

Soc. d'histoire naturelle de Toulouse. T. 95, Fasc. 3-4 pp. 287-302, 1960. — HERRMANN, S.: Wachstumsuntersuchungen an vegetativ vermehrten Bäumen sowie über ein Verfahren zur Gewinnung schnellwüchsiger Forstpflanzen. Allg. Forst- u. Jgdz. 132, 196-203 (1961). — KLEINSTÜCK, M.: Holz und Holzpflege bei den Japanern. Mitt. dtsh. dendrol. Ges. 1925, S. 312-313. — LANGNER, W.: Rückmutation bei Zwergfichte. Z. Forstgen. 3, 138-139 (1954). — MICHAELIS, P.: Über die Jugendform der Nachkommen von *Chamaecyparis pisifera* f. *squarrosa* und f. *typica* und ihre Stabilität. Deutsche Baumschule 3, 219-221 (1951). — MITTELSTAEDT, H.: Regelungsvorgänge in der Biologie. Beihefte zur Regelungstechnik. Oldenbourg, München 1956. — MOLISCH, H.: Pflanzenphysiologie als Theorie der Gärtnerei. 3. Aufl. Gustav Fischer, Jena 1920. — NEGER, F. W. / MÜNCH, E.: Die Nadelhölzer. Sammlung Göschen Bd. 355. Vierte Aufl. bearb. v. BR. HUBER. Walter de Gruyter u. Co., Berlin 1952. — PASSECKER, F.: Die Entwicklungsphasen der Gehölzpflanzen und

ihre praktische Bedeutung. Veröff. Kärntner Landesinst. angew. Pflanzensoziol. Klagenfurt 1954, 1. 88-102. — PASSECKER, F.: Gibt es eine Fixierung von „Jugendformen“ im Sinne Beijerinck? Mitt. dtsh. dendrol. Ges. 61, 45-49 (1959/60). — ROHMEDEK, E.: Das Problem der Alterung langfristig vegetativ vermehrter Pappelklone. Forstw. Centralbl. 75, 380-407 (1956). — ROHMEDEK, E.: Altersphasenentwicklung der Waldbäume und Forstpflanzenzüchtung. Silv. Gen. 6, 136-142 (1957). — SCHAFFALITZKY DE MUCKADELL, M.: Investigations on aging of apical meristems in woody plants and its importance in silviculture. Kandrups og Wunsch's Bogtrykkeri, København 1959. — VÖCHTING, H.: Über die Regeneration der *Araucaria excelsa*. Jb. wiss. Bot. XL, 1, 1904, S. 144. — WADDINGTON, C. H.: The strategy of the genes. George Allen and Unwin Ltd., London 1957. — WOYCICKI, ST.: On the origin of the *Retinospora* forms in *Thuja*, *Biota* and *Chamaecyparis*. Acta Soc. Botan. Poloniae Vol. XXIII Nr. 3, 1954, pp. 443-448.

Abies Karyotype Analysis¹⁾

By FRANÇOIS MERGEN and JEFFERY BURLEY
Yale University, School of Forestry
New Haven, Connecticut, U.S.A.

(Received for publication August 9, 1963)

Members of the genus *Abies* are found throughout the northern hemisphere and comprise over 40 species. Although of considerable importance commercially, little work has been done on the genetics of this group, especially on their phylogenetic and cytological relationships. Various aspects are currently under investigation at the Yale School of Forestry and results on treatment with colchicine (MERGEN and LESTER, 1961 b), and phenological and cytological studies (MERGEN and LESTER, 1961 a) have been published.

In a recent publication, KLAHN and WINIESKI (1962) presented a crossability pattern based on extensive natural and artificial hybridization but they did not discuss the cytological aspect of their hybrids. In 1933, SAX and SAX published camera lucida drawings of metaphase stages in the female gametophyte tissue of *Abies cephalonica* and *Abies concolor*. In both these species five of the 12 chromosomes are heterobrachial while the others are approximately isobrachial. MEHRA and KOSHOO (1956) found a similar condition for *Abies pindrow* and they observed that two of the isobrachial chromosomes bore subterminal secondary constrictions. For their determinations they used root tips of germinating seed that were pre-treated with colchicine, α -bromonaphthalene or 8-oxyquinoline. Although they took actual measurements in some critical cases to determine the morphology of the chromosomes, they gave no indication of the differential quantitative effect of the chemical pre-treatments. The examination of meiotic chromosomes of four species during microsporogenesis showed that three chromosomes of one of the species (*Abies sachalinensis*) had distinct satellites (MERGEN and LESTER, 1961 a).

With the exception of one instance, all of the reports on chromosome numbers give the value as $n = 12$, with 24 chromosomes in the sporophytic tissue. The only report of a natural polyploid *Abies* is by KANEZAWA (1949); he examined the chromosome number in three twin seedlings of *Abies firma* and two of them possessed 24 chromosomes while one seedling appeared tetraploid with 48 chromosomes.

Artificial polyploidy after treatment with colchicine has been induced in nine species of *Abies* (MERGEN and LESTER, 1961 b) but none of these seedlings survived for any considerable time after the treatment.

Because the chromosomes in the Pinaceae are long and have a tendency to become tangled up it is desirable to treat the dividing cells so as to shorten and separate the chromosomes for study. Previous published chromosome studies used a variety of methods and reagents to obtain better metaphase plates, but none of these reports presented quantitative estimates of the variation caused by these treatments.

The purpose of this study was to determine the karyotypes for several species of *Abies* and to observe the differential effects of several chemical treatments on chromosome size and structure.

Materials and Methods

1. *Karyotype*. For the karyotype analysis, female gametophyte tissue was used from cones collected during the early part of July, 1959. The fir trees that were used are growing in the George P. Brett Pinetum of Yale University, at Fairfield, Connecticut, and the following species were sampled: *Abies alba* MILL., *Abies X Borisii-regis* MATTF., *Abies cephalonica* var. *Apollinis* (LINK) BEISS., *Abies firma* SIEB. & ZUCC., *Abies lasiocarpa* (HOOK.) NUTT., and *Abies nobilis glauca* BEISS. The ovules were dissected out in the field and placed in either FAA or modified NAWASHIN's fixative. They were placed under vacuum overnight to insure good penetration of the fixative.

After hydrolysis in either IN HCl, or in 1:1 95% ETOH and concentrated HCl, the cells were squashed in BELLING's iron acetocarmine. Staining with crystal violet was also tried but with little success. After staining, the cover slips were sealed to the slide with paraffin to retard drying of squashed cells. Camera lucida drawings (X 1250) were prepared of a minimum of four complete metaphase plates per species. These drawings were projected at X 4, and the length of the chromosomes was measured with a mapping wheel with an accuracy of ± 0.25 mm on the projected enlarged drawing. Translated into absolute units, this in-

¹⁾ This study was supported by a grant from the National Science Foundation for research in forest genetics (G-8891). Laboratory assistance by Mrs. L. PIERCE SCANLON is acknowledged.

dicates actual chromosome measurements to the nearest 0.1 μ .

For the analysis, the chromosomes were arranged by increasing length of the short-arm, and the mean (average) haploid complement was determined for each species.

2. *Chemical treatment.* Seedlings of *Abies guatemalensis* REHDER were used throughout to evaluate the effect of chemical treatment on chromosome size. Root tips from 4-year-old seedlings growing in a greenhouse were excised during the early morning and placed in one of the following solutions:

- a) — 0.5% colchicine for 5 hours.
- b) — 1.0% colchicine for 5 hours.
- c) — 0.002 M 8-hydroxyquinoline (8-HQ) for 24 hours.
- d) — 1.0% colchicine for 5 hours followed by 0.002 M 8-HQ for 24 hours.
- e) Distilled water for 5 hours (Control).

Throughout the treatment period the solutions were aerated and kept under continuous illumination (3300 ft-candles) of a Fluomeric² light. After treatment the root tips were washed in distilled water and then were fixed in 3:1 acetic acid alcohol for 24 hours. Preliminary tests had shown that BENDA's fluid was unsatisfactory as the fats stained black with osmic acid and obscured the cell contents. They could be bleached with 3% H₂O₂, but this treatment reduced the stainability. After fixation the roots were hydrolyzed in 1N HCl for 16 minutes at 60° C and rinsed in distilled water. They were then placed in FEULGEN stain in the dark for two hours to stain and locate the meristematic region. This portion was dissected out and macerated in a drop of propionic carmine to intensify the stain. Heating to 70° C intensified the stain further. The macerated material was affixed to a cover slip with egg albumin and the cover slip was pressed to spread the material and bring the chromosomes more into one plane. The cover slip was then floated off in 45% acetic acid, dehydrated through alcohols and xylene, and mounted in one drop of Permount on a clean slide.

Enough slides were prepared to obtain at least four good metaphase figures per treatment. The chromosomes were measured with the aid of a filar micrometer eyepiece from the original slide, or by dividers and ruler from 3000 \times photomicrographs. When photographs were used at least two chromosomes per cell were measured by micrometer to verify the magnification.

The 24 chromosomes were arranged in pairs of the nearest corresponding arm lengths. The mean haploid complement was thus determined for each cell and these were pooled to obtain a mean for four cells per treatment.

Results and Discussion

1. *Karyotype.* In Figure 1 are illustrated the karyotypes of the six species of *Abies* studied, and the results confirm the uniformity of chromosome structure in this genus. The absolute lengths of the chromosomes are given also, and even by comparing the absolute lengths between the different species there appear to be no or few real differences in chromosome lengths among the six species. They confirm previous reports for *Abies* karyotypes, namely that five chromosomes are heterobrachial; however, the presence and positions of secondary constrictions are too variable to permit accurate classification, or the use of the

² "Fluomeric" is a trade name for a high intensity lamp which combines an arc tube with a filament bulb. Optimum range of rendition in visible spectrum: 400–750 millimicrons.

VARIATION BETWEEN SPECIES

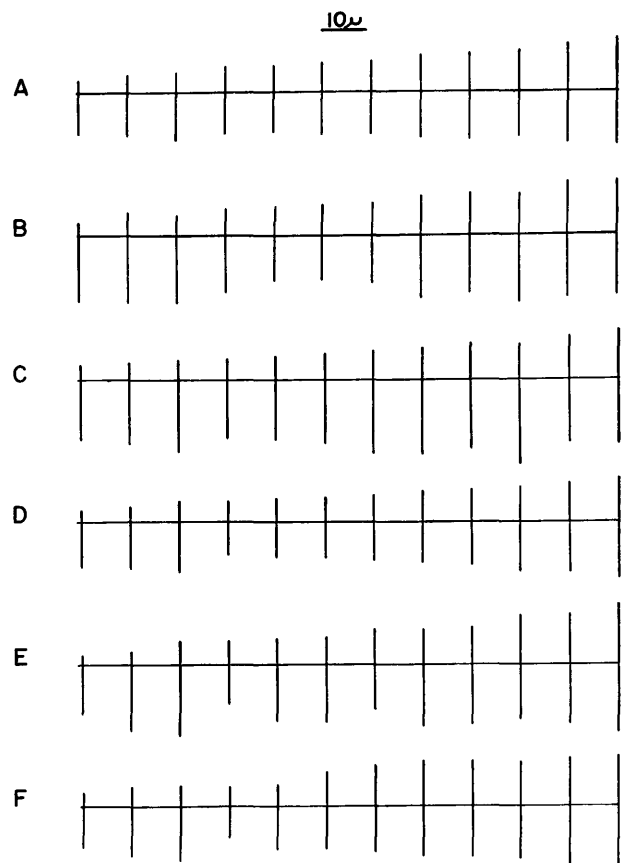


Figure 1. — Karyotype of haploid *Abies* cells. A = *Abies alba*; B = *Abies cephalonica* var. *Apollinis*; C = *Abies* \times *Boristi-regis*; D = *Abies lasiocarpa*; E = *Abies firma*; F = *Abies nobilis glauca*.

constrictions in diagnostic work. The variations within a tree, within the mitotic cycle, and in the method of measurement can easily obscure differences among species.

The karyotypes were determined on cells that had not been treated with spindle inhibitors, and the cells used were selected for being approximately at metaphase. However, because mitosis is a continuous process, it is virtually impossible to select the identical stage of mitosis in all of the cells. One way to overcome this difficulty would be to measure the chromosomes in several hundred cells per plant, and thus obtain a more reliable average along with meaningful statistics for the error term.

Because of the length of the chromosomes in *Abies*, a reliable measurement of a chromosome becomes difficult. This problem is illustrated by Figure 2, where a photomicrograph and a camera lucida drawing of the same cell at the same magnification are compared. This indicates the difficulty that arises in the measurement and representation of chromosomes, particularly when they are curved. An eyepiece micrometer becomes virtually useless with curved chromosomes, especially if the cell cannot be completely flattened. These mensurational problems make it difficult to detect minor differences among species in chromosome size and absolute position of secondary constrictions.

Researchers at times represent the chromosomes as a percentage of the total length of either the longest or the shortest chromosome. For genera with uniform chromosome complements, e. g., the conifers, it is felt that the

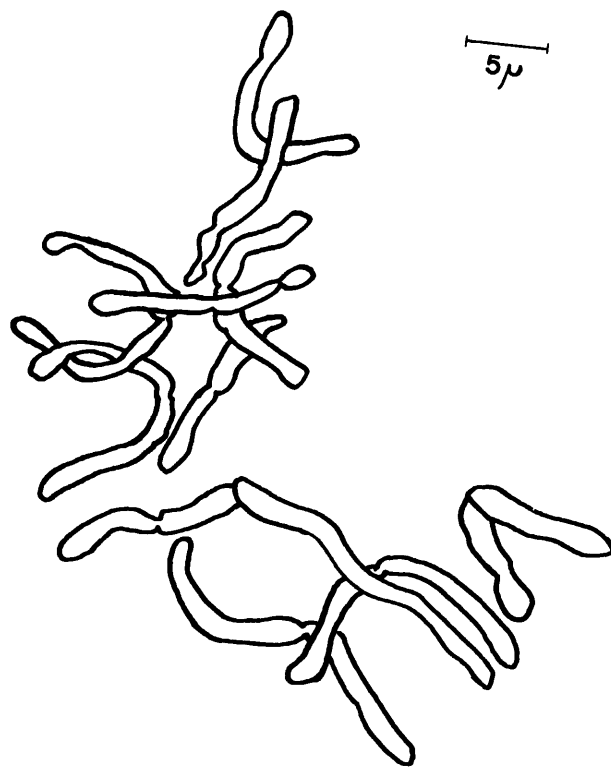


Figure 2. — Comparison of haploid chromosome complement in *Abies* when illustrated by a photomicrograph and a camera lucida drawing. Both illustrations were obtained through the same optical train of a Leitz microscope.

absolute measure is required to distinguish among species, and even with the techniques described in this paper it was not possible to obtain satisfactory and reliable measures for separation. Had the data been converted to percentages, even less separation would have been possible. To illustrate this point, the data from the six species were grouped together and considered as a number of samples from a single species. Coefficients of variation were calculated for the shortest and longest chromosomes, as represented by absolute and percentage data. For the shortest chromosome the coefficients of variation were 15.7 and 10.1, and for the longest 7.5 and 5.6. In each instance, a greater coefficient of variation was obtained when the measurements were in absolute units. This would indicate that if the values are given in absolute terms, a better separation of the species can be expected.

However, it is felt that the between-species differences deduced from the data obtained in this study are more probably differences brought about by position in the mitotic cycle and accuracy of the method of measurement rather than by specific species variation.

2. Effect of Chemical Treatment. Part of the difficulty of chromosome analysis in conifers is due to the high chromosome number and the extreme length of the chromosomes. To obtain a greater number of cells during metaphase for evaluation, the cells are treated with reagents that inhibit spindle formation. Colchicine has been used with success in *Pinus* by MERGEN and NOVOTNY (1957) to accumulate metaphase stages and also to constrict the chromosomes, thus facilitating chromosome spreading. 8-hy-

droxyquinoline was used with good success by SAYLOR (1962) to contract the individual pine chromosomes, but there are no reports of the quantitative effects of these reagents on individual chromosomes.

In this study the differential effects of the chemical treatments were determined on the mean haploid complement from four good metaphase plates for each treatment. Measurements were obtained and evaluated as for the karyotype analysis. The effects of contraction were quite pronounced and the results on an average haploid complement are represented in Figure 3. The treatment with 1% colchicine contracted the chromosomes slightly more than 0.5% colchicine, while 0.002 M 8-HQ produced an intermediate contraction. The strongest contraction was produced by a successive treatment of 1% colchicine for five hours followed by 8-HQ for 24 hours. The type of contraction and separation obtained by this combination treatment is illustrated in Figure 4. Figure 4 A is a metaphase plate of *Abies guatemalensis* showing secondary constrictions in several of the chromosomes, and 4 B is a composite cut-out of the paired chromosomes after treatment.

The quantitative effects of the treatments on the longest and shortest chromosomes are summarized in Table 1. The contraction was calculated by expressing the difference in length between untreated and treated chromosomes as a percentage of the untreated value.

These values indicate that at least for the longest and shortest chromosomes there is little differential contraction; however, there are several effects of 8-HQ that make its use unsatisfactory either alone or in combination with

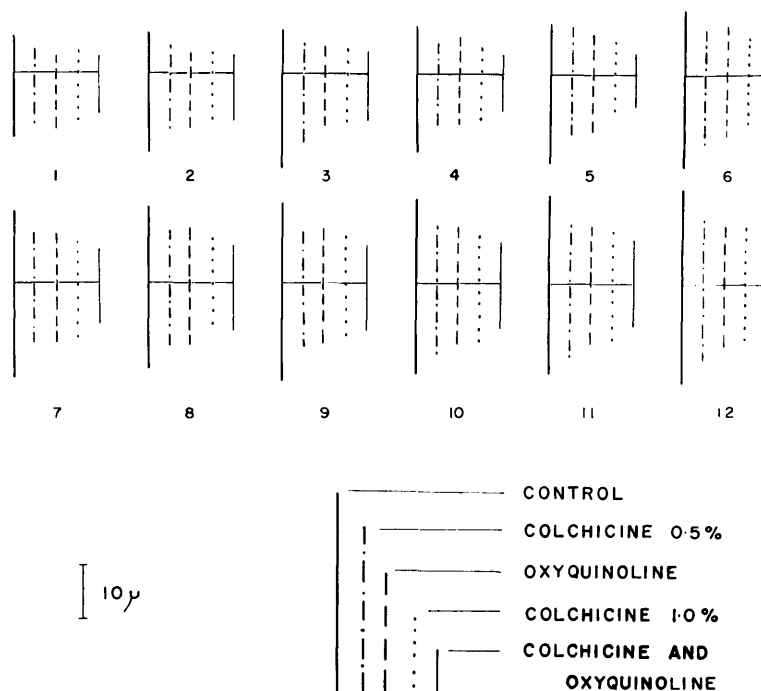


Figure 3. — Average effect of treatment on the individual chromosomes of *Abies guatemalensis*.

colchicine as a treating agent for *Abies*. Throughout these studies, the effects of 8-HQ were irregular, and the same degree of contraction was not found in all the cells on the same slide or in one species. On a slide there could be found metaphase plates that showed no effect of the treatment, while chromosomes in other cells exhibited considerable contraction. The high degree of contraction demonstrated in the photographs published by SAYLOR (1961) was only rarely observed in our *Abies* preparations.

After treatment with 8-HQ all stages of mitosis were visible in the treated material, and 8-HQ did not appear to inhibit spindle formation during mitosis in fir. We have observed the same phenomenon in several other coniferous species. This is in contrast to the observations of TJO and LEVAN (1950) and STÅLFELT (1950) who considered that 8-HQ inactivated the spindle.

On the other hand, the 8-HQ treatment appeared to inhibit mitosis. It allowed the cells past metaphase but held

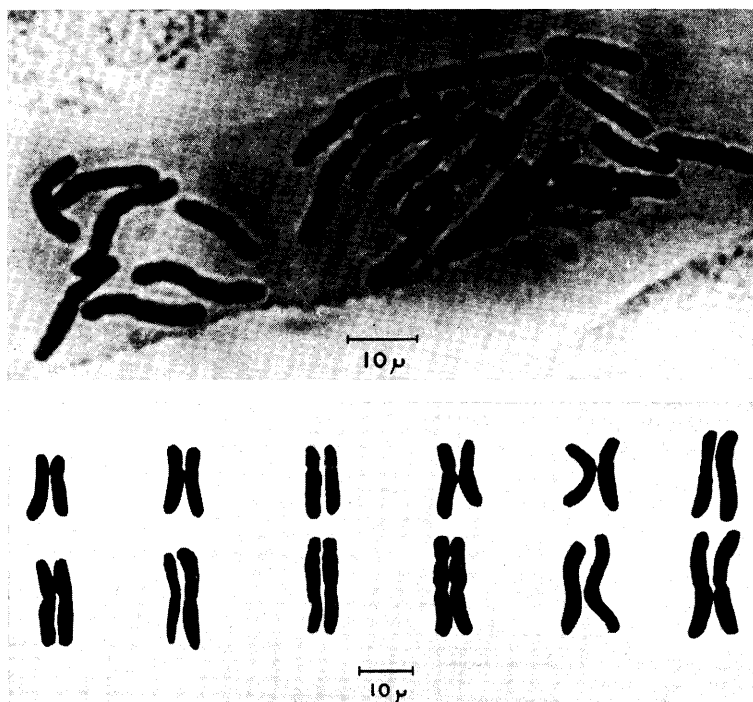


Figure 4. — Effect of colchicine and 8-hydroxyquinoline treatment on chromosome contraction and spreading. — A. Metaphase plate of *Abies guatemalensis*. — B. Composite cut-out of paired chromosomes of the same cell.

Table 1. — Effect of treatment on percentage contraction of the longest and shortest chromosome.

Treatment	Shortest Chromosome	Longest Chromosome
0.5% colchicine	33.0	29.0
1.0% colchicine	36.7	39.2
0.002 M 8 - hydroxyquinoline	31.2	34.4
1% colchicine + 0.002 M 8 - hydroxyquinoline	48.6	49.5

them in an interphase condition, and after a few hours' treatment few dividing cells were present. This counteracted the useful effect of colchicine in accumulating metaphase figures. As a result a considerably greater number of slides had to be prepared for the combination colchicine 8-HQ treatment to obtain the same number of measurable cells.

8-HQ induces a considerable number of achromatic bands on the chromosomes, and this makes the identification of normal secondary constrictions difficult. For this reason no secondary constrictions are indicated on the karyotypes that are given. This production of achromatic regions is due presumably to physicochemical interactions of 8-HQ with the chromatic material and this reduces the staining reaction in these regions. It is possible that the chromosomes become weakened at these points, and when placed under pressure during slide preparation they may stretch and, therefore, the contracting effect is reversed and the treatment loses some of its beneficial effects.

Besides the factors discussed that influence chromosome morphology (stage in mitotic cycle, and chemical treatment), one additional factor needs to be considered, namely that of cell size. Within a plant there are differences in cell size and nuclear volume; hence, the chromosome volume appears to be different in various tissues. To obtain an estimate of possible differences in the nuclear volume of meristematic cells in *Abies*, average cell and nuclear dimensions were determined for various tissues in the root tips of *Abies guatemalensis*. Root tips from 4-year-old seedlings were excised during the early morning, treated with 1% aqueous colchicine solution for five hours, and then placed in NAWASHIN's fixative. After dehydration they were embedded in paraffin, and longitudinal serial sections were cut at 10 μ . They were stained in safranin and counterstained with fast green. The median section was selected from 10 roots and the size of 10 cells, located nearest the three points (A, B and C) indicated in Figure 5 was measured. The locations corresponded to (A) the distal end of the

Table 2. — Variation in cell and nuclear size in relation to position of meristematic tissue. The values are given in microns and are based on 40 cells per tissue.

Material	Cell			Nucleus			
	Length	Width	Ratio	Length	Width	Ratio	Diameter
Distal end of central cylinder (A)	19.98	12.48	1.60	13.14	9.36	1.40	11.25
Distal end of rib meristem (B)	19.68	9.21	2.14	12.06	7.95	1.52	10.01
Central mother cell zone (C)	19.74	10.44	1.89	10.98	9.27	1.18	10.13

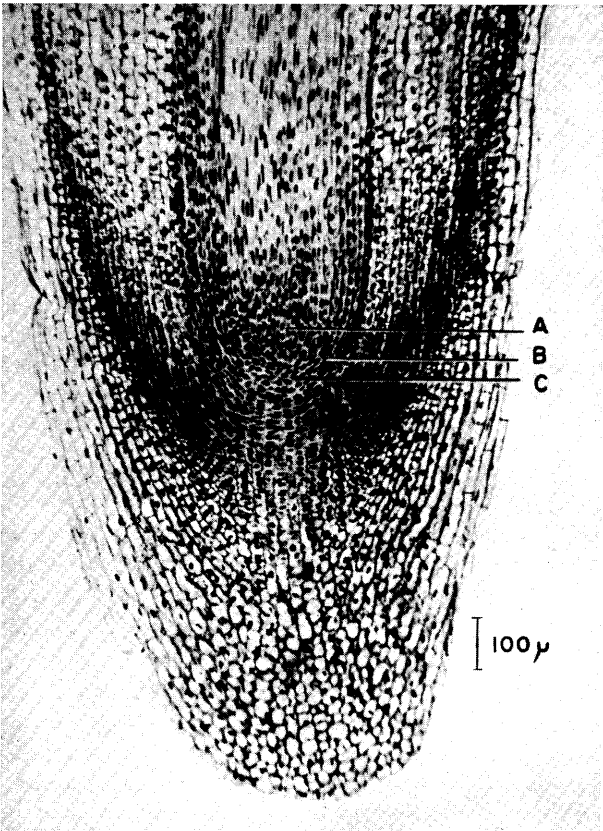


Figure 5. — Longitudinal median section through root of *Abies guatemalensis* showing location of (A) distal end of central cylinder (B) distal end of rib meristem; and (C) center of central mother cell zone.

central cylinder, (B) the distal end of the rib meristem, and (C) the center of the central mother cell zone. For these zones length and width of the cell and nucleus were measured to the nearest 0.1 μ with an ocular micrometer, and the measurements are summarized in Table 2.

Although there were differences in cell and nuclear size in the meristematic cells examined, this size difference was not as great as one might expect. Perhaps the uniformity of the plant material, and the fact that all of the root tips were collected at the same time, minimized the differences that one might encounter with more heterogeneous material in a general survey study.

Conclusion

The haploid karyotypes that were obtained with seven species of *Abies* confirm the uniformity of chromosome complements reported in conifers. Although the chromosomes were measured to the nearest 0.1 μ , it is felt that no diagnostic distinctions in karyotypes could be made among species. Because there are only minor differences between species, variations due to position in the mitotic cycle and due to measuring techniques may obscure specific differences. The *Abies* karyotype is characterized as follows: the three chromosomes with the smallest short-arm are heterobrachial; those with the next two smallest short-arm may or may not appear heterobrachial, depending on definition.

The use of 8-HQ to inhibit spindle formation, and to contract the chromosomes, has not proved as satisfactory in *Abies* as in other conifers. When 8-HQ was used after a colchicine treatment, it diminished the useful effect of colchicine. It is felt that for *Abies* 1% colchicine is a suit-

able reagent, causing complete spindle inhibition and repeatable chromosome contraction.

Summary

Karyotypes were determined for six species of *Abies* using female gametophyte tissue. No specific differences were observed and it is felt that these may be obscured by differences due to position in the mitotic cycle and to method of measurement.

The *Abies* karyotype is characterized by having three chromosomes that are distinctly heterobrachial. Two additional chromosomes may or may not be heterobrachial.

Solutions of 1% and 0.5% colchicine and 0.002 M 8-hydroxyquinoline were compared for their effects on spindle inhibition and chromosome contraction in root tips. 8-hydroxyquinoline alone and in combination with colchicine proved unsuitable for use with *Abies* chromosomes. The optimum treatment appeared to be five hours in 1% colchicine. This resulted in complete inhibition of spindle formation and in 37% and 39% contraction of the shortest and longest chromosomes.

Zusammenfassung

Titel der Arbeit: *Karyotyp-Analyse bei Abies*.

Von 6 *Abies*-Species wurden die Karyotypen am Gewebe des weiblichen Gametophyten festgestellt. Es wurden keine arteigenen Unterschiede beobachtet. Diese können aber auch infolge von Besonderheiten verborgen bleiben, die vom Mitosezyklus und der Meßmethode herrühren.

Der *Abies*-Karyotyp wird durch 3 Chromosomen charakterisiert, die distinkt heterobrachial sind. Zwei weitere Chromosomen können zusätzlich manchmal heterobrachial sein.

Verglichen wurden nun die Effekte von 1% und 0,5% Colchicin und von 0,002 M 8-Hydroxychinolin auf die Spindelhemmung und auf die Chromosomenkontraktion in Wurzelspitzen. 8-Hydroxychinolin allein und in Kombination mit Colchicin war nicht geeignet für *Abies*-Chromosomen. Dagegen war eine 5stündige Behandlung mit 1% Colchicin optimal. Dabei ergab sich eine komplette Hemmung der

Spindelbildung und eine Kontraktion von 37% bzw. 39% der kürzesten bzw. längsten Chromosomen.

Résumé

Titre de l'article: *Analyse des karyotypes de sapins*.

On a déterminé les karyotypes de six espèces de sapins à partir des tissus du gamétophyte femelle. On n'a remarqué aucune différence spécifique, mais on pense que celles-ci peuvent être masquées par des différences dues à la position dans le cycle mitotique et à la méthode de mesure.

Le karyotype du sapin est caractérisé par trois chromosomes qui sont nettement hétérobrachiaux. Deux autres chromosomes peuvent être hétérobrachiaux, mais pas toujours.

On a comparé les effets de solutions de colchicine à 1% et 0,5% et de l'hydroxyquinoline 0,002 M 8 sur l'inhibition du fuseau et la contraction des chromosomes dans les extrémités des racines. L'hydroxyquinoline 8, seule et combinée à la colchicine s'est révélée impropre pour les chromosomes de sapins. Le traitement optimum semble être cinq heures dans la colchicine à 1%. Ceci a entraîné l'inhibition totale de la formation du fuseau et une contraction à 37% et 39% des chromosomes les plus courts et les plus longs.

Literature Cited

- (1) KANEZAWA, R.: Polyploids appeared in twin seedlings of forest trees. (*Japanese with English summary*.) Jour. Jap. For. Soc. 31: 22-24 (1949). — (2) KLAHN, F. U., and WINIESKI, J. A.: Interspecific hybridization in the genus *Abies*. Silvae Genetica 11: 130-142 (1962). — (3) MEHRA, P. N., and KHOSHOO, T. N.: Cytology of conifers. I. Jour. Genet. 54: 155-180 (1956). — (4) MERGEN, F., and LESTER, D. T.: Microsporogenesis in *Abies*. Silvae Genetica 10: 146-156 (1961 a). — (5) MERGEN, F., and LESTER, D. T.: Colchicine-induced polyploidy in *Abies*. For. Sci. 7: 314-319 (1961 b). — (6) MERGEN, F., and NOVOTNY, H. M.: Squash technique for chromosome studies in pine needles and root tips of slash pine. For. Sci. 3: 56-60 (1957). — (7) SAX, K., and SAX, H. J.: Chromosome number and morphology in the conifers. Jour. Arnold Arb. 14: 356-375 (1933). — (8) SAYLOR, L. C.: A karyotypic analysis of selected species of *Pinus*. Silvae Genetica 10: 77-84 (1961). — (9) STÄLFELT, M. G.: The effect of oxyquinoline on protoplasmic viscosity. Appendix to TJIO and LEVAN, 1950. — (10) TJIO, J. H., and LEVAN, A.: Use of oxyquinoline in chromosome analysis. Anal. Estac. Exp. Aula Dei 2: 21-64 (1950).

The Heritability of Wood Characteristics of *Pinus radiata*

By J. W. P. NICHOLLS¹⁾, H. E. DADSWELL¹⁾ and J. M. FIELDING²⁾

(Received for publication September 16, 1963)

Introduction

Selection for wood characteristics in tree improvement programmes has been a feature of forest research during recent years and has stimulated widespread interest in the inheritance of wood properties.

Most of the studies have been based on species of the genus *Pinus* and this information has recently been summarized by ZOBEL (1961). He has warned of the confusion which may arise from quoting heritability results without indicating the method used in the calculation, the age of trees examined and the conditions of environment for

which the estimates were obtained.

It is useful to report estimates of gains as well as heritabilities from an inheritance study, but values of expected improvement may be of little significance if the results are derived from experimental material which is young compared with the harvest age. This follows because of the changes in heritability of a wood property with age (ZOBEL, 1961), but it is not implied that inheritance studies conducted on young trees are not of great importance; in fact they may be the only ones available until they can be supplemented by results from more mature subjects. Furthermore it is preferable to investigate a variety of properties in the material for examination so that genetic correlations between features may be calculated, as these too are of great interest in tree improvement work.

¹⁾ Division of Forest Products, C. S. I. R. O., Melbourne, Australia.

²⁾ Forestry and Timber Bureau, Department of Interior, Canberra, Australia.