its leaves and sporophylls, as well as in the character of its bud scales or cataphylls, *Metasequoia* is unique among the Taxodiaceae. Its leaves, although deciduous like those of *Glyptostrobus* and *Taxodium,* are perhaps more similar in structure to those of *Sequoia* than to those of any other genus in the family. In arrangement of the microsporangiate or pollenbearing cones, *Metasequoia* resembles *Glyptostrobus* and *Taxodium,* but the cones themselves, particularly with respect to the structure of the cone scales or sporophylls, are most like *Sequoia.* The racemose arrangement of the ovulate cones is likewise unique, but the cone scales, ovules, seeds, and young seedlings are much more like those of *Sequoia* and (in the case of the seeds) *Sequoiadendron* than those of *Glyptostrobus* and *Taxodium.*

Summing up the resemblances and differences in respect to 27 characters, including all those on which data could be obtained, and applying a simplification of Anderson and Abbe's (*1*) method of aggregate differences, the following conclusions were reached: *Metasequoia* resembles *Sequoia* in the largest number of these characters (18), differing from it in only 9. The next closest genus is *Taxodium,* with 14 similarities and 13 differences; then comes *Glyptostrobus,* with 12 and 15; and finally, *Sequoiadendron,* with 11 similarities and 16 differences with respect to *Metasequoia.* If spheres representing the five genera are joined by lines whose length is proportional to the number of differences separating each pair of genera, a three-dimensional figure of irregular shape is produced. This cannot be reproduced accurately on a sheet of paper, but its shape approaches that of a fusiform polygon with *Taxodium* and *Glyptostrobus* rather close together at one end, *Sequoiadendron* isolated at the other, and *Sequoia* and *Metasequoia* somewhere in the middle. The nearest genus to *Sequoiadendron* is definitely *Sequoia,* but the number of differences between these two (10½) is larger than that between *Sequoia* and *Metasequoia* (9). Since the striking differences between the latter two genera which have been mentioned above would preclude the possibility of uniting them into a single genus, the presence of *Metasequoia* strengthens the point of view adopted by Buchholz (*2, 3*) in recognizing the generic distinctness of *Sequoia* and *Sequoiadendron.* If the distinguishing characters are all considered to be of equal importance, *Sequoia* must be regarded as closer to *Metasequoia* than to *Sequoiadendron.* Furthermore, the only type of emphasis of characters which would lead to the opposite viewpoint would be one which considered certain vegetative characters (arrangement of leaves, deciduous vs. evergreen character) more important than differences in the structure of the reproductive organs (sessile vs. stipitate microsporangiate strobili, number of ovules per megasporophyll, length of time for maturation of seeds, shape of cone scales, number of cotyledons). In most groups of seed plants, these reproductive characters are considered more important than the vegetative ones. In regard to *Glyptostrobus* and *Taxodium,* the writer's opinion is that they are not related to *Metasequoia* more closely than is indicated by the placing of all of these genera in the family Taxodiaceae. The deciduous character of the leaves and the racemose arrangement of the cones, both of them clearly derived characters, have probably been acquired by *Metasequoia* independently of their appearance in *Glyptostrobus* and *Taxodium.* This opinion, of course, is subject to change as new evidence from additional characters is obtained. Of particular value would be a study of the development of gametophytes and embryos in *Metasequoia,* since these structures have been made classic botanical material by the researches of Chamberlain, Saxton, Looby and Doyle, Buchholz, and others.

Finally, the question of the origin of polyploidy in *S. sempervirens* should be considered. The configuration of the chromosomes at the meiotic metaphase in this species (Fig. 2) suggests that it is neither an autopolyploid or a typical allopolyploid, but one of the two intermediate types discussed elsewhere (*9*). It is either an autoallopolyploid with the genomic formula AAAABB, or a segmental allopolyploid with either $A_1A_1A_1A_1A_2A_2$ or $A_1A_1A_2A_2A_3A_3$. In any case, the trebling of the chromosome number was probably accompanied by hybridization between at least two, and perhaps three, distinct ancestral species. The comparison of characters summarized above has suggested to the writer that *S. sempervirens* could not have resulted from combining the characteristics of any two of the four diploid types mentioned and the other existing genera of the Taxodiaceae; *Cunninghamia, Taiwania, Cryptomeria,* and *Athrotaxis* do not seem to possess the characters found in *S. sempervirens* which are lacking in *Metasequoia, Sequoiadendron, Glyptostrobus,* and *Taxodium.* There is good reason to believe, therefore, that at least one of the diploid ancestors of this polyploid is extinct and has left no close relatives. If, however, one of these diploid ancestors is surviving or has left a close living descendant, this species would have to be one of the two most similar to *S. sempervirens* in their reproductive characteristics, namely, *Sequoiadendron* or *Metasequoia.* Of these two, *Metasequoia* seems to the present writer the most likely. If we imagine a species which, when its characters were combined with those of *Sequoiadendron,* would yield a plant resembling *S. sempervirens,* this imaginary species would possess the following characters: leaves rather large, broad, flat, needle-like, deciduous or with pronounced winter buds; ovulate cones very small, not over 1 cm long, and with 10–12 cone scales, each bearing 3 or 4 ovules; embryos dicotyledonous. This plant would have needles something like those of *Cunninghamia,* although smaller, and cones somewhat similar to those of *Chamaecyparis.* A plant like this seems unlikely to be found either in the living or fossil condition. On the other hand, if we postulate *Metasequoia* as one diploid ancestor of *S. sempervirens,* the other parent would have characteristics about as follows: leaves evergreen, spirally arranged, acicular and pointed, somewhat glaucous; winter buds absent; microsporangiate and ovulate cones terminal on the branchlets; ovulate cone scales with rather long prominent spines; mature cones about 12–15 mm long and half as broad; seeds with narrow, dark wing margins. This combination of characters is by no means an unusual or unexpected one; it is approached from various directions by *Sequoiadendron, Taiwania,* and *Athrotaxis.* There are grounds, therefore, on which to erect the working hypothesis that *S. sempervirens* originated as an allopolyploid from hybrids between an early Tertiary or Mesozoic species of *Metasequoia* and some probably extinct type of taxodiaceous plant not unlike the three modern genera mentioned above.

This hypothesis has value as a speculation, because it can form the basis for certain predictions which will test it. Buchholz (*2*) has pointed out that the embryology of *Sequoiadendron* is somewhat like that of *Athrotaxis* (and perhaps *Taiwania,* which appears not to have been studied), while that of *Sequoia* contains several peculiarities not found in any other genus of the family. On the basis of our present hypothesis, *Metasequoia* should also have these peculiarities (see summary in *3*). Furthermore, if the hypothesis is correct, then among the Mesozoic or early Tertiary fossils of Taxodiaceae which occur associated with *Metasequoia* there should be some which at least approximate the description given above. At any rate, the possibility that the "dawn redwood," *Metasequoia,* may actually be a direct descendant of an ancient ancestor of the present California redwood is a plausible and most intriguing one.

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