Möglichkeiten hinsichtlich der genetischen Konstitution der halb-immergrünen Pappel und der künftigen Verwendung als Elter für Züchtungszwecke werden erörtert.

References

(1) Bkown, C. S.: Australian Forestry 25 (2): 81–88 (1961). — (2) F. A. O.: Poplars in Forestry and Land Use. Rome, 1958. — (3) Maisenhelder, L. C.: Personal Communication, 1961. — (4) Pauley, S. S., and Perry, T. O.: Jour. Arnold Arboretum 35: 167–188 (1954). — (5) Pryor, L. D.: Aspects of Poplar growing and utilization in Australia. Appita Vol. 17: 5: 126–133 (1964). — (6) Schreiner, E. J.: Production of Poplar Timber in Europe. U. S. D. A. Agriculture Handbook no. 150, 1959. — (7) Vaartaja, O.: Ecol. Monog. 29: 91—111 (1959).

Karyotype Analysis of Sitka Spruce, Picea sitchensis (Bong.) Carr.[1])

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Introduction

Sitka spruce, Picea *sitchensis* (Bong.) Carr., occurs in a narrow coastal belt on the west coast of North America, ranging from Mendocino County, California (41° N latitude) to Kodiak Island and the Kenai Peninsula, Alsaka (61° N). It is an important timber tree, both in its natural range and as an exotic in northwest Europe.

In a series of reports, the author has attempted to describe the pattern of natural variation in development of Sitka spruce; emphasis was given to basic developmental characters and phenological responses that cause genetic variation in height growth (Burley, 1964; 1965 a; b; c). The underlying cause of this variation should be sought in the nucleus; differences in growth characteristics may be a reflection of differences in chromosome number, size or morphology. The purposes of the research described in this paper were to determine practical methods of chromosome analysis for Sitka spruce, to establish the basic karyotype of the species, and to investigate the variation in karyotype between ten selected provenances representing the natural range of distribution.

Literature review

The haploid chromosome number in the genus Picea has been reported as 12 for over 20 species (Miyake, 1903; Sax and Sax, 1933; Seitz, 1951; Darlington and Wylie, 1955; Mehra and Khoshoo, 1956; Little and Pauley, 1958; Gustafsson, 1960; Santamour, 1960; Khoshoo, 1961; Morgenstern, 1962; Packer, 1964). Studies of meiotic material confirm the basic haploid number as 12 in Norway spruce, Picea abies (Picea excelsa) (Miyake, 1903; Andersson, 1947 a, b, 194%). Report's of basic numbers other than 12 are rare. Kiellander (1950) found triploid, tetraploid and mixoploid seedlings among nursery stock of Norway spruce and he induced polyploidy with colchicine. Chromosome numbers between 24 and 70 were found in embryos and dwarf plants of Norway spruce by Illies (1958); she believed this was due to pathological polysomaty rather than endomitotic polyploid~Torok and White (1960) reported that somatic tumor cells of white spruce, Picea glauca, contained 22 chromosomes, but Risser (1964) used similar material and concluded that the diploid number was stable at $2n = 24$.

Darlington and Wylie (1955) cited Thomas as authority for the diploid number of 24 chromosomes in Sitka spruce. The same number was dbserved by Vabre (1954); she postulatod that differences in length and shape of chromosomes may assist in the classification of species but she gave no measurements for Sitka spruce. In a study of the unusual hybrid, Tsugo-Picea *hookeriana* (Picea sitchensis X *Tsuga* heterophylla) she again found a basic number of 24, but believed that one chromosome is doubled or 'polysomatic' (Vabre-Durrieu, 1954).

In view of the similarity in chromosome number, chroinosome size and morphology become of greater significance in comparative karyotypic studies. Previous reports agree that, in all species of spruce, nine chromosomes are isobrachial and three heterobrachial, but they have not specified the brachial indices (ratio of short arm length to long arm length), and are therefore purely subjective. For example, in the most recent and detailed analysis of spruce chromosomes, Morgenstern (1962) considered that both Picea *rubens* and Picea mariana have three heterobrachials in the haploid complement, but his ideograms suggest that possibly five chromosomes in Picea mariana are heterobrachial. The usual limits af the brachial index are 0.50—0.75 for heterobrachials and 0.75—1.0 for isobrachials. The use of the index in identifying chromosomes and describing karyotypes is of more value than qualitative terminology, particularly when the index is accompanied by statistical analysis of the variation in arm length (e. g. Saylor, 1961; Simak, 1962; Heneen, 1962; Moore and Gregory, 1963). It is of particular importance in the case of coniferous genera, in which species differentiation is generally recognized to have occurred by individual gene mutations rather than by gross changes in chromosomal structure (Sax and Sax, 1933).

Secondary constrictions have been reported for only three species of spruce. Santamour (1960) observed secondary constrictions on two chromosomes in Picea *jezoensis hondoensis*, a species closely allied to Sitka spruce (Wright. 1955). Three chromosomes in the haploid complement of Picea *smithiana* were shown to possess secondary constrictions (Mehra and Khoshoo, 1956). Morgenstern (1962) observed secondary constrictions in Picea rubens and Picea mariana but found that they could neither be consistently identified nor separated from other achromatic areas. Similar conclusions were reached for pines (Saylor, 1961) and firs (Mergen and Burley, 1964).

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The concept of racial diversity in the karyotype of forest trees is not new. As early as 1929, ROSSEELS proposed chromosome studies of tree races, particularly Scots pine. In view of the uniformity of karyotypes within coniferous genera, it is not surprising that statistically valid racial and specific differences have not been found. SAYLOR (1961) was unable to distinguish adequately among four out of five pine species examined, and SIMAK (1962) found no differences among eight larch provenances.

In previous published karyotype analyses, emphasis has generally been placed on the relative contribution of chromosome arms to the total chromosome complement and there have been few specific references to absolute length of chromosomes in conifers. As stressed by MERGEN and BURLEY (1964), there is a greater variability in the absolute measure and this may facilitate the separation of species, although in their study they were unable to distinguish among six species of fir. The length of the longest chromosome was approximately 20 μ for untreated material and 10 μ for material treated with colchicine and 8-hydroxyquinoline (8-HQ). DANGEARD (1941) reported variation in chromosome length within individual plants of *Pinus maritima;* 'normal root meristem' chromosomes averaged 22—24 μ while chromosomes in epidermal cells averaged 19 μ. In *Pinus sylvestris* the longest chromosomes varied from 20 to 21 μ in length according to the type of pretreatment reagent and fixative used (NATARAJAN, OHBA, and SIMAK, 1961). VABRE (1954) gave values of 8 and 9 μ for *Tsuga* and *Pseudolarix* respectively.

Many authors, without specifying chromosome lengths in their results, included tables or illustrations from which lengths may be determined. The accuracy of the scales supplied may be suspect but a selection of calculated lengths for longest chromosomes is given in *Table 1* to demonstrate the variability of absolute length.

In spite of the lack of critical data, it is apparent that somatic chromosomes are longer in gymnosperms than in angiosperms; during analysis they become entangled and they present more difficulty. Three groups of methods have been used to overcome this difficulty: (i) SAX and SAX (1933) utilized female gametophyte tissue as this has only the haploid number of chromosomes; (ii) the ability of colchicine to inhibit spindle formation, accumulate metaphase figures, and facilitate chromosome spreading has long been known (PERNICE, 1899; EIGSTI and DUSTIN, 1955) and it has been used successfully with a variety of conifers, e. g. *Metasequoia* (STEBBINS, 1948), *Larix* (CHRISTIANSEN, 1950) and *Pinus* (MERGEN and NOVOTNY, 1957); (iii) contraction of chromosomes is obtained with 8 hydroxyquinoline (STÅLFELT, 1950; TJIO and LEVAN, 1950) and it was found satisfactory for pine and spruce (MIKAELSEN, 1952; JOHNSSON, 1953; SAYLOR, 1961).

Table 1. — Length of longest chromosomes, calculated or measured from published results.

Species*)	Author, date	Length, μ
Chamaecyparis (4 species)	SHIBATA *et al.*, 1956	10—22
Cryptomeria japonica	SHIBATA *et al.*, 1956	17
Cupressus lusitanica	HUNZIKER, 1961	14—16
Larix (species and hybrids)	LARSEN & WESTERGAARD, 1938	12
Larix decidua (4n)	CHRISTIANSEN, 1950	10
Larix decidua (2n and 4n)	ILLIES, 1952	12
Libocedrus (4 species)	HUNZIKER, 1961	14—19
Metasequoia	STEBBINS, 1948	17
Picea abies	BEVILACQUA & VIDAKOVIĆ, 1963	10—20
Picea abies (2n and 4n)	ILLIES, 1952	20
Picea mariana	MORGENSTERN, 1962	11
Picea rubens	MORGENSTERN, 1962	11
Picea smithiana	MEHRA & KHOSHOO, 1956	10
Pinus sylvestris	AASS, 1957	13
Pinus (7 species)	SHIBATA *et al.*, 1956	13—17
Pinus (5 species)	SAYLOR, 1961	13—17
Pseudolarix kaempferi	MIYAKE & YASUI, 1911	3— 6
Taxus baccata	DARK, 1932	7
Tsugo-Picea hookeriana	VABRE-DURRIEU, 1954	10

*) Species names are those used by the original author.

Colchicine and 8-HQ have been used successfully in combination, either simultaneously or successively, for angiosperm material (e. g. BATTAGLIA, 1957). However, 8-HQ counteracted some of the useful effects of colchicine when the two reagents were applied successively to root tips of *Abies guatemalensis* (MERGEN and BURLEY, 1964). The combination of reagents caused nearly 50% contraction in the length of the chromosomes. No other report known to the author has specified the quantitative effect of substances causing c-mitotic effects in conifers.

Methods

Selection of seed sources and material

Seedlings from 47 seed sources distributed through the range were grown under uniform environmental conditions in a nursery at New Haven, Connecticut; it was shown (BURLEY, 1965 b) that the pattern of natural variation in phenological responses of Sitka spruce is basically continous with respect to latitude. Restricted ecotypes have developed in response to specific local environmental conditions. For the present study, a total of ten seed sources were selected;[4]) details of the locations are given in *Table 2.*

Table 2. — Data for locations of seed sources.

Latitude	State	Type of location
44° 30'	Oregon	
44° 40'	Oregon	Coastal, southern
46° 22'	Washington	
52° 24'	British Columbia	Coastal
54° 00'	British Columbia	Island, central
55° 05'	British Columbia	Inland
57° 00'	Alaska	Island
60° 06'	Alaska	Coastal, northern
60° 45'	Alaska	Inland
58° 23'	Alaska	Glacier valley

The southern, central and northern portions of the range were each represented by three provenances. In the northern and central samples three types of locations were selected, inland, coastal and insular. The tenth source was a specific phenological ecotype that had developed in response to the particular environment downslope from the Mendenhall Glacier in Alaska.

Seeds were soaked in water in a refrigerator for 48 hours. They were then sown on moist filter paper in petri dishes maintained at 23 ± 2° C. After germination, radicles were excised when they were approximately one centimeter long. To standardize the environmental effects and to reduce the physiological variability as much as possible, all collections were made at 8.00 a. m.; although no quantitative data were obtained, a peak of mitotic activity was commonly observed

[4]) Seeds were kindly supplied by W. H. VAN HEEK (Columbia Cellulose Co. Ltd.), R. M. HURD and R. R. SILEN (U. S. Forest Service) and J. D. MATTHEWS (United Kingdom Forestry Commission).

in the early morning. A similar mitotic rhythm has been suggested for slash pine (MERGEN and NOVOTNY, 1957) and firs (MERGEN and BURLEY, 1964).

Treatment of radicles

To determine a suitable technique for karyotype analysis and to study the c-mitotic effect of various reagents on the chromosomes of Sitka spruce, radicles from one provenance were used.

The radicles were transferred immediately after excision to glass flasks containing one of the following solutions:
(a) distilled water for 5 hours (control)
(b) 0.002M 8-hydroxyquinoline (8-HQ) for 24 hours
(c) 1.0% colchicine for 5 hours
(d) 1.0% colchicine for 5 hours followed by 8-HQ for 24 hours

During the first five hours of each treatment the solutions were placed under continuous illumination (3,300 foot candles from a Fluomeric[5]) light. A fan maintained the flasks at room temperature, and the solutions were kept agitated and aerated with compressed-air.

For the reasons discussed later, treatment with 1.0% colchicine for five hours proved satisfactory and this was used for radicles of the ten selected provenances.

Preparation of slides

Radicles were fixed in 3 : 1 acetic acid : alcohol under vacuum for 24 hours. After hydrolysis in 1N HCl for 15 minutes at 60° C they were rinsed in distilled water and placed in Feulgen stain in the dark for one or two hours. The stained meristematic region was dissected out and macerated in a drop of propionic carmine, with heating to approximately 70° C for a few seconds. The material was affixed to a cover glass with egg albumin and the cover glass was pressed firmly to spread the material and flatten the chromosomes. The cover glass was floated off in 45% acetic acid, dehydrated through alcohols and xylene, and mounted in a drop of Permount resin on a clean slide. Lead weights were placed on the cover glass for 24 hours to keep the material flat while the resin hardened.

Preparation of slides continued until at least four clear metaphase figures were obtained for each chemical treatment and for each seed source.

Measurement of chromosomes

Three types of measuring device were available, all based on the same microscope. The first involved direct measurement with a filar micrometer. Using an oil immersion objective, the micrometer measured in units of 0.032 μ. However, there was considerable variation in the observations made with this method, and the method can only be used with straight chromosomes. The second method utilized camera lucida drawings, and the third used photographs. Chromosomes were measured with dividers and engineer's rule to the nearest 0.25 mm. To determine the magnification of the drawing or photograph, at least two chromosomes in each cell were measured with the filar micrometer.

The 24 chromosomes in each cell were arranged in homologous pairs by inspection of individual arm lengths, total chromosome length, and brachial index. A mean haploid karyotype was calculated for each cell and for each chemical treatment or seed source.

[5]) 'Fluomeric' is a trade name for a high intensity lamp that combines an arc tube with a filament bulb. Optimum range of rendition in the visible spectrum is 400—700 mμ.

Results and discussion

Variation due to method of measurement

One chromosome was selected on one plate and measured six times by each of three observers on three days, using the micrometer at a magnification of 1,250×. An analysis of variance, based on units equivalent to 0.032 μ, indicated a significant difference between observers for the short arm measurements but no significant differences for the long arm, nor for the total length. There were no differences at the three days and it was concluded that the method is repeatable and accurate, with a coefficient of variation of 14.3%.

The same chromosome was measured from six camera lucida drawings at a magnification of approximately 2,000×; with this procedure the coefficient of variation was 3.9%. SAYLOR (1961) used a projection drawing in which the variation was 2.7% at 2,000×. He did not discuss the comparison of this method with direct measurement by micrometer, however.

Using photographs at 3,000× magnification, variation was less than 1%. The micrometer was used as a base to calculate magnifications and it was concluded that the three methods were comparable in accuracy to the nearest 0.1 μ.

The camera lucida method is laborious and subject to considerable human error, but it has the advantage that the chromosomes need not all be in one horizontal plane. The photographic method is more objective and forms a permanent record; it is particularly useful when all the chromosomes are in one plane, and it was the method used for this investigation.

Variation during mitotic cycle

Mitosis is a continuous process and there is a large part of the mitotic cycle within which chromosomal counts may be made. The chromosomes undergo contraction during these stages and differences are thus possible in relative length and position of constrictions.

As far as possible in this study, cells were selected approximately at metaphase *(Figure 1)*. However, to demon-

Figure 1. — Colchicine-metaphase (1300×).

strate the progressive contraction of chromosomes during mitosis, three cells at different stages were located on one slide. The lengths of the shortest and longest chromosomes were measured in each cell, and the decrease in length between prophase and metaphase was expressed as a percentage of the prophase measurement. There was no differential contraction between chromosomes (21.1 and 22.8%) nor between arms (22.2 and 21.8%), and absolute contraction was proportional to absolute chromosome length.

Variation due to chemical treatment

(i) Spindle inhibition:

An index of the efficiency of the various treatments was obtained by calculating the increased proportion of prophase and metaphase figures in random samples of mitotic cells. Ten slides per treatment were scanned systematically and the first 50 dividing cells on each slide were classified as prophase-metaphase or anaphase-telophase. For the controls, the numbers of cells in each class was similar (53.3 and 46.7%). After treatment for five hours with colchicine, there were 100% prophase-metaphase figures. An enumeration could not be made after treatment with 8-HQ for 24 hours, because no slide contained 50 cells in division. This decrease in the number of dividing cells suggests that 8-HQ inhibits mitosis. Colchicine caused a comparable inhibition in *Vicia* (Evans, Neary and Tonkinson, 1957).

(ii) Chromosome contraction:

The mean haploid karyotype for each chemical treatment is shown in *Figure 2;* it can be seen that the effects of the treatments were consistent over all chromosomes. In *Table 3* the contraction caused by each treatment is represented

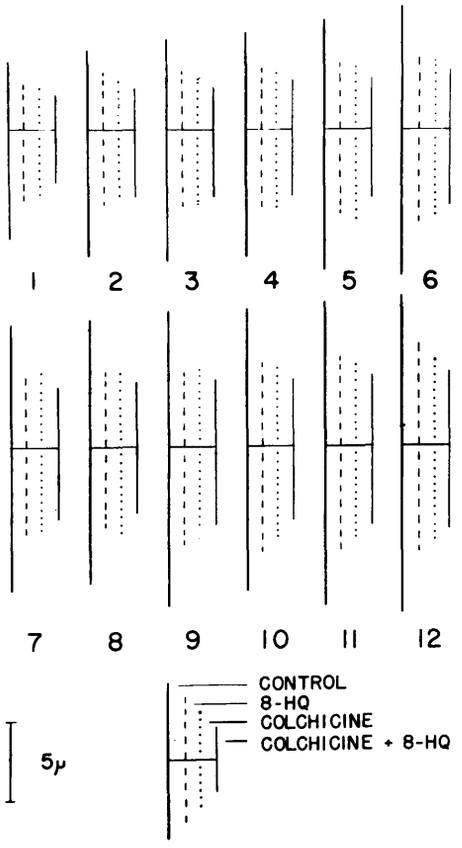

Figure 2. — Mean haploid karyotypes after four treatments, based on four cells per treatment.

Table 3. — Percentage contraction of shortest and longest chromosomes, and the total haploid complement after chemical treatment.

Treatment	Shortest chromosome	Longest chromosome	Total haploid complement
Distilled water 5 hours (control)	0	0	0
8-HQ 24 hours	33.9	34.3	37.2
Colchicine 5 hours	38.4	42.8	41.6
Colchicine and 8-HQ	50.0	50.2	49.5

as a percentage of the control for the longest and shortest chromosomes, and for the total haploid complement.

No account was taken of the variation within chromosomes within treatments, but the effects of the treatments were clear. Colchicine contracted the chromosomes slighly more than 8-HQ. Maximum contraction (to approximately half the original length) was obtained with the combination of colchicine and 8-HQ, and there was no differential contraction among chromosomes. The values for Sitka spruce corresponded closely to those obtained with *Abies guatemalensis* (Mergen and Burley, 1964).

(iii) Selection of the method for cytological analysis of Sitka spruce:

In view of the chromosome contraction caused by the combination treatment, it would appear reasonable to adopt this as the optimum method. However, certain additional effects decrease its value for mitotic analysis. As discussed earlier, 8-HQ inhibits mitosis and this reduced the number of cells available for analysis, consequently increasing the number of slides that must be prepared. In addition, one to several achromatic bands appeared on chromosomes after treatment with 8-HQ. These make the identification of secondary constrictions difficult and probably cause a weakening of the chromosome structure. This increases the incidence of chromosome breakage and possibly allows stretching at the weak points.

For the analysis of the ten selected provenances, treatment with 1.0% colchicine for five hours was adopted. Complete inhibition of the spindle was obtained and a chromosome contraction of approximately 40% was expected.

Variation due to provenance

Mean haploid karyotypes were calculated for each provenance. The chromosomes were numbered from 1 to 12 in order of increasing length of the short arm, and comparisons of provenance means were made for each chromosome arm in turn. At the 5% probability level, there were no significant differences among provenances and the data were pooled to determine the mean karyotype of the species.

However, total haploid complement length increased significantly with increasing latitude ($r = 0.576$; 5%), and this relationship was corroborated by the correlation between nuclear volume and latitude. According to Sparrow and Miksche (1961), differences in chromosome size or volume are reflected in comparable variation in nuclear size. Ten interphase nuclei were selected at random in three root-tip macerations for each of five provenances, and the nuclear diameter was determined with a filar micrometer to the nearest 0.1 μ in two directions mutually at right angles. The mean volume was calculated for each provenance in cubic microns (μ^3), and nuclear volume increased significantly with increasing latitude ($r = 0.879$; 1%). Nuclear volume ranged from 2310 μ^3 for the most southern source selected to 4445 μ^3 for the most northern source. Because these determinations were made on germinating

seed, they reflect the nuclear characteristics of material in its original habitat.

The number of provenances used here is not an adequate representation of the range of Sitka spruce (2,000 miles), and, further, the provenances were selected to include extreme types of habitat. However, the results are of considerable interest because they provide evidence for variations in nuclear and karyotype constitution that are associated with continuous variation in morphological and developmental characteristics of this species. In addition, no evidence has been published to date for patterns of karyotypic variation related to the seed origin of conifers. The relationships deduced with this material may reflect either variation in absolute karyotype structure or variation in the reaction with colchicine.

The karyotype of Sitka spruce

The basic haploid karyotype is shown in the form of an ideogram, based on absolute units, in *Figure 3*. Secondary constrictions did not appear in all cells and could rarely be identified with certainty. However, the most frequent location was in Chromosome 10 at the position indicated in the figure.

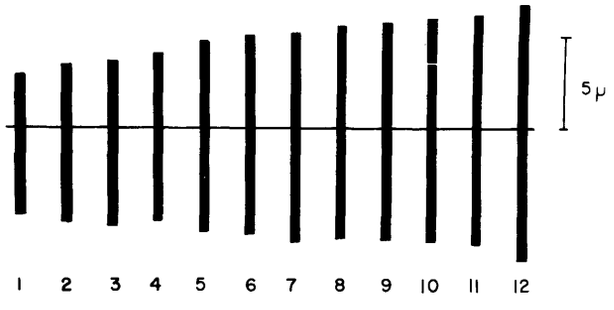

Figure 3. — Mean haploid karyotype based on four cells in each of ten provenances.

As discussed earlier, chromosomes contract during normal mitosis and it is difficult to identify the exact position of a cell in the mitotic cycle. To facilitate comparisons of different cells, treatments or observers, it is convenient to express an individual chromosome length as a percentage of the total chromosome length in a cell. In fact the majority of published ideograms have been based solely on relative length, giving no indication of absolute measurement.

The range, mean and standard error of the mean were calculated for the relative length of each chromosome arm in the haploid complement. These values are illustrated in *Figure 4*. The brachial ratios are given in *Table 4*. Three chromosomes may be seen to be heterobrachial.

The variability of the long arms of Chromosomes 3 and 4 is relatively great and there is a possibility that a true Chromosome 3 may be classified as a Chromosome 2 or 4. However, Chromosomes 1 to 5 and Chromosomes 11 and 12 are relatively easy to identify and classify accurately, because of the differences in short arm values. There is

Table 4. — Brachial ratios of chromosomes in haploid complement.

Chromosome	Ratio	Chromosome	Ratio
1	0.599	7	0.842
2	0.644	8	0.901
3	0.690	9	0.929
4	0.805	10	0.927
5	0.821	11	0.944
6	0.851	12	0.944

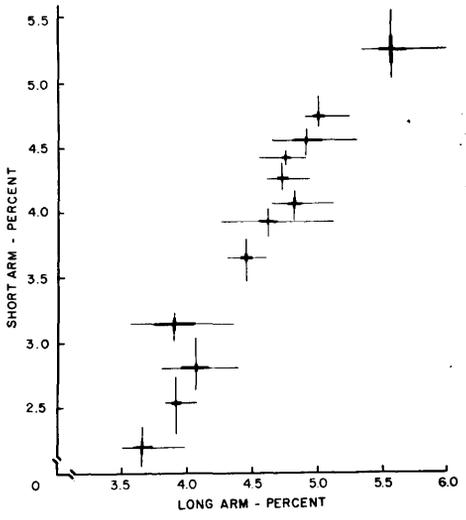

Figure 4. — Range, mean and standard error for short and long arms expressed as percentages of the total haploid complement.

a considerably greater possibility of misclassifying Chromosomes 6—10 because of the overlap of long arm values. The most probable errors are an interchange of Chromosomes 6 and 7 and a misclassification of Chromosome 9 as 8 or 10. These figures demonstrate the difficulty of accurate karyotype analysis and the need for a large sample.

In addition to having three heterobrachials, the karyotype of Sitka spruce is characterized by two other chromosomes. When the 12 haploid chromosomes are arranged in order of increasing short arm length, the length of the long arm also increases, with the exceptions of Chromosomes 4 and 7. These are shorter and longer respectively than the trend values. If the ideograms published by Morgentsern (1962) are rearranged into the order of increasing short arm lengths, Chromosomes 2, 4 and 7 for *Picea rubens* and 2, 5, 6 and 7 for *Picea mariana* interrupt the sequence of increasing long arm length. These differences between species may be an artifact of sampling intensity, but the results obtained in this study confirm and extend previous observations of chromosome number and karyotype in the genus *Picea*.

Summary

Solutions of 1.0% colchicine and 0.002M 8-hydroxyquinoline (8-HQ) were used to determine their quantitative effects on spindle inhibition and chromosome contraction in Sitka spruce, *Picea sitchensis* (Bong.) Carr. Maximum contraction (50%) was obtained with a successive application of both compounds but several undesirable effects of 8-HQ precluded its use in karyotype analysis. Colchicine caused complete inhibition of the spindle and contracted chromosomes by approximately 40%. There was no differential contraction between chromosomes nor between arms. The contraction undergone by chromosomes during normal mitosis was also evaluated.

No statistically significant differences were observed in the basic haploid karyotypes of ten provenances, and the data were pooled to obtain the karyotype of the species. However, there was a significant increase in total haploid complement length and nuclear volume with increasing latitude of seed origin. This is the first report of geographic variation in karyotypic constitution of conifers. Three chromosomes were heterobrachial and the karyotype was fur-

ther characterized by the arm lengths of two specific chromosomes; in these it differed from comparable karyotypes published for other species of spruce. The position of secondary constrictions was considered unreliable as an index of chromosome morphology. The variability of chromosome arm lengths was illustrated, emphasizing the difficulty of accurate analysis of karyotypes in conifers.

Résumé

Titre de l'article: *Analyse caryologique de l'épicéa de de Sitka (Picea sitchensis).*

Des solutions de colchicine à 1% et de 8-hydroxyquinoléine à 0,002 M ont été employées pour déterminer leurs effets quantitatifs sur l'inhibition du fuseau et la contraction des chromosomes chez l'épicéa de Sitka. Le maximum de contraction (50%) est obtenu avec une application successive des deux produits, mais certains effets défavorables de la 8-HQ interdisent son emploi dans l'analyse caryologique. La colchicine a amené une inhibition complète du fuseau et une contraction des chromosomes d'environ 40%. On n'a observé aucune contraction différentielle entre les chromosomes ou entre les bras. On a également évalué la contraction des chromosomes au cours d'une mitose normale.

Aucune différence significative n'a été observée dans les caryotypes haploïdes de base de dix provenances et les données ont été réunies pour obtenir le caryotype de l'espèce. Cependant, il y a une augmentation significative de la longueur totale et du volume nucléaire avec l'augmentation de la latitude de l'origine des graines. Ceci constitue la première observation de variation géographique dans la constitution caryotypique des conifères. Trois chromosomes sont hétérobrachiaux et le caryotype peut être caractérisé par la longueur des bras de deux chromosomes spécifiques. Cela constitute une différence par rapport aux caryotypes comparables publiés pour d'autres espèces d'épicéas. La position des constrictions secondaires ne peut constituer un index valable de la morphologie chromosomique. On insiste sur la variabilité de la longueur des bras des chromosomes qui rend difficile une analyse précise des caryotypes chez les conifères.

Literature Cited

Aass, I.: En cytologisk analyse of Skjåkfurna. (A cytological analysis of Scots pine *[Pinus sylvestris* L.] from Norway.) Medd. Norske Skogsforsøksvesen **14**: 93—109 (1957). — Andersson, E.: A case of asyndesis in *Picea abies*. Hereditas **33**: 301—347 (1947 a). — Andersson, E.: Pollen and seed setting studies of an asyndetic spruce and some normal spruces; and a progeny test of spruces. Svensk Papperstidning **4—7**: 1—22 (1947 b). — Andersson, E.: The association of forest tree breeding. Svensk Papperstidning **1—3**: 1—12 (1948). — Battaglia, E.: "Simultaneous" and "successive" pretreatments in chromosome analysis. Caryologia **9**: 370—371 (1957). — Bevilacqua, B., and Vidaković, M.: Effect of gamma rays on the chromosomes of the somatic cells in *Picea abies* Karst. Silvae Genetica **12**: 41—46 (1963). — Burley, J.: Variation in seed characteristics of Sitka spruce. Advancing Frontiers of Plant Sciences (New Delhi) **10**: 11—24 (1964). — Burley, J.: Genetic variation in *Picea sitchensis* (Bong.) Carr. A literature review. Commonw. For. Rev. **44**: 47—59 (1965 a). — Burley, J.: Genetic variation in seedling development of Sitka spruce, *Picea sitchensis* (Bong.) Carr. Forestry. (In press; 1965 b). — Burley, J.: Anatomy of the shoot apex of Sitka spruce, *Picea sitchensis* (Bong.) Carr. Forest Sci. (In press; 1965 c). — Christiansen, H.: A tetraploid *Larix decidua* Miller. Danske Biol. Medd. **18** (9): 8 pp. (1950). — Dangeard, P.: Sur les différences de taille entre chromosomes appartenant à différents tissus dans la plantule du pin maritime. C. R. Séances Soc. Biol. **135**: 581—583 (1941). — Dark, S. O. S.: Chromosome of *Taxus, Sequoia, Cryptomeria, Thuja*. Ann. Bot. **46**: 965—977 (1932). — Darlington, C. D., and Wylie, A. P.: Chromosome atlas of flowering plants. Allen and Unwin, London. 519 pp. (1955). — Eigsti, O. J., and Dustin, P. Jr.: Colchicine — in agriculture, medicine, biology, and chemistry. Iowa State College Press, Ames, Iowa. 470 pp. (1955). — Evans, H. J., Neary, G. J., and Tonkinson, S. M.: The use of colchicine as an indicator of mitotic rate in broad bean root meristems. Jour. Genet. **55**: 487—502 (1957). — Gustafsson, Å.: Polyploidy and mutagenesis in forest tree breeding. Proc. 5th World For. Congr., Seattle, 793—805 (1960). — Heneen, W. K.: Karyotype studies in *Agropyron*. Hereditas **48**: 471—502 (1962). — Hunziker, J. H.: Estudios cromosomicos en *Cupressus* y *Libocedrus (Cupressaceae)*. Rev. Invest. Agric., Buenos Aires, **15**: 169—185 (1961). — Illies, Z. M.: Colchicinversuche an *Larix decidua* Miller und *Picea abies* (L.) Karst. Z. Forstgenet. Forstpflanzenzüchtung **1**: 36—39 (1952). — Illies, Z. M.: Polysomatie im Meristem von Einzelbaumabsaaten *bei Picea abies*. Silvae Genetica **7**: 94—97 (1958). — Johnsson, H.: The oxyquinoline method — a useful method for chromosome studies in conifers. Tree Genetics News Letter **2** (2): 4 (1953). — Khoshoo, T. N.: Chromosome numbers in gymnosperms. Silvae Genetica **10**: 1—9 (1961). — Kiellander, C. L.: Polyploidy in *Picea abies*. Hereditas **36**: 513—516 (1950). — Larsen, C. S., and Westergaard, M.: Contributions to the cytogenetics of forest trees. I. A triploid hybrid between *Larix decidua* Miller and *Larix occidentalis* Nutt. Jour. Genet. **36**: 523—530 (1938). — Little, E. L. Jr., and Pauley, S. S.: A natural hybrid between black and white spruce in Minnesota. Amer. Midl. Nat. **60**: 202—211 (1958). — Mehra, P. N., and Khoshoo, T. N.: Cytology of conifers. Jour. Genet. **54**: 165—185 (1956). — Mergen, F., and Burley, J.: *Abies* karyotype analysis. Silvae Genetica **13**: 63—68 (1964). — Mergen, F., and Novotny, H. M.: Squash technique for chromosome studies in pine needles and root tips of slash pine. For. Sci. **3**: 56—59 (1957). — Mikaelsen, K.: A rapid method for chromosome studies on vegetative buds of spruce and birch. Medd. Norske Skogsforsøksvesen **11**: 597—523 (1952). — Miyake, K.: On the development of the sexual organs and fertilization in *Picea excelsa*. Ann. For. **17**: 351—372 (1903). — Miyake, K., and Yasui, K.: On the gametophytes and embryo of *Pseudolarix*. Ann. Bot. **25**: 639—647 (1911). — Moore, R. C., and Gregory, G.: Biometrics of the karyotype of *Protemnodon bicolor*, with reference to the limitations in accuracy of identifying human chromosomes. Nature, London, **200** (4903): 234—237 (1963). — Morgenstern, E. K.: Note on chromosome morphology in *Picea rubens* Sarg. and *Picea mariana* (Mill.) B. S. P. Silvae Genetica **11**: 163—164 (1962). — Natarajan, A. T., Ohba, K., and Simak, M.: Karyotype analysis of *Pinus sylvestris*. Hereditas **47**: 379—382 (1961). — Packer, J. G.: Chromosome numbers and taxonomic notes on western Canadian and Arctic plants. Can. Jour. Bot. **42**: 473—494 (1964). — Pernice, B.: Sulla cariocinesi delle cellule epiteliali e dell' endotelio dei vasi della mucosa della stomaco e dell' intestino, nella studio della gastroenterite sperimentale (nell' avvelenamento per colchico). (In Italian, cited by Eigsti and Dustin, 1955). Sicilia Med. **1**: 265—279 (1899). — Risser, P. G.: Somatic mitoses in cells of *Picea glauca* cultivated *in vitro*. Science **143** (3606): 591—592 (1964). — Rosseels, E.: A propos de la sélection des essences forestières. Bull. Soc. Cent. For. Belgique **36**: 291—298 (1929). — Santamour, F. S.: New counts in *Pinus* and *Picea*. Silvae Genetica **9**: 87—88 (1960). — Sax, K., and Sax, H. J.: Chromosome number and morphology in conifers. Jour. Arnold Arb. **14**: 356—375 (1933). — Saylor, L. C.: A karyotypic analysis of selected species of *Pinus*. Silvae Genetica **10**: 77—84 (1961). — Seitz, F. W.: Chromosomenzahlenverhältnisse bei Holzpflanzen. Z. Forstgenet. Forstpflanzenzüchtung **1**: 22—32 (1951). — Shibata, K., Ogoshi, Y., and Nakata, G.: Cytological studies on *Coniferae*. I. Chromosome numbers of some species in *Chamaecyparis, Cryptomeria* and *Pinus*. La Kromosomo (Senshokutai) **29**: 1025—1028 (1956). — Simak, M.: Karyotype analysis of *Larix decidua* Mill. from different provenances. Medd. Stat. Skogsforskningsinst. **51** (1): 1—22 (1962). — Sparrow, A. H., and Miksche, J. P.: Correlation of nuclear volume and DNA content with higher plant tolerance to chronic radiation. Science **134** (3474): 282—283 (1961). — Stålfelt, M. G.: The effect of oxyquinoline on protoplasmic viscosity. (Appendix to Tjio and Levan, 1950.) Anal. Exp. Aula Dei, Saragossa. **2**: 63—64 (1950). — Stebbins, G. L. Jr.: The chromosomes and relationships of *Metasequoia* and *Sequoia*. Science **108** (2796): 95—98 (1948). — Tjio, J. H., and Levan, A.: Use of oxyquinoline in chromosome analysis. Anal. Estac. Exp. Aula Dei, Saragossa, **2**: 21—63 (1950). — Torok, D., and White, P. R.: Cytological instability in tumours of *Picea glauca*. Science **131** (3402): 730—732 (1960). — Vabre, A.: Structure du noyeau quiescent de sept espèces de conifères. C. R. Acad. Sci (Paris) **238**: 382—384 (1954). — Vabre-Durrieu, A.: L'hybride *Tsugo-Picea hookeriana* et ses parents: étude chromosomique et caryologique. Trav. Lab. For. Toulouse, Tome I., Vol. 5., Art. 17: 4 pp. (1954). — Wright, J. W.: Species crossability in spruce in relation to distribution and taxonomy. For. Sci. **1**: 319—349 (1955).