# POLYPLOIDY IN GYMNOSPERMS

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Received April 9, 1958

The gymnosperms constitute a primitive group of seed plants which have a considerable evolutionary interest. A substantial amount of literature on the morphology of the group exists, but very little has been written about the various cytogenetic factors responsible for evolution within the group. Our knowledge of such factors is based solely on the studies of the present-day taxa which, indeed, offer but a mere apology for the glorious past of the group as a whole. In this communication the role of one such factor, polyploidy, has been evaluated. Polyploidy is found at three levels in gymnosperms: polyploid seedlings in the progeny of diploid species, isolated polyploid trees in otherwise strictly diploid species and lastly, polyploid species and genera. These will be discussed in turn.

## Polyploid Seedlings

These are stray seedlings that arise spontaneously in the progeny of diploid species and so far have been noted within five species whose details are given in table 1.

As is evident from the table these seedlings arise in very low percentages. Except in the case of *Welwitschia mirabilis* all the polyploid seedlings have been discovered in nurseries where they are conspicuous by their slow growth. Because of the aberrant growth of such seedlings, it appears that these were always discarded, until Kiellander (1950) first found that such aberrant seedlings were often aneuploid or polyploid. Nothing definite is known about their origin. It is likely that these seedlings arise either through a chance union of reduced and unreduced gametes or through chromosome doubling in embryonal initials during proembryo formation or early embryogeny. The former hypothesis would explain the origin of the triploid seedlings, but the more probable origin of the tetraploid seedlings is perhaps due to the chromosome doubling in embryonal initials. It is of interest to note that Illies (1953) discovered such seedlings in the progeny of the polyembryonic seeds of *Picea abies*.

In the opinion of the present writer these seedlings do not represent successful cases of polyploidy. They are comparable to the frequent reports of the origin of isolated polyploid seedlings within many of our crop plants. Furthermore, except for *Cryptomeria japonica* (Chiba, 1950; Zinnai and Chiba, 1951), all such seedlings are short, stumpy and very slow-growing. These characters do not equip them for competition in natural habitats where there are several competitors for any new niche opened for colonization. Under such conditions natural selection would favor fast-growing individuals. It is reasonable to assume that in nature such polyploid and aneuploid seedlings may be arising constantly in most of the diploid gymnosperms, but due to their defective growth such seedlings have no survival value whatsoever and are therefore constantly weeded out. The polyploid and aneuploid seedlings may be at best looked on only as "potentialities" of the diploid species. A parallel case has been reported by Jones (1954) in *Holcus*. He found only four races (4x, 5x, 6x, and 7x) occurring in nature, but seedlings raised in nurseries indicated that, in addition to these four races, there appeared several aneuploid types. The aneuploids were never found in nature, where they seem to be con-

TABLE 1. *Polyploid seedlings in* Gymnosperms

| Species | 2n-chromosome number of polyploid seedlings | Percentage | Author |
|---|---|---|---|
| *Pinus densiflora* (2n = 24) | 48 | 0.08 | Zinnai, 1952 |
| *P. radiata* (2n = 24) | 48 | — | Rodger, 1953–54 |
| *Picea abies* (2n = 24) | ±28, 36, 48<br>28–30, 36 | 0.00216<br>— | Kiellander, 1950<br>Illies, 1953 |
| *Cryptomeria japonica* (2n = 22) | 33, 44 | — | Chiba, 1950<br>Zinnai and Chiba, 1951 |
| *Welwitschia mirabilis* (2n = 42) | 84 | 2.127 | Fernandes, 1936 |

stantly wiped out. Therefore, it may be concluded: 1) that aneuploid and polyploid seedlings in gymnosperms, and also in other plants appear in nurseries because in such habitats there is hardly any competition among the individuals, and 2) that the breeders encourage these seedlings since they now focus more attention on such aberrants.

## POLYPLOID TREES

Two solitary cases of polyploid trees have been reported: one each in *Larix decidua* (Christiansen, 1950) and *Juniperus virginiana* (Stiff, 1951 and unpublished).

The investigations of Sax and Sax (1933) and of Christiansen (1950) have shown that the prevailing cytological situation in *Larix decidua* (syn. *L. europea*) is that it is a diploid (2n = 24). A single tetraploid tree (2n = 48) was found growing in an estate in Denmark and studied by Christiansen (1950). From its morphology and also from the cytological behavior the tree, as expected, was found to be an autotetraploid. A preponderance of quadrivalents in addition to such irregularities as bridges, laggards, micronuclei, polyads, pollen grains with irregular chromosome numbers and low fertility was observed. The trunk of this tree is thinner than that of the diploids. From a study of the growth rings, it is clear that the tree has had a poor start; then suddenly it got a fillip, and later the growth rate again became very low. No data are available regarding its sexual progeny, but it seems reasonably certain that the progeny may not be cytologically balanced because of the irregularities, and in particular because of the deviating chromosome numbers in the pollen grains.

*Juniperus virginiana* has been reported as diploid (2n = 22) by several workers (Sax and Sax, 1933; Mathews, 1939; Löve and Löve, 1948; Ross and Duncan, 1949; Mehra and Khoshoo, 1956a), and also by Stiff (1951). The latter found one triploid (2n= 33) plant (about 4 ft in height) in the Orland E. White Arboretum of the Blandy Experimental Farm. Furthermore, he noted that the triploid does not differ in any qualitative character from the diploid, but shows gigas characters, as evidenced by the larger dimensions of stomata and nuclear volume. In view of this, it is more or less clear that the triploid plant is autotriploid.

It is certain that both instances are solitary cases of autoploidy in otherwise strictly diploid species. Furthermore, it appears that what is dealt with here are those polyploid seedlings which have been able to thrive and in one case (*Larix decidua*) even grow to maturity.

The reason for their success lies in the fact that both of these plants happen to have occupied protected habitats (an estate or an experimental farm) where, as indicated earlier, selective forces are not against such individuals. These two cases are comparable to the several reports regarding the occurrence of sporadic autoploid individuals in experimental fields in many of our crop plants.

With all their inherent limitations, both the polyploid seedlings and polyploid trees undoubtedly merit a very careful study of their morphology, cytogenetics and particularly of their economic properties. More important is the possibility that they could in time be utilized for raising auto- or allotriploid strains which are useful at least in some angiosperm tree species (Müntzing, 1936b; Johnsson, 1945, 1950, 1953). In *Populus tremula* the triploids are more vigorous than the diploids and tetraploids (Johnsson, 1953), which is perhaps due to hybridity rather than polyploidy itself. In this connection it is of interest to note that the triploid *Larix* raised after interspecific hybridization by Syrach-Larsen and Westergaard (1938) is also fairly vigorous.

## Polyploid Species and Genera

A species or a genus can be regarded as polyploid only if most (if not all) of its members are polyploid. In this sense, isolated polyploid seedlings and trees are only fortuitous cases. A perusal of the existing cytological literature on gymnosperms reveals that there are two types of gymnosperms that have been regarded as polyploid. In one class polyploidy is only of an apparent kind, being due to the increase in chromosome number as a result of causes not associated with the origin of polyploids. On the other hand, in the other class the taxa are genuinely polyploid. To the first category belong *Pseudolarix amabilis* (Sax and Sax, 1933) *Podocarpus* species (Flory, 1936; Mehra and Khoshoo, 1956b), and, perhaps, also *Welwitschia mirabilis* (Florin, 1932; Fernandes, 1936).

*Pseudolarix* possesses $n = 22$, which number is unique for Pinaceae, since most of its genera contain $n = 12$. Out of the 22 chromosomes in *Pseudolarix,* there are 20 terminal or subterminal and two nearly median chromosomes. By comparing this karyotype with the karyotype of the allied genera like *Pinus, Cedrus* and *Larix* (all $n = 12$ and with no terminal chromosomes) it is easy to imagine that the karyotype of *Pseudolarix* has arisen by the fragmentation of ten (out of 12) median-submedian chromosomes through the region of centromere. This would result in 20 terminal and two median-submedian chromosomes. The centromere of the terminal chromosome could become subterminal through an inversion involving the centromere. Such a suggestion was rejected by Sax and Sax (1933), in view of the well known postulate of Navashin (1932) about the centromere. Instead, Sax and Sax (1933) put forward the suggestion that *Pseudolarix* is a hypotetraploid ($4x - 4$) with 12 as the basic number. In this connection it is pertinent to note that the original postulate of Navashin (1932) has lost much of its rigidity, and data have accumulated which go a long way to show that fragmentation of median-submedian chromosomes into terminal chromosomes is possible. Such a mechanism has been inferred directly or by implication in several instances involving plant material by various workers (cf. Levan, 1932, 1935; Levan and Emsweller, 1938; Beal, 1939; Darlington, 1939, 1940; Rhoades, 1940; Garber, 1944; Chakravorty, 1948; Darlington and LaCour, 1950; Sundar Rao, 1950; Sears, 1952 a, b; Darlington, 1956; see these papers for further references). In view of this fact, the present writer feels that a more reasonable explanation of the origin of *Pseudolarix* is in the fragmentation of median chromosomes into terminal chromosomes, and not in autoploidy followed by a loss of four chromosomes. The

mechanism of fragmentation is probably misdivision (Darlington, 1939, 1940; Darlington and LaCour, 1950). The material basis for the process of misdivision is to be looked for in the compound nature of the centromere so clearly brought out by Lima-de-Faria (1949, 1956) and, particularly, by Tjio and Levan (1950).

Similarly, *Podocarpus* species with n = 19 may have been derived by fragmentation of median-submedian chromosomes of the species with lower numbers (cf. Flory, 1936; Mehra and Khoshoo, 1956b). However, a future cytogenetic investigation of the family Podocarpaceae may reveal the true situation. At the moment, the second suggestion of Flory (1936) that *Podocarpus* species with n = 19 are triploids from n = 13 cannot be regarded as valid. The simple reasoning here is that to date we do not know of any sexual triploid species ordinarily breeding true for its chromosome number. However, in view of the great antiquity of both Pinaceae and Podocarpaceae, one cannot be very sure of the manner in which chromosome number has increased in *Pseudolarix* and *Podocarpus* species, since there is no certainty regarding the probable ancestors of these genera. At any rate, no living genus can be regarded as the ancestor of these genera. The present evidence clearly reveals that the karyotypes of both *Pseudolarix* and *Podocarpus* species (n = 19) are neither exact multiples nor show any qualitative resemblance to their low-numbered relatives, and are therefore not polyploids of the latter.

Further support for the suggestion that *Pseudolarix* and *Podocarpus* species are not polyploids can be derived from the consideration of a comparable case in cycads. Sax and Beal (1934) have found that in the genus *Microcycas* (n = 13) out of 13 chromosomes, 11 are terminal, one subterminal and one has a median centromere. There is, however, a complete unanimity of opinion that *Microcycas* is not a polyploid of cycads with low numbers and a symmetrical karyotype. We are therefore hardly justified in regarding *Pseudolarix* or *Podocarpus* species as polyploid, because karyotypically both these cases are comparable to *Microcycas*.

*Welwitschia* has 2n = 42 (Florin, 1932; Fernandes, 1936), and is often interpreted to be a hexaploid because of the basic number of the genus *Ephedra* (x = 7). The analysis of the karyotype of *W. mirabilis* given by Fernandes (1936) clearly indicates that the 42 chromosomes of the somatic complement can only be classed in two basic sets of 21 chromosomes each. There is, however, no indication that the basic karyotype is composed of seven chromosomes, since in that case it should have been represented six times. At this stage it is pertinent to note that except *Podocarpus* many of the gymnosperm genera worked out to date show in general a stability of the basikaryotype not only within their species (cf. Sax and Sax, 1933; Sax and Beal, 1934; Flory, 1936; Mehra, 1946; Mehra and Khoshoo, 1956a, b) but also in their polyploids (Mehra, 1946a; Christiansen, 1950; Knaben, 1953; Hunziker, 1955). In strong contrast to *Welwitschia,* in the genus *Ephedra,* Mehra, (1946a) and Hunziker (1955) have found that in the tetraploid species the basikaryotype is represented four times. Therefore there is a complete lack of a basikaryotype of seven chromosomes in *Welwitschia,* unless we believe that in this genus alone there were radical alterations in the karyotype following polyploidy. The investigation of Fernandes (1936) has further shown that in *Welwitschia* the number of nucleoli is only two as is expected of a diploid. Furthermore, some of the chromosomes are telocentric. All these facts, when taken together, strongly point that the situation in *Welwitschia* should not be interpreted on the basis of *Ephedra.* However, a more important argument in support of the above conclusion is that the two genera (*Ephedra* and *Welwitschia*) are quite different morphologically (using the word in its widest sense) and

phytogeographically (cf. Arnold, 1948; Eames, 1952; Takhtajan, 1953; Florin, 1955; see these for further references). In view of these facts it is not as yet certain whether the resemblances between the two genera are of any real significance. They may only indicate parallel evolution in two otherwise more or less unrelated stocks. The chromosome number of *Welwitschia* is fairly high (2n = 42), and surely the higher the number of chromosomes, the more ways there are in which it could be compounded (cf. Stebbins, 1950). Thus, one cannot be very sure as to how the higher number has arisen, especially when the genus is not only monotypic but even the order Welwitschiales is monogeneric. With the present state of knowledge of the genus *Welwitschia,* the question of its ploidy level should be kept open.

*Gnetum* (n = 22) is yet another gymnosperm whose number is apparently indicative of polyploidy, especially because Fagerlind (1941) observed 11 smaller and 11 bigger bivalents in pollen mother cells of *G. gnemon.* It is interesting to note that according to Cook (1939) there are some resemblances in embryogeny between *Gnetum* (n = 22) and *Juniperus* (n = 11, Cupressaceae). However, such resemblance may be far-fetched, and the question of polyploid nature of *Gnetum* should wait till more species of the genus have been worked out.

There are only 11 gymnosperm species which can be regarded definitely polyploid in constitution. These are: *Sequoia sempervirens* (Hirayoshi and Nakamura, 1943; Yasui, 1946; Stebbins, 1948), *Juniperus chinensis pfitzeriana* (Sax and Sax, 1933), *J. squamata meyeri* (Jensen and Levan, 1941); the remaining eight cases occur in the genus *Ephedra* (Florin, 1932; Resende, 1937; Mehra, 1946a; Hunziker, 1953, 1955; Krapovickas, 1954). We may now proceed to comment critically on each of these.

The cytology of *Sequoia sempervirens* was correctly worked out for the first time by Hirayoshi and Nakamura (1943) and by Yasui (1946). Stebbins (1948), unaware of these publications also studied the species having n = 33, a number now confirmed by M. L. Stiff (personal communication) who counted 66 chromosomes in somatic tissue. The numbers n = 33 and 2n = 66 indicate that the species is a hexaploid, when compared with n = 11 found in all the genera of the family Taxodiaceae (cf. Mehra and Khoshoo, 1956a, table 2). That this species has in all probability remained a hexaploid since Tertiary (Pliocene) is apparent from the work of Miki and Hikita (1951). These writers have correlated the epidermal and guard-cell dimensions with chromosome number in the present day *Sequoia* (6x) and *Metasequoia* (2x). On the basis of this correlation they have deduced that the probable somatic chromosome number in fossil Sequoias of Japan was 66 and that of fossil *Metasequoia japonica* was 22. The observations of Hirayoshi and Nakamura (1943) and of Stebbins (1948) indicate that there are hexa-, quadri-, bi- and univalents at meiosis in *Sequoia.* Stebbins (1948) is of the opinion that it is a case of auto-allohexaploidy which has arisen from at least two if not three parental species. He made a thorough comparative character-analysis of the species in relation to other Taxodiaceous genera. He rightly rejected the idea that *Sequoiadendron giganteum* is one of the parents because of the fundamental differences between it and *Sequoia.* Yasui (1946) is also of the same opinion. On the other hand Doyle (1945) believes that *Sequoiadendron giganteum* (*Sequoia gigantea*) is the type from which *Sequoia sempervirens* has been derived. Apart from the morphological evidence given by Stebbins (1948), it may be pointed out that, if Doyle's interpretation is correct, then the autotetraploid of *Sequoiadendron* raised by Jensen and Levan (1941) should have shown rather a closer resemblance to *Sequoia sempervirens* (6x). Actually that is not the case. Instead, Stebbins (1948) has convincingly come to the con-

clusion that *Metasequoia glyptostroboides* is likely to be one of the parents of hexaploid *Sequoia*.

The multivalent associations indicate that the sexual progeny of *Sequoia* would not be balanced cytologically. In this connection it is of interest to note that Doyle (1945) has observed a considerable gametophytic and embryological sterility in this species. Furthermore, Buchholz and Kaieser (1940) and Doyle (1945) have also noted a good deal of seed abortion. In view of the observations a question arises as to how *Sequoia* has been able to reproduce with fidelity from times immemorial. Available data shows that in this species there exists a capacity for vegetative reproduction (cf. Bailey, 1933; Buchholz, 1939a, b,; Dallimore and Jackson, 1948; Platt, 1953). It is a matter of common observation that this species not only has suckers from stumps which often produce "merchantable lumber" (Bailey, 1933) but even the burls sprout (Platt, 1953). This capacity for vegetative reproduction may explain to some extent the lack of diploidization in *Sequoia*. However, according to G. L. Stebbins (personal communication) there is no doubt that sexual reproduction in *Sequoia* is rare in the mature forests of the species, though he has several times observed, "literally thousands of seedlings of the species in the raw, gravelly soil of stream margins in the redwood belt, which appear in great numbers after landslides, where they actually occupy pioneer stages in ecological succession." He further remarks that, "although a considerable proportion of the seeds produced by *Sequoia* may be inviable, the actual number of seeds produced each year by a single large tree is so enormous that there are always plenty available to colonize every suitable ecological niche which occurs in the vicinity of old trees. Under these conditions, selection pressure for increased fertility would be very low, and this explains the persistence for millennia of meiosis with multivalents." Furthermore, because of prolific seed formation, there are greater chances of production of individuals containing parental genomes in full. Those that are unbalanced may be eliminated by selection during embryo development or during germination or, even later, in the seedling stage. In view of abundant seed formation such embryonal selection can occur without any harmful effect on the reproductive capacity of the species.

In conclusion, *Sequoia* is the solitary gymnosperm species which is hexaploid. It is unique, since it maintains itself in spite of the persistence of multivalents (Stebbins, 1948) and sterility (Buchholz and Kaeiser, 1940; Doyle, 1945). This is possible because of its capacity for vegetative reproduction, prolific seed production, and subsequent selection of genetically balanced individuals in new and unstable habitats. It may also be suggested that the wide geological distribution of this species throughout northern hemisphere may be in part due to its capacity for both sexual and vegetative reproduction.

The second case of polyploidy is *Juniperus chinesis pfitzeriana*, which possesses 22 bivalents and has 6% of pollen sterility (Sax and Sax, 1933). These writers are of the opinion that the species is an autotetraploid in which diploidization has taken place. It is quite possible that this line of argument may be the correct one. However, one point needs emphasis. The species has rather long chromosomes and yet shows normal meiosis. It has all the cytological characteristics of a perfect allotetraploid. May it not be that this species arose after an intercenospecific cross? Perhaps the only objection to such a suggestion is that, so far, all the *Juniperus* hybrids discovered are fertile and therefore exhibit intra- or interecospecific relationship.

The third polyploid in coniferales is *Juniperus squamata meyeri* reported by Jensen and Levan (1941). They have investigated only the root-tips of the species and therefore it is not possible to

comment on the nature of the polyploidy (personal communication from A. Levan).

Out of the eight polyploid species in *Ephedra,* five (*E. altissima, E. intermedia, E. likiagensis, E. saxatilis* and *E. sinica*) have been analyzed by Mehra (1946a). He has shown that all these species are allotetraploid as indicated by karyotypic analysis. Though every tetraploid species contains a basikaryotype represented four times, yet, due to the differences in number and nature of nucleolar organizers, the entire complement can be divided into only two sets of 14 chromosomes each. Furthermore, meiotic analysis of a few tetraploid species has revealed that there is bivalent pairing, although some cytological disturbances at various stages and also some degree of sterility occur (Mehra, 1946b). *E. distachya* (Florin, 1932; Resende, 1937) is a tetraploid but has not been so far analyzed. *E. americana* (sensu lato, cf. Index Kewensis) contains not only *E. americana* proper, but also *E. andina* and *E. rupestris.* Out of these, *E. americana* (sensu stricto) and *E. rupestris* are diploids, while *E. andina* has been reported as diploid, tetraploid and hypertetraploid with $2n = 30$ chromosomes (Hunziker, 1953, 1955). Only the tetraploid form has been on karyotypic grounds deduced to be an allotetraploid (Hunziker, 1955). *E. breana,* though reported as diploid by Hunziker (1953, 1955), has, however, been found to be a tetraploid by Krapovickas (1954). It therefore seems that *E. americana* (sensu lato) and *E. breana* contain intraspecific chromosomal races, and need a closer cytotaxonomic study.

The following is the analysis of the natural polyploids reported so far in gymnosperms:

| | |
|---|---|
| (i) Auto-allohexaploidy: | *Sequoia sempervirens* |
| (ii) Allotetraploidy: | *Juniperus chinensis pfitzeriana, Ephedra altissima, intermedia, likiagensis, saxatilis, sinica* and the tetraploid form of *E. americana* (= *E. andina*) |
| (iii) Undetermined: | *Juniperus squamata meyeri, Ephedra breana* (tetraploid form) and *E. distachya* |
| (iv) Apparently intraspecific polyploidy: | *Ephedra americana* (sensu lato) and *E. breana* |
| (v) Further cytogenetic studies are desired on: | All *Juniperus* and *Ephedra* polyploids |

## Causes of Rarity of Polyploids

The foregoing survey has revealed that so far there have been only eleven authentic cases of polyploidy distributed in various orders reported. The data on the number of cytologically determined species within the various orders from an unpublished manuscript of the writer are summarized in table 2, which reveals that, in strong contrast to other plant phyla, the gymnosperms contain a very low percentage of polyploids. The question as to why polyploidy is rare in gymnosperms then arises. Various explanations have been suggested from time to time.

The first hypothesis was advanced by Sax (1932). She found a preponderance of interstitial chiasmata at meiosis. This fact was later confirmed by Sax and Sax (1933) and by Andersson (1947). According to Sax, quadrivalents with such chiasmata are likely to cause irregularities in meiosis, which would in turn cause sterility. Polyploids then would have a "small chance of survival." Heilborn (1934) has criticized this hypothesis since

TABLE 2. *Polyploids in* Gymnosperms

| Order | Total number of species | Number of species worked out | Number of polyploids | Percentage of polyploids |
|---|---|---|---|---|
| Cycadales | ca. 85 | 23 | — | — |
| Ginkgoales | 1 | 1 | — | — |
| Coniferales | ca. 483 | 195 | 3 | ca. 1.5 |
| Ephedrales | ca. 35 | 18 | 8 | 44.4 |
| Welwitschiales | 1 | 1 | — | — |
| Gnetales | ca. 30 | 2 | — | — |
| Total | ca. 635 | 240 | 11 | ca. 4.6 |

interstitial chiasmata are not universally present in gymnosperms. Dark (1932) found many of the chiasmata to be terminal, and similar observations have been made by Khoshoo (1957) on normal plants of *Cephalotaxus*.

Multivalents are a constant feature of autoploids (like *Larix decidua,* cf. Christiansen, 1950) or auto-alloploids (*Sequoia sempervirens,* cf. Hirayoshi and Nakamura, 1943; Stebbins, 1948) in gymnosperms. In these cases multivalents contribute to the sterility. On the other hand, if the polyploid is alloploid (*Juniperus chinensis pfitzeriana,* Sax and Sax, 1933; *Ephedra* species, Mehra, 1946b), there is only bivalent pairing and sterility is absent or low. Therefore, while Sax's hypothesis may explain the rarity of auto- or auto-alloploids in gymnosperms, it cannot explain the rarity of alloploids, which is more a question of cytogenetic differentiation of species.

Another hypothesis was put forward by Müntzing (1933, 1936a), who is of the opinion that double fertilization is the factor involved in the preservation of polyploidy in angiosperms. He states that the cytological ratio between the ovular tissue, endosperm and embryo normally is 2:3:2. This ratio is seriously disturbed if a diploid and its polyploid cross. In support of his concept, he noted that there is rarity of polyploidy in a group without double fertilization. Apparently this hypothesis works very well with the gymnosperms, since this group is strictly cross-pollinated and has no double fertilization. Therefore, a polyploid could easily cross with its diploid progenitor or progenitors. Generation after generation a polyploid race would break up and lose its identity, unless it developed some kind of barriers. However, this concept cannot explain the preponderance of polyploidy in Ephedrales where there is no double fertilization either. Furthermore, it cannot explain the occurrence of polyploidy in other plant phyla lacking both endosperm and double fertilization, viz. Pteridophyta (cf. Manton, 1950; Manton and Sledge, 1954), mosses (cf. Steere, 1954; Steere, Anderson and Bryan, 1954) and liverworts (cf. Tatuno, 1949; see also the list by Delay, 1953). Some of these groups have a very high frequency and grade of polyploidy.

Sax and Sax (1933) rightly believe that Müntzing's hypothesis could instead very well explain the occurrence of fewer genera and species in gymnosperms, because there would be free hybridization, so that, due to free recombination, distinctions between groups would break down. No doubt the cause of fewer genera and species may also lie in the extinction of taxa.

Darlington (1937) put forth the view that the relationship between the number and size of chromosomes and cell size acts as a limiting factor for polyploidy. He states that with polyploidy the chromosome number multiplies but the area of the metaphase plate does not increase proportionately. Evidently the ratio be-

tween the two is not maintained in polyploids. As a result, the chromosomes cannot arrange themselves properly during cell division, because of the small cell plate. Therefore, chromosome number and size would be limited by the smallest cell in the life history of the plant. In plants with secondary growth these are the cambial cells. In support of his concept, Darlington (1937) states that polyploidy is rather rare in genera like *Lilium* and *Fritillaria,* which possess the largest chromosomes and have no secondary growth. He considers the gymnosperms to be at the "upper limit," since these possess fairly large chromosomes and have secondary growth.

It is possible that in gymnosperms this ratio has reached an equilibrium. Any change (in the optimum chromosome number and cell size) by polyploidy proves deleterious to growth and development of the species. Indirect support for this contention comes from the observations on the polyploid seedlings occurring spontaneously and on those produced artificially by colchicine. In general, in both cases the growth is retarded and the seedlings are short and stumpy. The reason, perhaps, lies in the disturbance in size relationship of nucleus and cell.

Stebbins (1938, 1950) has substantiated the above concept and has pointed out that fiber cells are formed in angiosperms. These, being very small, must be formed from similarly very small cambial cells. In gymnosperms, in general, fibers are not present and the chromosome size is larger in comparison to most woody angiosperms.

It may then be said that gymnosperms are a group with secondary growth, without fibers and with long chromosomes. Therefore, the concept, substantiated by Stebbins, can explain the presence of long chromosomes in the group but cannot explain the rarity of polyploidy in general. It cannot explain the occurrence of the hexaploid *Sequoia sempervirens* (which is one of the gigantic trees of the world), of other successful cases of polyploidy in conifers and the preponderance of polyploidy in Ephedrales. It may be remembered that all these taxa have large chromosomes and secondary growth.

Yet another hypothesis could be developed, taking into consideration the cytogenetic relationship of the diploid species of gymnosperms.

In an unpublished manuscript, the writer has concluded that, in general, the gymnosperm genera (except notably *Podocarpus*) are homoploid, and speciation is a matter of gene mutation and/or repatterning of chromosomes. Furthermore, due to the lack of double fertilization and the presence of wind pollination, hybridization in gymnosperms is to be expected to be very common. This naturally explains the numerous reports on the natural and artificial intra- and interspecific hybridization within several gymnosperm genera: *Araucaria, Taxus, Pinus, Picea, Larix, Abies, Tsuga, Cupressus, Chamaecyparis,* and *Juniperus* (cf. Forestry Section, Plant Breeding Abstracts). Besides these, there are also intergeneric hybrids on record: *Ceratozamia* × *Zamia* (Chamberlain, 1926), four hybrids involving *Tsuga, Picea* and *Keteleeria* (Campo-Duplan and Gaussen, 1949) and *Cupressus* × *Chamaecyparis* (Osborn, 1940).

Many of the above hybrids (intra- and interspecific, and inter-generic) are fairly fertile. So far, the writer has not found a report of a case of a completely sterile interspecific or intergeneric hybrid in gymnosperms in which sterility is chromosomal due to a lack of homology of chromosomes. On the other hand, generally bivalent pairing has been found in hybrids wherever such studies have been made (Sax, 1932; Sax and Sax, 1933; Hirayoshi, Nakamura and Kano, 1943; Ross and Duncan, 1949). In many cases, because of the homology of the chromosomes, there is not only fertility but also regular gene flow. These facts will be considered in detail in a subsequent publication; for the present discussion the

only important point is that, wherever hybridization is successful, the relationship between the two parents is below or at the ecospecific level, no matter whether the taxa involved are full fledged genera in the morphological sense. Therefore, hybridization is likely to result in "homogamic hybrid complexes" (cf. Grant, 1953). Keeping in mind the important postulate of Stebbins (1947, 1950) that polyploidy is almost always associated with hybridization, it appears certain that polyploids ensuing from "homogamic hybrid complexes" would be autoploid or, at best, segmentally alloploid. At any rate, it is more or less certain that the polyploids would have many autoploid characteristics. Naturally, such polyploids would possess multivalents, particularly because in gymnosperms chromosomes are fairly long. The validity of this statement is borne out by the cytological studies on true autoploids (*Larix decidua,* Christiansen, 1950) and also on those in which hybridization is involved (*Sequoia,* Stebbins, 1948; triploid *Larix,* Knaben, 1953). In all these cases there are multivalents and sterility, which in all probability is the result of irregular disjunction of multivalents, univalents, although it also may be due to physiological causes (cf. Stebbins, 1947). Such polyploids would neither breed true morphologically nor cytologically. Almost all gymnosperms reproduce predominantly by sexual means and because of this, it is doubtful if the "raw" gymnosperm polyploids with all their inherent limitations would be able to pass the "bottleneck" of initial sterility before they could establish themselves as fertile and true-breeding lines. The case of autotetraploid maize (Gilles and Randolph, 1951) is apparently an encouraging example, since, being a sexual annual, it showed progressive bivalent formation within ten years. However, the question remains whether autotetraploid maize can have survival value in nature and it is only then that a reduction of quadrivalents may be expected to take place. It may be pointed out that the situation in experimental fields is far too different from the one in nature. It is also pertinent to mention here that autoploids are not at all good competitors. This conclusion is borne out by the work of Sakai and Suzuki (1955a,b), and of the present writer (unpublished data on the *Sisymbrium irio* complex). According to all these workers, genomic alloploids are much superior in competitive ability to auto- or autoalloploids.

This explanation apparently does not hold for *Sequoia,* which is an auto-allohexaploid and possesses multivalents. As already indicated, the persistence of multivalents can be partly explained by the capacity of *Sequoia* for vegetative reproduction and by its exceptionally high seed production. Because of the latter, there is a greater probability not only for production of genetically balanced individuals but also of low selection pressure for increased fertility. It is because of such a combination of factors that *Sequoia* still retains autoploid characters. In the light of these statements *Sequoia* therefore does not contradict the above hypothesis.

In sexual pteridophytes and angiosperms almost all successful and vigorous polyploids have alloploid characteristics. Their origin is directly possible from intercenospecific crosses with chromosomal sterility, a situation singularly lacking in gymnosperms. Furthermore, in gymnosperms the origin of alloploids from auto- or segmental alloploids is problematic, perhaps, because of sterility and low competitive ability. The very fact that polyploids have not established themselves in gymnosperms (even though polyploid seedlings arise constantly) is a clear proof that alloploids cannot arise from auto- or segmental alloploids in this group. The few cases of alloploids occurring in gymnosperms may very well have arisen after rare intercenospecific crossing.

In support of the above contention, the examples of genera like *Aquilegia, Ceano-*

*thus Quercus,* etc., which are well known for ecospecific differentiation of species (cf. Stebbins, 1950), could be cited. In all these genera there are either no polyploids or their percentage is very low (cf. Darlington and Wylie, 1955).

The low frequency of polyploidy is also explained by the fact that agamospermy is, perhaps, totally lacking in the group (at any rate, so far, there is no authentic case reported in literature). Furthermore, the incidence of vegetative reproduction is also very low. It is not necessary to mention the fact that raw polyploids have a better chance of survival if potentialities for agamospermy and/or vegetative reproduction are present in diploid species. It may be pointed out here that the observation of Land (1913) regarding the possible vegetative reproduction in *Ephedra* are significant, in view of the high incidence (44.4%) of polyploidy in this genus.

The gymnosperms (especially *Ginkgo* and Coniferales) resemble woody angiosperms in their habit, and, in general, lack cases of polyploidy for reasons enumerated above (cf. also Stebbins, 1938, 1950). Both groups show stability not only in their habit and chromosomes but also in that both occupy relatively stable mesophytic habitats where they form great forest belts. It is true that the basic numbers of several woody angiosperms may be themselves of a polyploid origin (Stebbins, 1947, 1950), and this could very well act as an additional factor accounting for a general lack of polyploids. However, there is so far no evidence for the polyploid origin of the basic numbers of the majority of gymnosperms. Furthermore, the cytological stability of gymnosperms and also of woody angiosperms is not a modern attribute but appears to have been handed down from geological times, Paleozoic in the former and Cretaceous in the latter. Keeping these points in mind, it should be emphasized that the lack of polyploids, at least in conifers, is not due to any inherent features of this group alone, but that conifers are woody plants and like other woody plants (whether gymnosperms or angiosperms) lack polyploids for reasons discussed earlier. Furthermore, Stebbins (1950) has already made the important generalization that polyploids occupy geologically newer habitats, and, it appears, that the stable and constantly favorable habitats in which woody-plants grow, do not offer such opportunities in abundance. As a result, in general, polyploid races in trees are unlikely to establish themselves.

In brief, the rarity of polyploidy in gymnosperms is chiefly due to the woody habit itself and to ecospecific differentiation of species. Polyploids which might arise would have autoploid characteristics. Such polyploids are usually sterile and have low competitive ability. The net result is that these polyploids lack survival value, especially because other supporting factors such as vegetative reproduction and agamospermy are lacking in gymnosperms. Before the validity of these statements is accepted, studies along the following lines are needed. Artificial polyploids from interspecific and intergeneric crosses should be raised and studied cytogenetically. Search for completely sterile hybrids needs to be intensified. Polyploids from such hybrids should also be studied. Furthermore, competitive ability of tetraploids should be tested against their diploid parents. Incidentally, some of these studies would give very useful information about the extent and nature of cytogenetic differentiation of species and genera.

## Conclusions

The foregoing survey has revealed that polyploids are rare in gymnosperms. The genus *Ephedra* represents the only group in which this type of evolutionary change is common. Primarily, the causes of rarity of polyploidy in gymnosperms are to be looked for in the woody habit and ecospecific differentiation of taxa (between which hybridization is possible), even though two genera may be involved.

Perhaps, in *Ephedra* the diploid species are strongly differentiated cytogenetically, and hybridization is still possible between them. It appears that in most of the other gymnosperms hybridization is not possible with increased morphological and cytogenetical differentiation of species.

As a whole, gymnosperms constitute a group in which hybridization is quite common, frequency of polyploids is low and apomixis is almost absent. Only one "closed system"—*Sequoia sempervirens* (6x)—is found in the group. In strong contrast, there are several cases of closed systems in pteridophytes and angiosperms, notably in the former (cf. Manton, 1950; Stebbins, 1950; Abraham and Ninan, 1954; Verma, 1956). The evolutionary future of all such closed systems is very limited. One could therefore reasonably expect more progressive evolution in gymnosperms, since the taxa are predominantly at diploid level. However, such evolution is not possible at present because the group is an antique one and therefore several taxa (notably cycads and *Ginkgo*) have been subjected to selection from times immemorial. Thousands of mutations may have occurred ever since their origin, so that their genotypes are now depleted and senescent even though they have stayed on the diploid level.

In pteridophytes and angiosperms hybridization, polyploidy and apomixis have been so rampant as to have caused a great amount of reticulation in the phylogeny of taxa, and to date there is no agreed classification of these groups (Stebbins, 1947, 1950). It may be remarked that ever since their origin, the incidence of hybridization, polyploidy and apomixis seems to be so common that in future it would be very difficult to obtain a truly phylogenetic classification of these groups. On the other hand, in gymnosperms only the incidence of hybridization is high. The genera and species are fewer than in pteridophytes and angiosperms. The problems of classification are relatively simple. This is partly due to the heavy extinction that has taken toll of the group in the past, thus creating discontinuities. Therefore, taxa that were once morphologically connected and genetically related became well separated when the "links" perished. This is why genera in gymnosperms are generally well recognized and clear cut. The only other difficulty in erecting a truly phylogenetic classification lies in parallel mutations and convergent evolution. These factors might connect taxa that were otherwise unrelated.

## Summary

The role of polyploidy in gymnosperms has been evaluated. Polyploidy is found in the progeny of diploid species, in stray trees of otherwise strictly diploid species, and, finally, entire species or genera may be of polyploid constitution.

So far, polyploid seedlings have been discovered in very low percentages in the progeny of only five species (table 1). Such seedlings are only "potentialities" of the diploid species and, because of their short, stumpy habit and slow growth, cannot establish themselves in nature where fast growing individuals would be at a selective advantage.

Only two polyploid trees, one each in *Larix decidua* (4x) and *Juniperus virginiana* (3x), have been discovered so far. Both of these are autoploid in nature and appear to have arisen from polyploid seedlings which happen to have occupied protected habitats and were thus able to thrive.

Two types of gymnosperm species and genera have been regarded as polyploid. In one group, the increase in chromosome number is not due to causes associated with the origin of polyploids, i.e., the polyploid condition is only apparent and not real. *Pseudolarix amabilis* (n = 22), *Podocarpus* species with n = 19 and *Welwitschia mirabilis* (2n = 42) belong to this group.

The second group contains eleven cases of true polyploids. Three of these (*Sequoia sempervirens,* and two *Juniperus*

species) belong to Coniferales, while the remaining eight are found in Ephedrales. Only one (*Sequoia*) is auto-allohexaploid, seven (*Juniperus chinensis pfitzeriana* and six *Ephedra* species) are allotetraploids, while the remaining three species (*Juniperus squamata meyeri* and two species of *Ephedra*) are so far undetermined. Taking all gymnosperms together the frequency of polyploidy is only 4.6%.

The various hypotheses advanced to explain the rarity of polyploidy in gymnosperms have been reviewed. It appears that the chief causes of such rarity are the stability in habit and habitat and ecospecific differentiation of all the taxa between which hybridization takes place; even when the two taxa may be two genera in morphological sense. The resulting polyploids from such hybrids are expected to possess autoploid characteristics. Due to multivalents, other meiotic irregularities and physiological causes, a good deal of sterility is expected. Associated with this is the low competitive ability found in autoploids in general. All these facts, coupled with the total lack of apomixis in gymnosperms, would make the success of polyploids highly unlikely. In gymnosperms such polyploids do not seem to evolve into perfect alloploids.

The problems of classification are relatively simple in gymnosperms because of the lack of reticulate phylogeny which is so characteristic of pteridophytes and angiosperms, because of rampant hybridization, polyploidy and apomixis in the latter two groups. The only difficulties in erecting a sound classification of gymnosperms lie in the discontinuities caused by extinction of taxa. Thus, related taxa appear to be distinct and unrelated, and because of parallel mutations, unrelated taxa appear to be related.

## Acknowledgments

I owe a deep debt of gratitude to Prof. P. N. Mehra for inspiring me to take up cytology of gymnosperms and for his keen interest, to Prof. G. L. Stebbins, Jr. (Davis) for his valuable criticism of the manuscript and inestimable help, and to Dr. M. L. Stiff (Lafayette) for kindly allowing me to quote his unpublished observations. To Profs. P. Maheshwari (Delhi), O. E. White, W. S. Flory (Virginia) and Dr. Z. M. Illies (Schmalenbeck) I am grateful for their help with the literature.

## Postscript

After the above paper was submitted for publication, a valuable work appeared on the "Chromosomal Evolution in the Podocarpaceae" by J. B. Hair and E. J. Beuzenberg (Nature 1958, **181**: 1584–1586). Fifty-two species have been studied which cover all the seven genera of the family. The haploid chromosome number ranges from 9 to 19. There is a "regular numerical relationship" between the number of metacentric and subtelocentric chromosomes. The wide range in the chromosome number is the result of "fragmentation" of metacentrics and/or "fusion" of subtelocentrics. Furthermore, as usual, true polyploidy is conspicuous by its absence. The results of this investigation have been incorporated in table 2 of the present paper.

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