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BIOLOGISKE MEDDELELSER, BIND XVIII, NR. 9

A TETRAPLOID *LARIX DECIDUA* MILLER

BY

H. CHRISTIANSEN

KØBENHAVN
I KOMMISSION HOS EJNAR MUNKSGAARD
1950

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Due especially to the excellent contributions by SAX & SAX (1932, 1933) the chromosome number and chromosome morphology of many coniferous trees are known.

The great majority of the species of coniferous trees agree in being on the same chromosome level, having either 2n = 24, or 2n = 22 or 2n = 26. Polyploidy apparently is an exceedingly rare phenomenon in this group of plants, strictly polyploid numbers being known only for *Sequoia sempervirens, Pseudolarix amabilis* and *Juniperus Chinensis* (see the chromosome list in DARLINGTON & JANAKI AMMAL, 1945).

These facts justify the conclusion that polyploidy has not been effective in the evolution of the conifers, and have also led to the view that a breeding scheme in conifers based on experimentally produced polyploidy would have little chance of success.

The few polyploid or mixoploid plants of *Picea abies*, raised by colchicine treatment or found by measurements of stomata among seedlings in nurseries, as described in the annual reports of the Swedish Forest Tree Breeding Institute at Ekebo (1946, 1948), proved to be rather dwarfy and far behind the diploid plants in rate of growth.

Much better is the triploid larch hybrid (*Larix decidua* Miller × *Larix occidentalis* Nutt.) described by SYRACH LARSEN & WESTERGAARD (1938), but in this case it is open to discussion to what extent the fairly satisfactory growth is due to polyploidy or to heterosis.

Thus the chance of finding in nature a large, old autopolyploid specimen of a coniferous tree would *a priori* seem rather remote, but nevertheless such trees exist, the first one, a tetraploid *Larix decidua*, having been found last year (1949) in the park of the estate Gisselfeld in Sealand, Denmark.

The tree (plate I, fig. 1) stands in a small glade, but a nearby group of deciduous trees to the south gives much shade and has hampered the development of the lower branches of the larch and probably also its growth.

The tetraploid larch is 15.2 m in height and has a straight trunk, which measures 97.5 cm in girth at a height of 1.3 m above the ground.

The branches of the crown are rather sparse, but long and drooping-curved. The lower branches, especially, are very drooping, and the side branches and twigs, which are but sparsely ramified, hang down vertically, giving to the tree a pendulous habit (plate I, fig. 2).

Borings with a sampler near the ground showed the tree to have 54 annual rings, and the total age of the tree may thus be estimated at 56—58 years.

The annual rings in the inner and outer part of the trunk differ much in size as will be seen from the following table:

Mean size of annual rings for periods of 10 years:

(1895—1898	0.7 mm)
1899—1908	3.4 —
1909—1918	3.3 —
1919—1928	2.7 —
1929—1938	1.9 —
1939—1948	0.8 —

It appears from the table, that the tree had a very slow start, but at the age of 8—9 years suddenly put up a much better growth with annual rings measuring on an average more than 3 mm. The good growth continued till the age of about 27 years, and within this period the minimum and maximum size of the annual rings was 0.3 mm (1899) and 6.6 mm (1906), respectively. In later years the growth gradually decreased, and in the last ten years it fell to the level of the starting years. The reason for this decline is not known. A direct comparison with the growth of normal, diploid trees of *Larix decidua* is not possible, since trees of the same age and under similar conditions are not growing in the park of Gisselfeld. The measurements of the growth of larch found in the Danish forestry literature can hardly be used, partly because they refer to stands, not to single trees, and partly because the soil conditions, exposure, etc., vary much in the different parts of Denmark. It is evident that the dimensions of the trunk of the tetraploid larch tree are inferior to those of well-developed diploid larches; if, however, the former had not slowed down about 1918, but continued to grow at the fast rate till 1948, this would have meant an increase of its diameter by about 40 per cent. and a substantial improvement. In the present circumstances the relative growth rate of diploid and tetraploid larch cannot be determined until comparable plants are cultivated side by side.

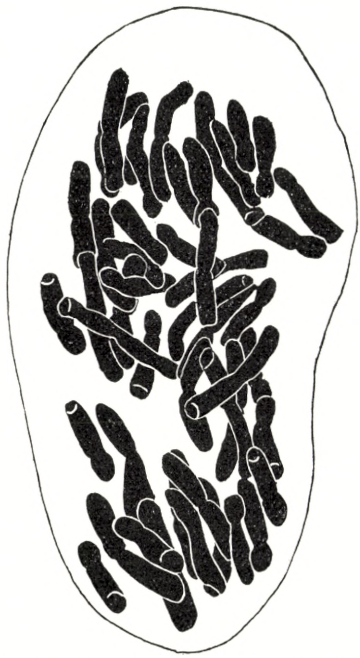
The botanical characters described below first made me suggest the tetraploid nature of the tree, and modern methods of plant cytology made it a relatively easy task to confirm the assumption.

Mitosis in very young needles was studied in iron-aceto-carmine smears by means of the following technique: Tips of young shoots were stripped of all needles except the very youngest at the end, cut in two to ensure a better penetration of the fluid and submersed in 0.3 per cent. colchicine for 5 hours in order to attain a contraction and separation of the otherwise long and tangled chromosomes. Thereafter fixation in Carnoy for 14 hours, maceration in 1 part 96 per cent. alcohol + 2 parts conc. hydrochloric acid for 20 min. and boiling in iron-aceto-carmine for 8 min. If young needles, taken from buds in early spring, are used, boiling in iron-aceto-carmine after Carnoy is usually sufficient.

The best metaphase plate obtained is shown in text-fig. 1. 48 chromosomes are seen, which on closer examination group themselves in two classes of 24 each, one having median to submedian, the other subterminal constrictions. This is in agreement with the idiogram for *Larix* given by Sax & Sax (1933). Due to the large number of 48 chromosomes it was rather difficult to get plates, in which an exact statement of the number and morphology was possible. In fact only 2 plates were completely analysed.

On account of the total absence of male inflorescences in the spring of 1949 no study of the meiosis of the tetraploid larch could be made at that time. In the spring of 1950, however, male inflorescences are abundant, and a preliminary examination has been made from buds forced at room temperature.

Certain irregularities of meiosis are observed, although fewer than might be expected on account of the apparent seed sterility mentioned below. At diakinesis and metaphase I a number of



Text-figure 1: Mitotic metaphase from young needle after colchicine treatment, 48 chromosomes (× 2000).

tetravalents are found (in the few cells examined the number varies from 10 to 12). At telophase I chromatin-bridges and lagging chromosomes are present, but their occurrence is not very frequent. The "tetrads" of the tetraploid larch are rather irregular. The number of cells varies from monads (often giant cells) to hexades. Micronuclei are observed. There is also, however, a considerable number of apparently normal tetrads, and a quantity of normal pollen may therefore be expected. Pollen-grain mitoses are frequently observed at the tetrad stage, and chromosome counts seem to indicate that cells undergoing mitosis at this stage have irregular chromosome numbers.

The tetraploid larch differs in its botanical characters from typical *Larix decidua* in much the same way as most autotetraploids differ from their diploid ancestors. This will be evident from comparative measurements and illustrations of needles, stomata, and cones. The mean length of the needles of the tetraploid larch is 33.1 mm (350 measurements), that of a nearby diploid 17.6 mm (379 measurements). The tetraploid tree thus has needles of about the double length of the diploid as is clearly seen in the photo plate II, fig. 1. Note also the much bigger size of the terminal bud of the tetraploid.

Measurements of stomatal length of 4n and 2n larches gave the following values: 4n 71 μ (63 measurements), 2n 48 μ (63 measurements).

The cones of the tetraploid tree are large, but vary much more in size than those of normal trees. The greater variation in the case of the tetraploid may be assumed to be due to a deficient seed production, the sparse seeded or seedless cones being more or less dwarfy. The largest cone of 25 measured had a length of 42 mm, the smallest 28, the mean value being 36 mm. The corresponding figures for normal *Larix decidua* are for 25 cones: 38, 22, and 30 mm.

The shape and shape variation of cones of the tetraploid larch will appear from plate II, fig. 2. The cones of this tree are generally broader than those of normal trees and more conical. Often the tetraploid cones are more or less flattened, and not unfrequently twin cones are found (see the second row of cones in the figure). The twin cones are usually curved towards each other.

Scales of the tetraploid cones (left) and diploid (right) are shown in plate II, fig. 2. As will be seen, they are of equal length, but very different in breadth, the broader tetraploid scales often being somewhat emarginate. It should be noted that the tetraploid scales are of a more shiny appearance than the normal ones.

In the record books of the Gisselfeld Gardens the tetraploid larch was first mentioned in 1907 under the name of *Larix europaea,* var. *pendula.* Although the tree does not show all the characters of *L. decidua,* it no doubt belongs to this species. As regards the varietal name, the situation is much more intricate. In the record books of Gisselfeld no author to var. *pendula* is given, and this name has been used profusedly for species and varieties characterized by a more or less drooping habit of growth. Most of the trees of larch generally referred to as var. *pendula* are apparently simple mutants with drooping growth. The tetraploid var. *pendula* at Gisselfeld, on the other hand, presumably owes most of its pendulous habit to its tetraploid constitution, and if specimens identical with this tree exist, they are likely to be found in English parks. The Gisselfeld estate at the time of the planting of the tetraploid larch had an English head gardener, who is known to have introduced trees from English nurseries.

The seeds of the tetraploid tree appear to be larger than normal seeds, but as most of them, if not all, are empty, it has not been possible to make a true comparison. Whether the sterility (or very low fertility) of the tetraploid larch is due to self-sterility or is caused by the tetraploid nature of the tree, cannot be decided at present.

In order to secure the future existence of the interesting tetraploid larch tree, graftings from it have already been made by Dr. Syrach Larsen at the Forest Tree Breeding Institute at Hörsholm. An additional number will be made next summer and also some rooted cuttings. This material will make it possible to carry out the aforesaid comparison of the growth of tetraploid and diploid young trees. Later, when the graftings start flowering, they will, together with the original tree, be valuable for breeding purposes.

The employment of the tetraploid tree of *L. decidua* may take place in the following two ways:

(1) A direct utilization presents itself if the tree proves superior to diploid larches. In this case vegetative propagation by cuttings or graftings as well as propagation by seeds should be performed. The seedling-method, if practicable, would offer the advantage of producing some variability in the offspring.

(2) Indirectly the tree should prove valuable as a parent in crosses with other larches, *L. decidua* as well as other species. Species hybrids are well known in the genus *Larix*, and the hybrid *L. leptolepis* × *L. decidua* has already proved valuable in Danish forestry (Syrach Larsen, 1937), its growth qualities being superior to those of both parent species.

The present investigation has been made at the Laboratory of Genetics of the Royal Vetr. and Agricultural College, Copenhagen, and the author is much indebted to Prof. C. A. Jørgensen for help and advice.

Literature cited.

Medd. från Föreningen för Växtförädling av Skogsträd, Ekebo. No. 44, 1946, p. 9, and No. 52, 1948, p. 5.

SAX, H. J. (1932). "Chromosome pairing in *Larix* species". J. Arnold Arbor. XIII, pp. 368—73.

— (1933). "Chiasma formation in *Larix* and *Tsuga*". Genetics 18, pp. 121—28.

SAX, K. & SAX, H. J. (1933). "Chromosome number and morphology in the conifers". J. Arnold Arbor. XIV, pp. 356—75.

SYRACH LARSEN, C. & M. WESTERGAARD (1938). "A triploid hybrid between *Larix decidua* Mill. and *Larix occidentalis* Nutt." Journ. of Genetics XXXVI, No. 3, pp. 523—30.

SYRACH LARSEN, C. (1937). "The employment of species, types and individuals in forestry". Roy. Vet. Agr. Coll. yearbook.

— (1947). "Estimation of the Genotype in forest trees". Roy. Vet. Agr. Coll. yearbook.

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PLATE I.

Fig. 1. The tetraploid *Larix decidua* var. *pendula* in the park of Gisselfeld, Denmark (photo C. SYRACH LARSEN 1949).

Fig. 2. The lower part of the tree, showing the drooping side branches.

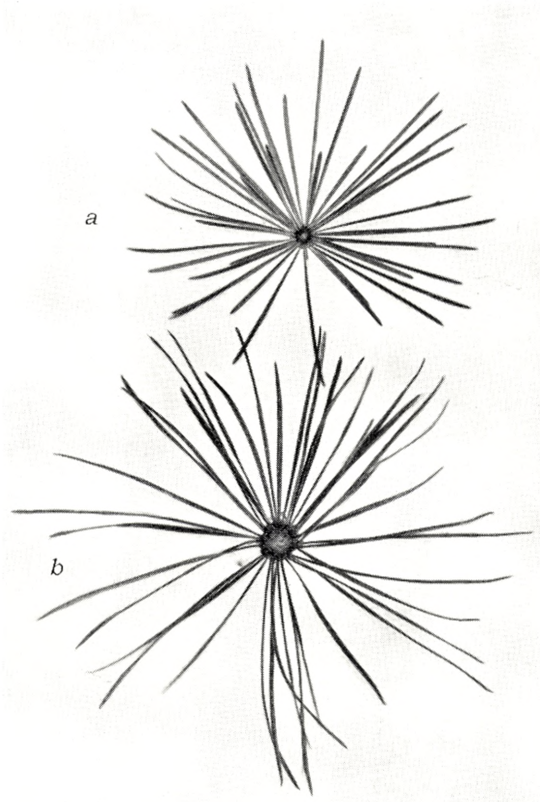

Fig. 1. Dwarf shoot of diploid (*a*) and tetraploid (*b*) larch ($\times$ 1).

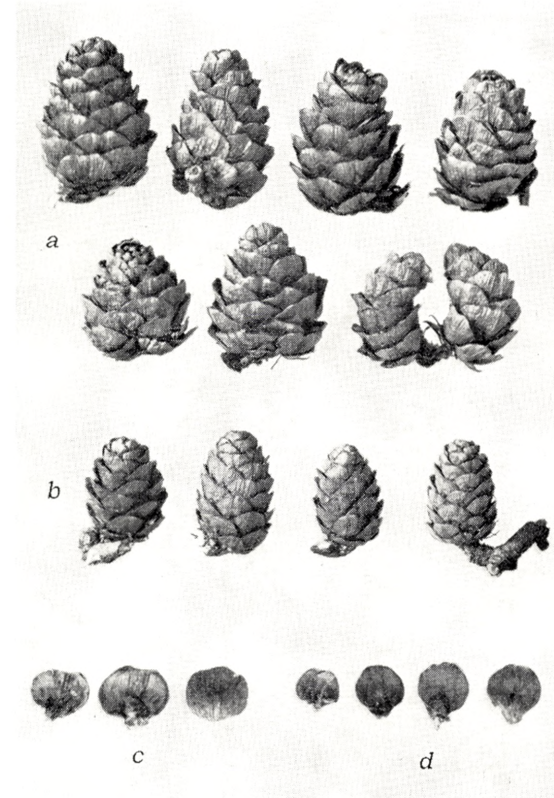

Fig. 2. Cones and scales of diploid and tetraploid larch; (*a*) cones of tetraploid; (*b*) cones of diploid; (*c*) scales of tetraploid; (*d*) scales of diploid. (*a* and *b* $\times$ $^3/_5$, *c* and *d* $\times$ $^2/_3$).

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