Pollen Grains of Pinus edulis With More Than the Haploid Number of Chromosomes

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In 1962 we observed during the germination of pollen that *Pinus edulis* produced pollen grains of various sizes. There were two concrescented, round, four concrescented and other forms of pollen grains *(Fig.* 1). These pollen grains seemed to be non-viable during germination on 1 percent agar and distilled water. However when verifying the viability of the abnormal pollen grains by determining the hydrogenase activity (CHIRA, 1963), we observed that the pollen was alive.

We gave further attention to these abnormalities during the years 1963—1964. We were especially interested in the reasons for the deformations, how they originate, to what extent these pollen grains are viable and what the main differences are between the normal and abnormal grains.

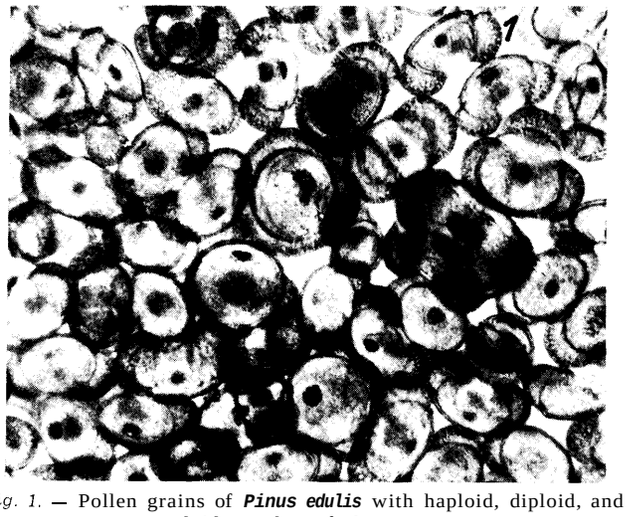

Fig. 1. — Pollen grains of ***Pinus edulis*** with haploid, diploid, and tetraploid number of chromosomes.

Material and Methods

To find out when and under what conditions the various irregularities in pollen grains of *Pinus edulis* originate, we followed in detail the development of the pollen from the establishment of the archesporial tissue to the release of pollen from the anthers. This was followed by rapid as well as classical methods, controlling the changes of Pollen Mother Cells (PMCs) during meiosis two or three times within twentyfour hours. For the observation we used 300 developing PMCs. The temperatures are given on the graph *(Fig.* 25).

For pollen germination a different system was used to that applied for other pine species. We had to do this, because the method of establishing the degree of dehydration of the pollen of *Pinus edulis* does not permit observations on the growth of the pollen tube. Therefore we tested other treatments to assess the activity and influence of those pollen grains with more than the haploid number of chromosomes on the growth of the pollen tubes. We therefore tested various concentrations of agar with saccharose, glucose, ribose, galactose, arabinose, fructose, betaindolyl, butyric acid, nicotinic acid, boric acid and gibberelline. Of these media only one was suitable for the growth of the pollen tube of P. *edulis* and this consisted of 1 percent agar t 2 percent of saccharose and 0.01 percent boric acid. It proved possible to germinate the pollen of this species on such a medium at a relative humidity of 96 percent and a temperature of 30° C. *(Fig.* 24).

To determine the vitality of such pollen after one year's storage, we stored it under the most suitable conditions determined by experiments. After one year's storage the viability of the pollen was good.

Results

This study of the development of pollen in *Pinus edulis* revealed a number of interesting facts. During the division of cells in the archesporial cells it was not possible to find any deviations from the normal division of cells. At temperatures above +5° C the cells continued to divide. Lower temperatures stopped the division of cells. At temperatures higher than 10 to 15° C it was possible to observe a gradual differentiation of cells in the archesporial tissue. These temperatures after 3 to 4 days resulted in larger sizes of the PMCs. and after 5 to 6 days they took on a round ball shape *(Fig.* 2). After this differentiation it was possible to observe the initial prophase, which also passed normally as in other pine species *(Fig.* 4). From the total number of PMCs. about 20 percent of them when forming bivalents and chiasmata achived a more compact shape. They showed no spiralization and the chiasmata originated at the ends of the homologous chromosomes. In some cases it was possible to observe fragments of single bivalents *(Fig.* 3). From such PMCs., damaged by low temperatures of 2 to 4° C during prophase and at other developmental stages during the telophase I, PMCs. with four abnormal nuclei of various irregular sizes originated. They originated from the above-mentioned single bivalents *(Fig.* 5, 6, *and 7).* After the formation of the dyad such PMCs. did not continue to divide *(Fig.* 6). It was possible to observe in their further development the formation of the exine and intine of the pollen body. In this way the developed pollen grains were of conspicuously different sizes when compared with normal grains *(Figs.* 1, 13, *and* 14). In some cases the formation of dyads did not take place *(Fig.* 5). Similarly it was possible to establish the origin of chromosome bridges during metaphase II and telophase II *(Figs.* 15 *and* 16). During the testing of the pollen grains developed in this way, and especially by establishing the viability of pollen by special methods, it was possible to say, that they are not viable. Such PMCs. as we observed had been exposed during these stages for about 4 hours to low temperatures of +3° C (see *Fig.* 25).

The same stages, being exposed at their later development to a temperature of 3° C for a shorter period of 2 to 3 hours were not damaged. Under such conditions the PMCs. from the metaphase I and anaphase I produced dyads with a partition *(Fig.* 12). For comparison we give a normal dyad *(Fig.* 11). The PMCs. with a partition did not pass to the metaphase II. From such dyads, after the origin of two independent cells, rounded pollen grains with a ring form

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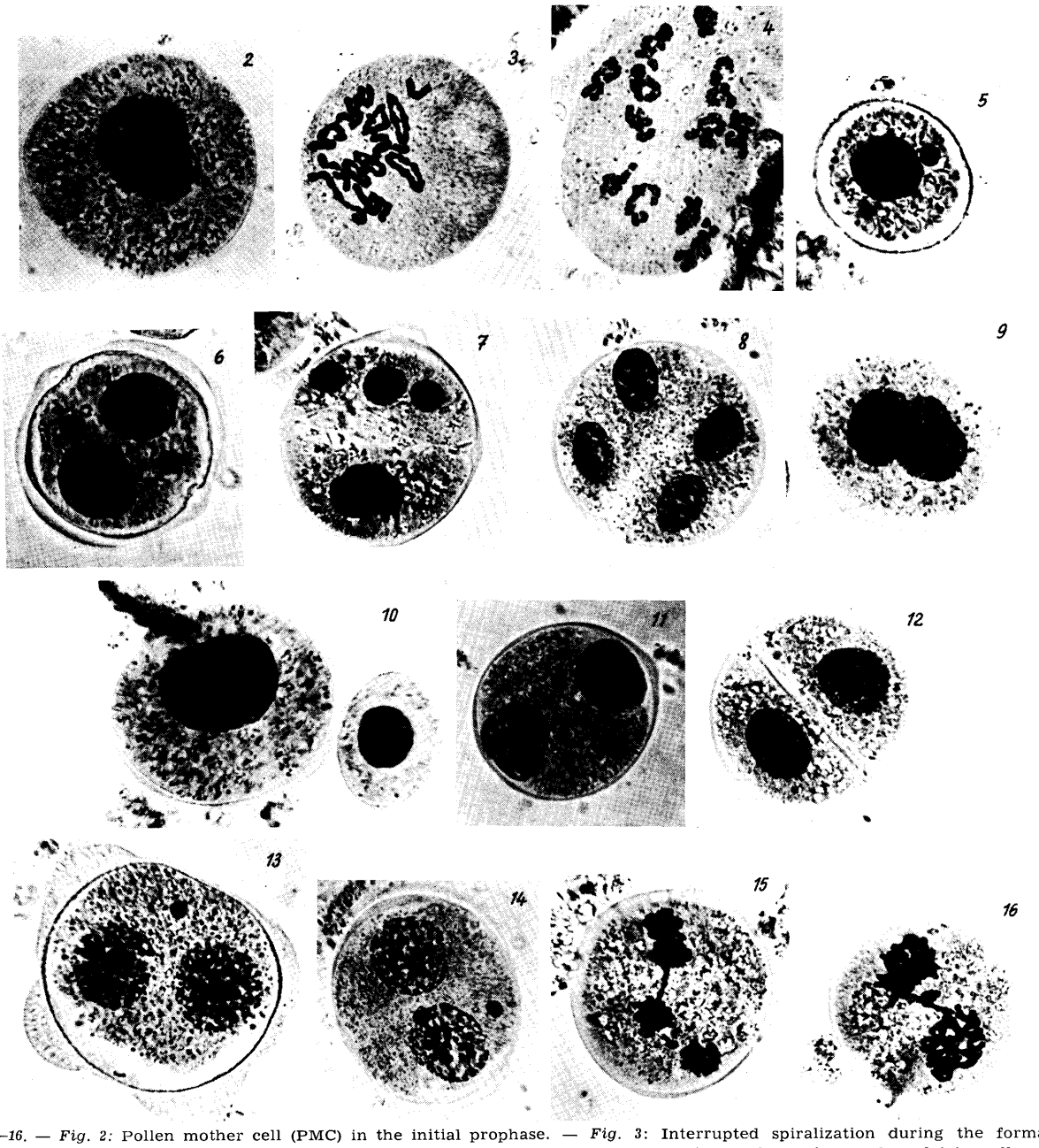
to the air-sacs developed by gradual differentiation. According to our observations these pollen grains had an unreduced number of 2n chromosomes *(Figs. 17 and 21)*.

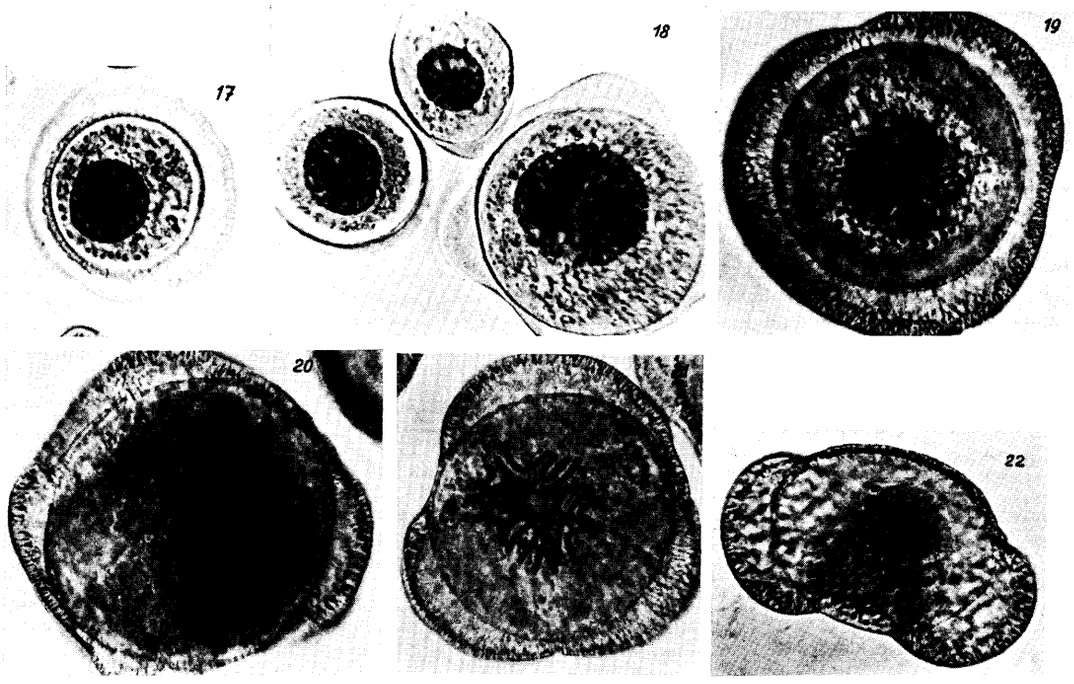
Similarly from the stages in metaphase II and anaphase II, which had been exposed to a short period of 2 to 3 hours to the effects of temperatures of 3° C and which due to these conditions had a partly damaged spindle mechanism, pollen grains developed with 4n complement chromosomes *(Figs. 18 and 20)*. During the effect of the spindle mechanism the normal development of tetrads did not take place *(Figs. 9 and 10)*, but at the beginning it was the regressive coalescence of chromosomes during the dyad and later the coalescence of both nuclei with 2n chromosomes in one nucleus, which gave 4n chromosomes *(Fig. 10)*. For comparison we give a normally developed tetrad of this pine *(Fig. 8)*. A third mitotic division took place in pollen grains observed in such a way *(Figs. 19 and 21)*. The PMCs., which were not exposed to the low temperature during their development, developed normally without any changes. For comparison we give normally developed pollen grains with n chromosomes *(Fig. 22)*.

Discussion

The occurrence of abnormal pollen grains represents about 40 percent of the total observed. At the same time we established that during the period when temperature ef-



Figs. 2—16. — *Fig. 2:* Pollen mother cell (PMC) in the initial prophase. — *Fig. 3*: Interrupted spiralization during the formation of chiasmata as a result of low temperature. — *Fig. 4:* Normal chiasmata in a PMC. — *Fig. 5:* Three abnormal nuclei in pollen showing the condition after telophase I. — *Fig. 6:* Four abnormal nuclei in pollen; interrupted formation of tetrads. — *Fig. 7:* Four abnormal nuclei and interrupted formation of tetrads in PMC. — *Fig. 8:* Normal tetrad of PMC. — *Fig. 9:* Coalescence of nuclei after telophase II of PMC.; tetrad did not develop. — *Fig. 10:* A "Giant" nucleus in pollen; after telophase II. — *Fig. 11:* Normal diakinesis. — *Fig. 12:* PMC. in diakinesis with a partition. — *Fig. 13*: Abnormal pollen grain with three nuclei; pollen was non-viable. — *Fig. 14:* Abnormal pollen grain with three nuclei; pollen was non-viable. — *Fig. 15:* Chromosome bridges during anaphase II to telophase II. — *Fig. 16:* Bridges during methaphase II. (× 625).



Figs. 17—22. — Fig. 17: Developed pollen grain with diploid nucleus; pollen was viable. — *Fig. 18:* Pollen grains with haploid, diploid, and tetraploid nuclei; they are viable. — *Fig. 19:* Pollen grain with the diploid number of chromosomes. — *Fig. 20:* Pollen grain with the tetraploid number of chromosomes. — *Fig. 21:* Pollen grain with the diploid number of chromosomes. — *Fig. 22:* Normal pollen grain with the haploid number of chromosomes. (× 625)

fected the development of PMCs., about 20 percent were at metaphase I and anaphase I and about 20 percent were at metaphase II and anaphase II. These results show that these stages of meiosis are very sensitive to external influences and especially to temperature.

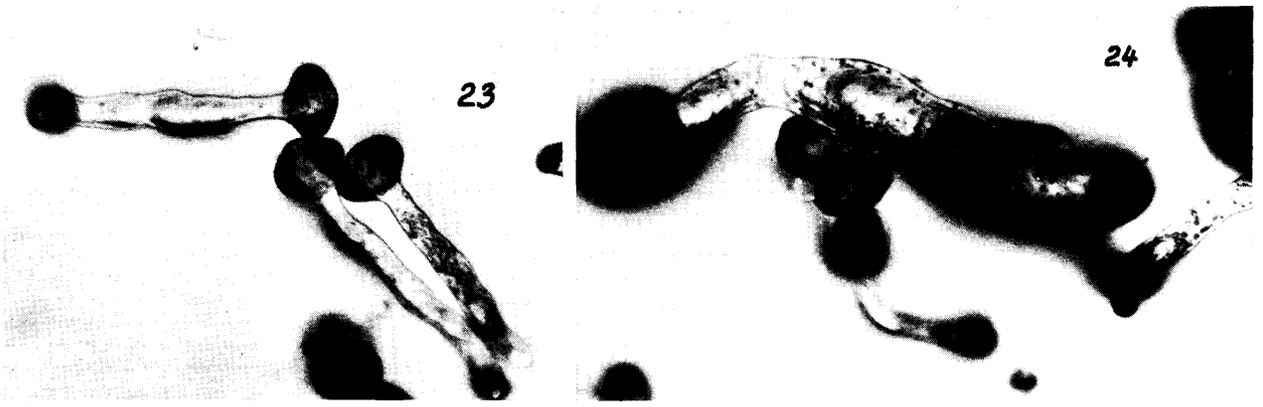
CHRISTIANSEN (1960) records a similar observation during his study of the causes of sterility of pollen in *Larix decidua* MILL., yielding a 4 to 8 percent occurrence of abnormal pollen grains with more than the haploid number of chromosomes and being recorded as non-viable when tested by classical methods, the pollen at the same time being completely sterile. There is no reference in the literature available to us of the mode of origin of tetraploid pollen grains. It is generally known that when low temperatures occur during the development of pollen occasional diploid pollen grains occur which are of great importance for practical selection work. By using such pollen it is possible to obtain high-quality triploid organisms. HRUBÝ (1961) states, that in certain locality conditions, the occurrence of polyploid organisms is more frequent than in conditions favourable to the plants. He considers that this is the reason for the natural variability of certain species and in this way some individuals of such species become better adapted to severe locality conditions than individuals with no tendency to polyploidy. HRUBÝ assumes that the polyploid condition conveys a certain advantage for a plant in unfavourable conditions and that polyploidy has an important place in the evolution of new species and in the occupation of new areas by these species (see VINCENT, 1962).

In *Pinus edulis* both diploid and tretraploid pollen grains are produced. This species originates from the warm areas of north Mexico, Colorado and Arizona and our conditions in Czechoslovakia are really very severe and unfavourable for it. NOVÁK (1953) states that *Pinus edulis* hardly bears our conditions and can be grown only on the warmest and most protected places, but even in these it may get damaged by cold during severe winters.

We decided to pay greater attention to the problems described here because determination of the origin of pollen grains with more than the haploid number of chromosomes and the study of the causes of this phenomenon may be of great importance for the selection of species. LARSEN and WESTERGAARD (1938) found triploid individuals in *Larix europaea,* and they assumed that they originated by pollination of the normal ovum cell with diploid pollen of *Larix occidentalis.* ZINNAI (1952) established 5 tetraploid trees of *Pinus densiflora* SIEB. and ZUCC. in the nursery. MERGEN (1958) observed a polyploid of *Pinus elliottii* ENGELM. Because these observations were made on plants two to three years old it has not yet been possible to make a detailed evaluation of heterosis from polyploids.

Polyploid pollen grains originated in the manner described may be of great importance for the study of the incompatibility of pines and therefore we suppose that it is correct to pay great attention especially to those changes occurring during the development of PMCs.

It is of interest that pollen from *Pinus edulis* with more than the haploid number of chromosomes, which we tried to germinate in distilled water and on agar with saccharose at various concentrations appeared to be non-viable. By the method of determining the activity of dehydrogenase of pollen we established that the pollen is vital. Because it is not possible to observe the growth of the pollen tube by this method, we tried to find new methods such that we could observe the growth of the pollen tube. As we have mentioned this was achieved with the medium consisting of 1 percent agar + 2 percent saccharose and 0.01 percent boric acid. On such a medium with a relative humidity of 96 percent and at 30° C the pollen grains with more than the haploid number of chromosomes germinated normally. We made sure that these pollen grains are able to germinate and that the growth of their pollen tube is greater than the growth of the pollen tube of normally developed grains *(Figs. 23 and 24).*



Figs. 23—24. — *Fig. 23:* Growth of pollen tube with the haploid number of chromosomes. — *Fig. 24:* Growth of pollen tube with the tetraploid number of chromosomes. *(× 140)*

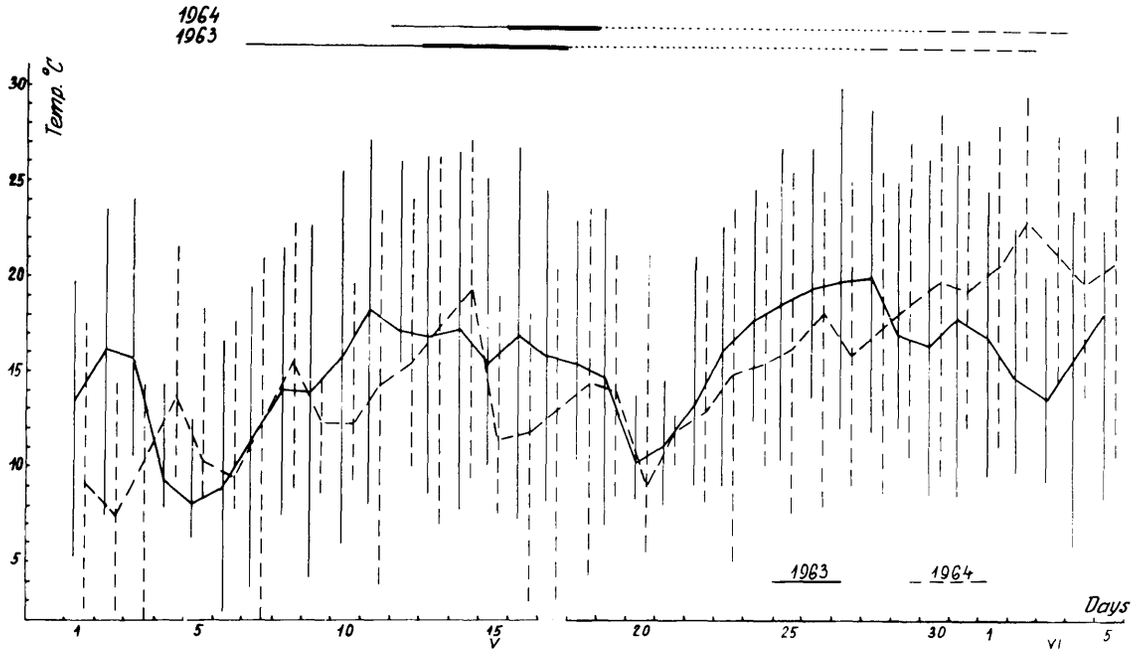


Fig. 25. — The influence of the mean maximum and minimum temperatures on the course of meiosis of PMCs on *Pinus edulis*. — ——— The course of prophase. ——— Metaphase — anaphase I, II, telophase and tetrads. . . . . The development of the airfilled bladders and of the exine of the pollen grain. - - - - - The third mitotic division till the full maturity of the pollen grain.

According to these facts it is evident that those pollen grains with more than the haploid number of chromosomes, which are quantitatively as well as qualitatively different from the normal pollen grains, were not able to grow under the same conditions as normal pollen grains. This example may serve as the basis for advice on the judgement of polyploid plants generally. It often happens, that on the basis of the first experiments, without proper knowledge of the influence of environmental conditions on the development of the organism, polyploid plants are often prematurely condemned for practical use.

We assume that the present ideas for the exploitation of polyploidy in conifers are not clear. Stebbins (1947), for example, states that polyploidy in plants other than the Gymnosperms is of economic importance. On the other hand some good polyploid Gymnosperm species are known, such as *Pseudolarix amabilis* Gord., *Juniperus chinensis* var. *Pfitzeriana* Spaeth (Sax and Sax, 1933), *Sequoia sempervirens* Endl. (Buchholz, 1939) and others.

Finally we should like to mention that in future we shall pay great attention to the origin of diploid and tetraploid pollen grains with the object of exploiting the physical effects of temperature during meiosis on the origin of polyploid pollen grains. Finally, we shall also pay great attention to the exploitation of polyploidy in the selection of forest tree species.

Summary

During a study of the development of the pollen of *Pinus edulis*, under the conditions of the Mlyňany Arboretum in the years 1963-64 we established the following facts:

1. The pollen grains of *Pinus edulis* develop normally if the temperatures are not lower than $+4^0$ C during metaphase I—II and anaphase I—II.

2. Temperatures of $+3^0$ C for more than 4 hours effect the function of spindle fibers during metaphase I—II and anaphase I—II. Pollen grains develop with a abnormal shape and they are non-viable.

3. Temperatures of 3^0 C for only 2 to 3 hours during metaphase I—II and anaphase I—II caused the development of PMCs. of *Pinus edulis* pollen grains with more than the

haploid number of chromosomes (2n and 4n) and these were viable.

4. Pollen grains with more than the haploid number of chromosomes (2n and 4n) did not germinate on the media normally used for pines. Their viability was expressed on a medium consisting of 1% agar + 2% of saccharose and 0.01% of boric acid, at a temperature of 30° C and relative humidity of 96%.

5. Pollen tubes with 2n and 4n chromosomes reached "giant" sizes.

Literature Cited

(1) Buchholz, J. T.: The embryogeny of *Sequoia sempervirens* with a comparison of the Sequoias. Amer J. Bot. 26 (1939). — (2) Hrubý, K.: Genetika. Praha (1961). — (3) Chira, E.: Rýchla metóda na zisťovanie životaschopnosti pel'u pri niektorých druhoch rodu *Pinus*. Biológia 5, 390—395 (1963). — (4) Christiansen, H.: On the effect of low temperature on meiosis and pollen fertility in *Larix decidua* Mill. Silvae Genetica 9, 72—78 (1960). — (5) Klika J., Novák, F. A., Šiman, K., Kavka, B.: Jehličnaté. Praha (1953). — (6) Larsen, C. S., and Westergaard, M.: Contribution to the cytogenetics of Forest trees. I. A triploid hybrid between *Larix decidua* Miller and *Larix occidentalis* Nut. Jour. Genetics 36, 523—530 (1938). — (7) Mergen, F.: Natural polyploidy in slash pine. Forest Sci. 4, 283—295 (1958). — (8) Sax, K., and Sax, H. J.: Chromosome number and morphology in the conifers. J. Arnold Arboretum 14, 356—357 (1933). — (9) Stebbins, G. L., jr.: Types of polyploids: their classification and significance. Adv. Gen. 1, 403—429 (1947). — (10) Vincent, G.: Výběr a šlechtení v lesním hospodářství. Praha (1962). — (11) Zinnai, I.: Tetraploid plants of Japanese red pine (*Pinus densiflora* Sieb. et Zucc.) discovered in transplant bed. Jour. Jap. For. Soc. 34, 185—187 (1952).

Preliminary Observations on the Change With Age of the Heritability of Certain Wood Characters in Pinus radiata Clones

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Introduction

Wood characters have come to be regarded as important and necessary selection factors in tree improvement programmes and therefore it is essential to know how they are transmitted from parent to progeny. Qualitative descriptions of inheritance are far from adequate for this purpose and information may be more precisely presented by some unambiguous measure of the intensity of genetic control.

The most commonly used measure utilizes a ratio called heritability (Lush, 1937), employed in either a broad or a narrow sense. Broad sense or gross heritabilities can be estimated following the examination of vegetatively propagated material since the genotypes of individuals are transmitted unchanged. Narrow sense heritabilities are determined using seedling material where non-additive genetic effects cannot be transferred from the parents to the progenies. Besides being used to measure expected progress resulting from selection, heritability has also been used to describe the "degree of rigidity of genetic control" of characters (Mergen, 1960).

Several methods have been proposed for estimating heritabilities, but a commonly used approach separates the variance of a character into components attributable to different causes. The calculations may be based either on progeny means or on individual tree observations. However, few existing experiments are suitable for heritability studies and less than ideal material has had to be used in many of the studies conducted to date.

The heritability of wood characters changes with the age of the experimental material and different characters follow different trends (Stern, 1958, 1960; Zobel, 1964). It is useful to establish the form of the relationship of heritability with age for important wood characters. Heritabilities of wood characteristics derived from young trees might then be extrapolated to obtain estimates appropriate to harvest age. In addition, such patterns may assist towards an understanding of the mechanism of genetic control as applied to wood characters. Data used to calculate heritabilities could also be submitted to analyses of covariance to determine genetic correlations between selected pairs of characters. These correlations provide some indication of the change in one character due to a change in another as a result of selection.

The experimental material used for such an investigation should be old enough to provide a clear picture of any worth-while trends of heritability, and individual determinations should not be subject to large standard errors. There are at least two clonal plantations in Australia which would satisfy the requirements regarding age, and vegetatively propagated material eliminates any uncertainty in the relationship between progenies from a given parent group.

Mature clonal material of *Pinus radiata* was used to determine gross heritabilities at successive growth rings from the pith for ring width, percentage late wood, average tracheid length, basic density, and incidence of grain deviation from the tree axis.

Material

The specimens were obtained from a clonal plantation of *Pinus radiata* which had been established at Mt. Burr, South Australia, in 1940. The clones were planted in adjacent rows, or, in some instances pairs of rows, without replication, at a spacing of 2¾ m. between rows, and 2⅛ m. between trees. They were propagated from cuttings taken from the same location within the parents and raised under the same methods and conditions, so that all the trees were of the same physiological age. The stand was silviculturally untreated apart from the pruning of dead limbs to a height of 2½ m. The site was without appreciable slope, of practically uniform quality and located on a transitional, volcanic soil described as a coarse, sandy, valley type.

Originally 20 clones were planted, but 1 died and the remainder are represented by 12—30 trees in each case. From each of the 19 clones, 3 trees were chosen at random, within the limits imposed by avoiding trees of low vigour (resulting from early dead tops and competition).

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