Fällen zur Befruchtung der Eizellen führen und daß es von dem Grad der „Unverträglichkeit" abhängt, ob lebensfähige Samen erzeugt werden oder nicht. Dieser Grad der „Unverträglichkeit" kann, auch an Samen, die normalerweise zur Klasse 0 zu rechnen sind, mit Hilfe der Röntgenphotographie bestimmt werden.

Résumé

Titre de l'article: *Etude par radiographie* des croisements *artificiels entre* Picea *abies* (L.) Karst. *et* Picea *glauca (Moench)* Voss.

La technique de radiographie, mise au point en Suède pour l'évaluation de la qualité des graines a été essayée depuis 4 ans au »State University College of Forestry« a l'université de Syracuse. L'emploi des techniques d'évaluation de la qualite des graines et de la faculté germinative presente des voies interessantes pour la recherche. Cette étude montre une application possible de cette methode en génétique forestière pour l'examen des graines résultant des croisements et des autoféconda tions en se basant sur le developpement de l'embryon et de l'endosperme, cela même si la graine n'est pas capable de germer.

On estime, d'accord avec le travail realise par Orr-Ewing que l'autopollinisation et les croisements entre espèces d'épicéa normalement incompatibles conduisent dans beaucoup de cas a la fertilisation de l'oeuf; c'est le »degré d'incompatibilité« qui détermine s'il se produit ou non une graine viable. Ce degre peut être évalué par radiographie egalement sur les graines qui seraient normalement réunies dans la classe 0.

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A Karyotypic Analysis of Selected Species of Pinus

By L. C. Saylor[1])

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Introduction

Genetics and breeding have received considerable emphasis in recent years as means of improving commercial pine species. Although the value of such programs has been amply demonstrated, their progress is often hindered by the lack of basic information from such disciplines as physiology, cytology, plant breeding, and genetics.

Lack of cytogenetic information in the genus Pinus can probably be attributed to at least two factors. First, it is likely that the results of early karyological investigations discouraged additional studies in this field. These findings indicated that the genus is homoploid ($2n = 24$)[2]) with very little if any interspecific variation in karyotype. As a consequence it has been assumed that evolution has occurred almost exclusively at the genic level without involving detectable chromosomal alterations. To date no major evidence has been found to contradict this opinion, even though it is based on information from less than one-third of the species. Secondly, attempts to make detailed studies of chromosome morphology have been hampered because the numerous and long chromosomes, characteristic of this genus, frequently overlap and become obscured. Before comprehensive cytological investigations can be undertaken, therefore, techniques must be perfected that enable a thorough examination of the chromosomes. Then, if structural rearrangements of the chromosomes have occurred during the course of evolution of this genus, cytogenetic studies may prove useful in elucidating species relationships.

The present study is part of a continuing cytogenetic investigation of the entire genus *Pinus*. This paper describes: (1) new or modified techniques for obtaining critical data on the chromosomes of this genus; (2) results obtained from a karyotypic analysis of a selected group of species.

Review of Literature

The few investigators who have examined the genus *Pinus* for karyotypic variation have stressed the uniformity of chromosomes within karyotypes, as well as the general similarity of the karyotypes among the different species. Sax and Sax (1933) contributed the first significant information concerning this genus. These authors concluded: "The chromosomes of all species of Pinus seem to be very similar. One of the 12 chromosomes is somewhat heterobrachial, and the others have approximately median

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2) Polyploid and mixoploid seedlings occasionally occur naturally (Khoshoo, 1959; Mergen, 1958), although these seedlings rarely if ever reach maturity because of defective growth.

fiber attachments." In a meiotic analysis of several white pine hybrids, SAX (1960) found additional evidence of similar chromosome morphology and genetic compatibility among pine species. The nearly normal meiotic behavior of the F_1 species hybrids was considered an indication that structural changes in the chromosomes have not played an important part as an isolating mechanism in pine speciation.

Although the results of other investigations tend to confirm the general karyotypic pattern presented by SAX and SAX, indications of possible karyotypic differences also occur. In their cytological examination of the conifers, MEHRA and KHOSHOO (1956) critically examined the karyotypes of several species of pine. Although no satellites were detected, as many as eight secondary constrictions were described in the diploid complement of a single species. SANTAMOUR (1960), in a recent article listing new chromosome counts in *Pinus* and *Picea*, also recorded a prominent secondary constriction for a species of pine (*P. parviflora*).

AASS (1957) examined the karyotypes of twenty-four morphologically atypical *Pinus sylvestris* trees in an attempt to determine if the morphological deviations were correlated in any way with polyploidy or other cytological irregularities. Athough no cytological abnormalities were detected, secondary constrictions were described, plus the fact that two pairs of chromosomes appeared smaller than the others (both possible distinctive features of this karyotype).

Further evidence of interspecific karyotypic variation is presented by VIDAKOVIĆ (1958). This study included examination of both meiotic and mitotic material of three trees presumed to be natural hybrids between normal *Pinus sylvestris* and *Pinus nigra*. Somatic chromosomes of the alleged parental species, as well as the intermediates, were studied for variation in size, as determined by differences in length between the smallest and the largest chromosomes. The results indicated this difference in size to be greater in *P. sylvestris* than *P. nigra*, while the three questionable specimens were intermediate between the parental types.

In a general study of the Coniferales, STIFF[3]) has examined the somatic chromosomes of a large number of pine species and has described karyotypic variation. This work, however, remains unpublished.

Materials and Methods

The species selected for this investigation, *Pinus strobus* L. (eastern white pine), *P. taeda* L. (loblolly pine), *P. palustris* MILL. (longleaf pine), *P. virginiana* MILL. (virginia pine), and *P. resinosa* AIT. (red pine), were chosen to provide information about species both distantly and closely related.

All cytological work was done with somatic chromosomes obtained from root-tip meristems. Seeds, obtained from various sources, were germinated in trays under a water mist, and the seedlings were potted and kept in optimum growing conditions in a regulated greenhouse.

The procedures needed to produce accurate and consistently repeatable observations and measurements were developed and tested for reliability. Because new and generally satisfactory methods were devised, a detailed description of these techniques is presented.

[3]) The geographical distribution and cytology of the Coniferales. Unpublished Ph. D. Thesis, University of Virginia, Charlottesville.

Figure 1. — *P. taeda* chromosomes from a root-tip subjected to 33 hours pretreatment in oxyquinoline. Arrows denote the smallest chromosomes with submedian centromeres. 1650 ×.

Pretreatment and Staining Techniques

Various pretreatments have been developed to shorten and straighten chromosomes as well as to arrest cell division at the metaphase stage. MERGEN and NOVOTNY (1957) tested several methods in their search for a satisfactory pretreatment for *Pinus elliottii*. Their procedure was not completely satisfactory for the present study, however, because the recommended colchicine treatment did not produce the desired contraction and the chromosome arms were poorly defined because of the disrupted condition of the chromatin material. The standard solution of 8-hydroxyquinoline (TJIO and LEVAN, 1950) proved to be a most statisfactory pretreating agent, although its effectiveness was found to be dependent upon the length of the treatment and the temperature at which it was used.

Aceto-carmine proved slightly superior to aceto-orcein because its staining action was more consistent. It should be emphasized, however, that the condition of the root-tips is extremely important, because actively growing root-tips not only provide large numbers of dividing cells, but they also provide optimum conditions for the staining and contraction reactions.

The steps in the technique found to be most satisfactory are:

1. Pretreat in oxyquinoline (0.3 g/l.) for 24—36 hours at 12° C.
2. Fix in alcohol-acetic, 3:1, for 1—4 hours.
3. Hydrolyze in 1N HCl for 10—15 minutes at 60° C.
4. Rinse thoroughly in water.
5. Stain in aceto-carmine or aceto-orcein for 30—60 minutes.

This technique is easily performed and it provides several desirable features that other procedures have failed to produce. The chromosomes can be consistently shortened to lengths that allow them to be easily separated for detailed observation. At the same time, the chromatin material remains intact so that when properly stained the chromosome arms and constriction regions are sharply defined (*Figures 1 and 2*).

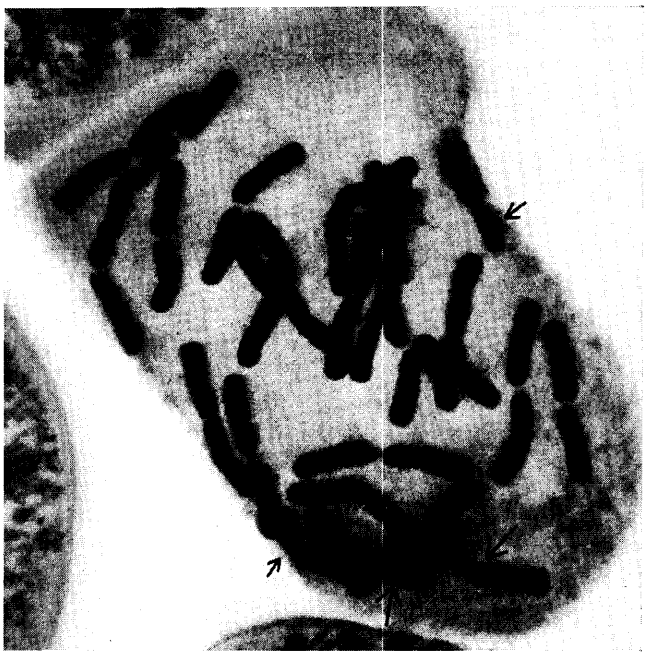

Figure 2. — *P. resinosa* chromosomes from a root-tip subjected to 26 hours pretreatment in oxyquinoline. The short and long arrows denote respectively the smallest and second smallest chromosomes with submedian centromeres. 1650 ×.

Drawing Procedure

Because of the large number of measurements deemed necessary to analyze the chromosomes, a method combining both accuracy and speed was desired. A projection drawing method was devised to meet these requirements.

A compound research microscope was mounted on a base plate with a standard 6-volt ribbon-filament lamp, and the entire unit was placed on blocks eight inches from the floor to afford ease in operating the microscope. A specially designed glass-top table was placed over this unit, and the normal image from the slide was projected directly upward from the eyepiece to tracing paper securely fastened on the glass surface of the table. A projection distance of 15 inches was used in combination with a 90 × oil immersion objective and a 15 × eyepiece to provide a magnification of 2000 × for all drawings. Top quality tracing paper was used because its homogeneous translucence, often lacking in cheaper paper, provided superior definition of the chromosome images and pencil lines. To increase the clarity of the image, the tracing was done in a darkened room.

Although the projection distance was short, a relatively high energy light source was needed because of the optics used. In trying different light sources, an unexpected problem developed whereby the projected image moved slightly across the tracing paper. Leveling the microscope substage and securely clamping the slide reduced but did not eliminate this creeping. Later tests proved that heat from the light source caused differential expansion of certain microscope parts, and this in turn caused most of the movement noticeable at this high magnification. To help eliminate this undesirable situation, a "warm-up" period of 30 minutes was used to allow all parts of the microscope to reach a constant temperature.

The projection arrangement described above contains several desirable features not previously combined in one system. The focusing advantage of the camera lucida method is retained, which reduces the necessity of having all chromosomes in the same focal plane. At the same time, the shadow problem inherent in most projection systems is eliminated, because the image is traced on the side of the paper opposite the light source and there is no interruption of the light rays. These advantages combined with freedom of movement and ease in drawing make the method highly satisfactory.

Photomicrographs were unsatisfactory for a majority of the measurements because of the difficulty of obtaining all chromosomes in the same focal plane. They were used for permanent records, however, whenever a satisfactory plate was obtained. The chromosomes of these photomicrographs were also measured to provide data for checking the accuracy of the projection method.

Measurement and Analysis

Once drawn, the chromosomes of a given plate were arbitrarily numbered from 1 to 24, and the individual arms were measured using an engineer's dividers and an accurately calibrated steel rule. When curved, the chromosome arm was measured along a series of straight lines tangent to the arc described by the arm. The measurements were made from the extremities of the arms, and, therefore, the recorded lengths do not include primary constrictions, although secondary constrictions are included unless obviously stretched. The short and long arms of each chromosome were recorded respectively as the *a* and *b* arms, while the secondary constrictions were designated according to their distance from the proximal end of the arm.

The 24 chromosomes were arranged in 12 pairs by matching the chromosomes with the most similar *a* and *b* arm values. In determining the pairs, the shorter (*a*) arm was considered the more accurate length, because it was less susceptible to stretching. The positions of secondary constrictions were used when possible as an aid in determining pairs, but they could not be used as a major criterion because they were not consistently detectable. Once paired, the lengths of the corresponding arms of each pair were averaged to obtain the values for the haploid karyotypes of the individual plates.

In analyzing the individual karyotypes, the chromosomes were arranged from 1 to 12 according to a descending order of *a* arm lengths. (Position 1 has the largest chromosome, while the shortest is in position 12.) Variation in the length of the *b* arm according to its position in the normal descending sequence was then used as a diagnostic feature of the karyotype. (See *Table 1, P. strobus* chromosomes 6, 8, and 10.) Attempts were also made to use the number and position of secondary constrictions as an additional means of determining specific karyotypes, but these proved less successful.

Statistical Estimates of Error

There is danger of introducing errors during several phases of the preparation and analysis of this material. To test the accuracy and observational repeatability of the procedures, a statistical estimate of error was calculated in two ways.

One method evaluated the magnitude of the variation that occurred among measurements when a given plate was drawn three different times. Standard deviations were determined for three lengths, *a* arm, *b* arm, and *a* + *b* (the total chromosome length), using conventional methods.

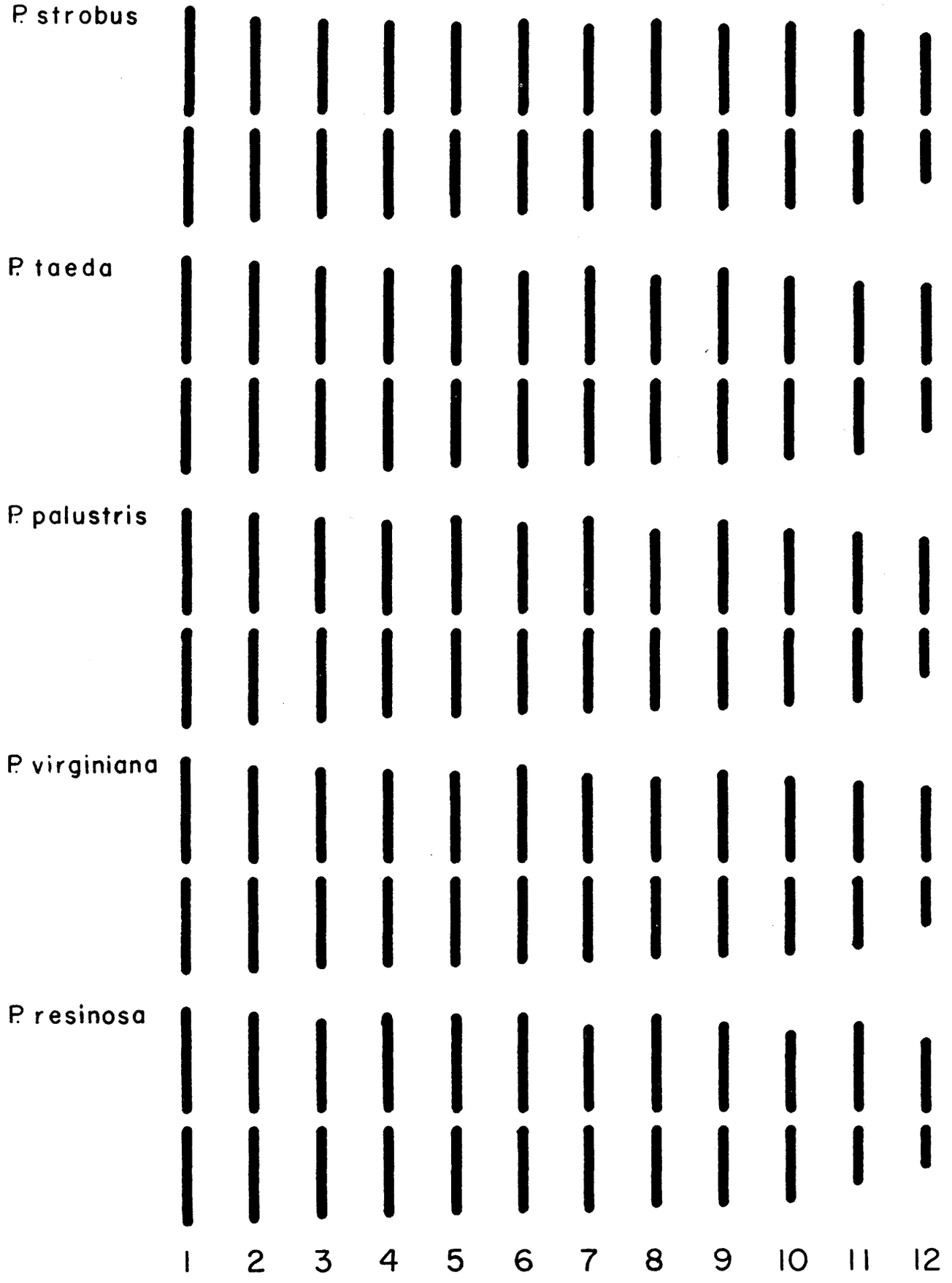

Figure 3. — Idiograms of five species of *Pinus*. Chromosomes arranged in descending order of length of the shorter arm.

The following values were obtained:

Arm	s (in mm.)
a	0.240
b	0.175
a+*b*	0.363

These data provide an estimate of the variation introduced during the interpretation, drawing, and measurement of the chromosome arms. A combined error of less than 0.37 mm. (C. V. = 2.7%) indicates that this phase of the method is very accurate.

The second method was similar to the first except a more inclusive estimate of the error was provided. The difference in arm lengths between members of a given chromosome pair (pair no. 12) was used to estimate the total variance $s^2 = \frac{s_D^2}{2}$ of the *a* and *b* arm lengths. The chromosomes of the twelfth pair were chosen because they are easily recognized in all species.

The following values were provided by this method:

Arm	s (in mm.)
a	0.403
b	0.479

This test evaluates the variation that results from the three factors previously mentioned, plus that caused by stretching and differential contraction of the chromosome arms. The small error term (0.48 mm.) again confirms the precision of this procedure. It indicates that two-thirds of the time the measured values are not expected to vary more than the width of a pencil line because of errors introduced by the techniques used in determining the arm lengths. The coefficient of variation, often a more meaningful statistic, indicates that this error is approximately four per cent. According to these estimates, therefore, the total error introduced by the procedures has been controlled as well as is practicable.

Results and Discusion

One of the first problems encountered in this experiment involved determining the magnitude of the intraspecific karyotypic variation and thus the number of plates needed to assess correctly a given species. *Pinus taeda* was examined most thoroughly for intraspecific variation. Forty-eight plates from twenty seedlings of four different geographical areas were studied. The data represent only thirty-four plates from sixteen seedlings, however, because the plates drawn while the projection techniques were first being tested were considered inaccurate and were discarded. As no intraspecific variation was detected, a total of twelve plates from at least four seedlings was considered adequate for this analysis.

The numerical values *(Table 1)* used in making the idiograms *(Figure 3)* represent mean values from at least 12 plates. The data provide no critical information about actual differences in chromosome lengths that possibly exist among the various species. In this study these differences can most likely be attributed to unequal pretreatment periods. To present the chromosomes on a standard basis, therefore, the chromosomes of a given species are expressed in the idiograms as percentages of the longest chromosome in the complement.

As the photomicrographs *(Figures 1 and 2)* and idiograms *(Figure 3)* show, the chromosomes within any given karyotype appear similar, except for the smallest chromosome. They show a gradual decrease in size, and possess either median or submedian centromeres. (Chromosomes with submedian and median centromeres are delimited by short-arm : long-arm ratios between .500—.750 and .750—1.00 respectively.) These results are in agreement with the basic karyotype presented for the genus *Pinus* by Sax and Sax (1933), with the smallest chromosome being the heterobrachial chromosome described by these authors. This chromosome noticeably interrupts the size sequence, and can be readily recognized in all species studied.

The karyotypes of all the species are much alike, except that one differs from the others by a single chromosome. *Pinus resinosa* possesses a second chromosome with an obvious submedian centromere *(Figure 2)*. As shown in *Table 1,* this chromosome has a short-arm : long-arm ratio of 0.664 compared to 0.875 for the corresponding eleventh chromosome of the species *(P. strobus)* having the next smallest ratio. Although it is the second smallest chromosome, it is sufficiently larger than chromosome number 12 to be easily recognized.

The relative lack of karyotypic variation between species is not surprising in view of the results of previous studies. As the following comparisons show, however, the detectable variation is less than was anticipated at the outset of this investigation.

Pinus taeda and *Pinus palustris* occur together in the *Australes* group of the section *Diploxylon* (Shaw, 1914). Although morphologically distinct, these two species cross freely in nature forming fertile hybrids. This suggests a

Table 1: — Mean arm lengths (in. mm.) of chromosomes of five species of pine. (Values obtained from projection drawings 2000 ×.)

	P. strobus			*P. taeda*			*P. palustris*			*P. virginiana*			*P. resinosa*		
Position	Arms			Arms			Arms			Arms			Arms		
	a	*b*	*a*/*b*	*a*	*b*	*a*/*b*	*a*	*b*	*a*/*b*	*a*	*b*	*a*/*b*	*a*	*b*	*a*/*b*
1	13.9	14.8	.939	14.7	15.8	.930	13.5	14.1	.957	12.7	13.7	.927	16.4	17.1	.959
2	13.3	13.8	.964	14.2	15.3	.928	13.0	13.7	.949	12.3	12.7	.968	15.7	16.5	.951
3	12.7	13.6	.934	14.0	14.7	.952	12.7	13.2	.962	11.9	12.3	.967	15.1	15.5	.974
4	12.6	13.1	.962	13.7	14.3	.958	12.1	12.6	.960	11.7	12.1	.967	14.7	16.2	.907
5	12.3	12.9	.953	13.5	14.8	.912	12.0	13.2	.909	11.5	11.8	.975	14.5	16.0	.906
6	12.1	13.5	.896	13.4	14.0	.957	11.8	12.3	.959	11.3	12.6	.897	14.3	16.5	.867
7	11.9	12.5	.952	13.2	14.7	.898	11.7	13.2	.886	11.1	11.6	.957	14.0	14.3	.979
8	11.6	13.4	.866	13.0	13.5	.963	11.4	11.6	.982	10.8	11.2	.964	13.4	16.1	.832
9	11.5	12.5	.920	12.7	14.4	.882	11.2	12.6	.889	10.5	11.9	.882	12.9	14.8	.872
10	11.2	12.7	.882	12.3	13.0	.946	10.9	11.6	.940	10.2	11.0	.927	12.4	13.3	.932
11	10.5	12.0	.875	11.5	12.5	.920	10.2	10.8	.944	9.7	10.6	.915	9.7	14.6	.664
12	7.8	11.3	.690	7.9	12.2	.648	6.9	10.6	.651	6.7	9.8	.684	7.0	11.9	.588

high degree of genetic compatibility, so the agreement in their karyotypes, as shown in *Figure 3,* is not unexpected.

Data are also available from limited observations of another species of *Australes, Pinus echinata* MILL. (shortleaf pine). Several plates of this species were drawn and measured while testing the projection equipment. These data were not considered sufficiently accurate to be presented with the other results, but they do indicate that the karyotype of this species closely resembles that of loblolly and longleaf pine.

Pinus virginiana is another member of the hard pine section and is placed by SHAW in the *Insignes* group. No natural hybrids between this species and any member of the *Australes* group have been reported in the literature, although field workers have suggested that natural hybridization possibly occurs between *Pinus virginiana* and *Pinus echinata.* This infrequent formation of hybrids suggests that partial genetic barriers may exist between species of the *Australes* and *Insignes* groups. As can be seen from *Figure 3,* however, the karyotypes of *P. taeda, P. palustris* and *P. virginiana* are quite similar.

The most surprising result of this study is the similarity of the karyotypes of *Pinus strobus,* a member of the *Strobi* group of the section *Haploxylon,* and the species of *Diploxylon.* The separation of the hard pines from the soft pines is based on convincing morphological and anatomical characteristics. A strong genetic barrier also seems to exist, because natural or artificial hybrids have never been reported between species of these two sections. Because of this genetic isolation and the known variation in other characteristics, karyotypic divergence was considered most likely between species of these two sections. However, no distinctive karyotypic feature was found to differentiate the two sections. Even though these findings were not expected, it should be noted that studies in other disciplines occasionally produce comparable results. In his chemical analyses, MIROV (1953) found no sharp distinction in the composition of the turpentines of these two sections.

Pinus resinosa, a species assigned to SHAW's *Lariciones* group of the *Diploxylon* section, was the only species that showed obvious divergence from the general karyotypic pattern. This fact is quite significant because it indicates that karyotypic information may still be helpful in determining species relationships within a section, even though the sections themselves are not karyotypically distinct.

The basic similarity in length and centromere position shown by the chromosomes of the five species studied is in general agreement with earlier investigations. This evidence again suggests that structural rearrangement of the chromosomes has not been a major process in the evolution of this genus. This does not mean that it has not occurred, however, but rather that detectable rearrangements seem to have taken place infrequently.

The symmetry of the chromosomes within any given karyotype found in this study also supports the concept that long-lived perennials occupying relatively stable habitats should be characterized by sexual reproduction, cross-fertilization, relatively high chromosome numbers, and free recombination of genes (STEBBINS, 1958). Pines possess these traits, and their numerous, similar chromosomes are a feature consistent with the high recombination index postulated for plants occupying closed, stable habitats.

More subtle differences, than have thus far been discussed, possibly exist between the karyotypes of the five species studied, but the small magnitude of the variation makes their validity rather doubtful at this stage. If the individual chromosomes are arranged according to a descending order of the *a* arm lengths, the lengths of the *b* arms do not form a continuous descending sequence. Some of the *b* arms are longer than the arms in preceding positions and, therefore, irregular patterns are obtained.

The *b* arm patterns and the location of the chromosomes with the most median and submedian centromeres (all possible diagnostic features of the karyotype) are presented in *Table 2.* According to this analysis, four different karyotypes exist. The karyotypes of *P. taeda* and *P. palustris* agree in all three features and thus can be considered similar. The others, however, appear to differ sufficiently from this type and among themselves to be regarded distinctive.

Table 2: — Karyotypic patterns obtained by arranging the individual chromosomes according to a descending order of the shorter *(a)* arm lengths. Data obtained from *Table 1.* (See text for further explanation.)

Species	Position		
	Chromosomes with most median centromere	Chromosomes with most submedian centromere	Exceptions to the descending order in *b* arm lengths
P. strobus	2,4	12	6,8,10
P. taeda	8	12	5,7,9
P. palustris	8	12	5,7,9
P. virginiana	5	12	6,9
P. resinosa	3,7	11,12	4,6,8,11

The value of the above classification is obviously limited, because of the similarity in lengths of *a* arms across several positions in a given species. Three and four positions may sometimes be included in a range of 0.5 mm., and the statistical analysis indicated the error term to be approximately this large. In such a situation, therefore, it is incorrect to assume these positions can be positively delimited from each other. The data have been presented with this in mind; the only basis for the arrangement being that the above patterns were found in more than fifty per cent of the plates examined for a given species.

Even though the karyotypes of four species (*P. taeda, P. palustris, P. virginiana,* and *P. strobus)* can be made nearly identical by slightly rearranging the order of the chromosomes, there is little doubt that *P. resinosa* possesses a distinctive karyotype when compared to the others. Because of the additional heterobrachial chromosome, this karyotype can be immediately recognized. It will be extremely interesting to see if all of the members of the *Lariciones* group contain karyotypes similar to *P. resinosa;* such a survey is now in progress. Although the study has just been initiated, the writer has examined plates of three other members of this group, *Pinus nigra* ARNOLD (Austrian pine), *Pinus thunbergii* PARL. (Japanese black pine), and *Pinus densiflora* S. & Z. (Japanese red pine), and all appear to possess the second submedian chromosome characteristic of the karyotype of *P. resinosa.*

It is possible to use the number and location of secondary constrictions as a source of additional karyotypic information on species differences (STEWART, 1947), and such an attempt was made in this study. The results were rather ambiguous, however, because the pattern of constriction location was inconsistent in all of the species studied.

The sporadic appearance of the secondary constrictions may be the result of several confounding factors. Clarity of secondary constrictions varied from plate to plate and even between chromosomes of a given plate. In some cases

the constrictions were easily recognized, but borderline achromatic discontinuities appeard in the arms in sufficient numbers to make interpretation difficult and uncertain. As a result some plates appeared to have few, if any, while others had one and sometimes two constrictions in nearly every arm *(Figure 1)*. Such inconsistent results might be attributed to the pretreatment, since excessive contraction of the chromosomes during the oxyquinoline treatment could obscure the smaller achromatic regions and thus lead to inconsistent observations.

Artifacts, caused by breaking the chromosome arms, could also cause the ambiguity regarding constrictions, so attempts were made to determine the likelihood of causing breaks in the chromosome arms during the microtechnical procedure. Pressure tests were conducted, and the results indicated that the chromosomes tended to stretch or separate at the centromere region (and other obvious achromatic regions, if present) before the arms would break.

Smears were also made of fresh, untreated material to study the chromosomes in an uncontracted condition. Fixation in alcohol-acetic was omitted, but the root-tips were hydrolyzed in 1 N HCl. Three different stains, aceto-carmine, aceto-orcein, and Feulgen were used, and in all three cases clear achromatic areas were evident in several chromosome arms. Because of the size of several of these regions, there is little reason to doubt that they exist. What remains to be determined, however, is the constancy of their position and their possible functions.

The situation in *Pinus* may be comparable to that found in *Polygonatum* (Solomon's-seal). THERMAN-SUOMALAINEN (1949) found a wide variation in both the thymonucleic acid charge and the degree of spiralization of secondary constriction regions in the species of this genus. She concluded that these regions tend to be very labile both as to staining and contraction reactions.

It is also worth noting that cases of normal relational coiling were observed in several of the chromosome arms of the untreated material. If this coiling persisted during the contraction phase of the pretreatment, the crossed chromatids might possibly cause a faint achromatic discontinuity to appear in the shortened chromosome arm.

The lack of observational repeatability concerning the secondary constriction regions may possibly be caused by inadequate techniques. More importantly, however, these results tend to indicate that a more thorough study of secondary constriction behavior is needed before these achromatic regions can be used unreservedly as a diagnostic feature of the karyotype in the genus *Pinus*. This seems especially pertinent since secondary constrictions are often described as part of the karyotype on the basis of very few observations.

Summary

Microtechniques, using a prolonged pretreatment in oxyquinoline, were developed which consistently shorten and clarify the morphology of the long chromosomes characteristic of the genus *Pinus*. A method of projection drawing that combines speed with accuracy was devised for use at high magnification.

A detailed karyotypic analysis, based on relative arm lengths, is presented for five species of pine. The number and position of secondary constrictions have thus far proved unreliable as diagnostic features of the karyotypes.

No intraspecific variation was detected in any of the species. Although four species were shown to have remarkably similar karyotypes, positive evidence of interspecific variation was found. The haploid karyotpe of *P. resinosa* differs from the others by possessing two heterobrachial chromosomes instead of one.

Zusammenfassung

Titel der Arbeit: *Eine Kernanalyse bei einigen ausgewählten Kiefernarten.*

Durch eine längere Vorbehandlung mit Oxyquinolin wurden die für die Gattung *Pinus* charakteristischen langen Chromosomen erheblich verkürzt und ihre Morphologie aufgeklärt. Es wurde eine Projektionszeichenmethode entwickelt, die auch bei starken Vergrößerungen schnell zu genauen Ergebnissen führt.

An 5 Arten der Gattung *Pinus* wurden detaillierte Kernanalysen auf der Grundlage der relativen Länge der Chromosomenarme durchgeführt. Dabei erwiesen sich für den Untersuchungsbereich die Anzahl und Lage der sekundären Einschnürungen als diagnostisch ungeeignete Merkmale der Kerntypen.

An keiner Art wurde eine intraspezifische Variation der Chromosomenform festgestellt. Dagegen konnte trotz großer Ähnlichkeit der Kerntypen von 4 Arten ein positiver Beweis für eine interspezifische Variation gefunden werden: *Pinus resinosa* besitzt im Gegensatz zu den anderen Arten statt nur ein heterobrachiales Chromosom deren zwei.

Résumé

Titre de l'article: *Analyse caryologique de quelques espèces de pins.*

La mise au point de microtechniques basées sur un traitement prolongé de l'oxyquinoleïne a permis d'abréger et de simplifier la morphologie des caractères des chromosomes longs du genre *Pinus*. Une méthode rapide de dessins par projection permet l'emploi de forts grossissements.

Une analyse détaillée du noyau basée sur la longueur relative des branches des chromosomes est présentée pour 5 espèces de pins. Le nombre et la position des constrictions secondaires s'est révélé un caractère peu sûr pour l'étude des noyaux.

On n'a trouvé dans les espèces étudiées aucune variation intra-spécifique. Bien que 4 espèces aient des noyaux remarquablement semblables, on a pu mettre en évidence des différences interspécifiques. Le noyau haploïde de *Pinus resinosa* diffère des autres par le fait qu'il possède deux chromosomes heterobrachiaux au lieu d'un.

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Effect of X-rays on Seeds of Scots Pine from Different Provenances (Pinus silvestris L.)

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Introduction

The experiment reported in this paper was undertaken to obtain information about the radiation sensitivity of pine seeds from different provenances.

Variation of radiation sensitivity among strains within particular species has already been studied in some agricultural plants. Thus, JOHNSSON (1933) found a greater sensitivity to radiation in the red and the white varieties of *Atriplex hortensis* than in the green ones. SMITH (1942) discovered a mendelian factor decisive for the sensitivity of *Triticum monococcum* to X-rays as expressed by seedling growth and survival. MÜNTZING (1941) concluded in his experiment with autotetraploid and diploid *Hordeum distichum* that "the autotetraploid line is more resistant to X-rays because it has four quite homologous genomes instead of only two as in the diploid". The genetic damages in a chromosome of the tetraploid barley can therefore be counterbalanced by a normal condition in the other homologous chromosomes. TEDIN and HAGBERG (1952) found that the X-ray sensitivity differed between the sweet and the bitter lupine, *Lupinus luteus*. GREGORY (1956 a and b) observed a variation in sensitivity to X-rays in experiments with *Arachis hypogea*. SARIĆ (1957) reported the tetraploid form of *Petkus winter* rye to be less sensitive to X-rays than the diploid one, and LAMPRECHT (1956, 1957) described certain pea varieties which were more sensitive to X-rays than others. GELIN *et al.* (1958) published a report concerning genetically conditioned influences on the radiation sensitivity of peas. They found that the reaction to radiation is determined by the moisture content of the seeds, germination temperature, and other factors important for plant growth. Recently ŠMÁLIK *et al.* (1960) have found variation in the X-ray sensitivity of four potato varieties. The authors stated that long established varieties (date of origin) and varieties with high protein content are more sensitive to X-rays. LAFFÈRS (1960) stated that germinant seedlings of *Picea abies* from high elevations were more resistant to gamma rays than seedlings of lowland origin, i. e. principally a result similar to that of this experiment.

Table 1. — Description of the material

No.	Provenance	Altitude (m)	Latitude	No. trees
1	Kojkul	175	66°20'	10
2	Bölensberget	398	63°55'	10
3	Hissjö	90	63°55'	8
4	Olingsjön	490	61°45'	10
5	Ovanåker	300	61°20'	4
6	Sunne	150	59°50'	9
7	Sanda	54	59°10'	8
8	Långenäs	75	57°40'	8
9	Byarum	295	57°30'	8
10	Bjärges	45	57°25'	8

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Material and methods

Pine seed lots (*Pinus silvestris* L.) representing ten provenances in various parts of Sweden were used. *Table 1* describes the origin of these seeds and presents the number of harvested trees in each provenance.

After collection in the autumn of 1959, the cones were sent to the Forest Research Institute where the seeds were extracted. Since the radiation sensitivity of pine seeds depends on the developmental stage of the embryo (GUSTAFSSON and SIMAK, 1958), only fully developed seeds were used (IV A). Seeds from each of the provenances were examined and divided into embryo classes by the X-ray photo method (SIMAK and GUSTAFSSON 1953).

Table 2 shows that embryo class IV predominates in all the seed lots. Thus, the selective use of these seeds provides samples that rather faithfully represent the population stuctures. The high percentage of well developed seeds in northern Sweden is unusual (cf. GUSTAFSSON and SIMAK 1956), and it was probaly caused by the exceptionally good conditions for seed ripening which prevailed in this part of the country in 1959 (cf. EHRENBERG *et al.* 1955).

Since the moisture content of seeds determines their radiation sensitivity (EHRENBERG 1955, GUSTAFSSON and SIMAK 1958), it was standardized by equilibrating the seeds for one week with an air current of 45 per cent relative humidity, obtained by passing the air through 9.8 molar KOH.

Table 2. — Analysis of the seed material

No.	Weight of 1000 seeds in class IVA	Embryo and endosperm spectrum, per cent								
		Empty seeds	IA	IIA	IIIA	IVA	IIB	IIIB	IVB	Abnorm. seeds
1	3.602	28	1	1	3	64	—	—	1	2
2	4.177	6	1	10	18	61	3	—	—	1
3	3.399	9	1	2	3	79	1	1	4	—
4	5.139	13	—	2	5	77	—	—	1	2
5	4.600	20	1	2	2	74	—	—	1	1
6	5.103	1	—	—	2	97	—	—	—	—
7	3.693	5	—	1	1	90	—	—	1	2
8	4.968	4	—	—	1	94	—	—	1	—
9	4.823	7	—	—	3	90	—	—	—	—
10	3.469	16	—	4	3	69	1	2	5	—