seedlings per progeny. This improvement, along with prevention of patchy inoculation, would go far toward increasing overall reliability of mixed-pollen crosses.

Summary

Recults and analyses given in this paper show that mixed-pollen crosses which involve 10 or more pollens can be used to obtain relatively reliable estimates of general combining ability and breeding value of blister rust resistant plus trees. Mixed-pollen cross results fell within ± 3 percent of those obtained with more conventional tests using four individual tester crosses, in 95 out of 100 cases. This level of accuracy is low by experimental standards, but it is perfectly adequate for large-scale, practical plus tree testing. Prevention of patchy inoculation of progeny test plots, as well as an increase in numbers of plots tested in mixed-pollen cross progenies, would further improve accuracy.

Literature Cited

BARTLETT, M. S.: The square root transformation in analysis of variance. J. Roy. Statist. Soc. Suppl. 3: 63—78, with 2 p. 73 (1936). — BARTLETT, M. S.: The use of transforrnations. Biometrics 3: 39—52 (1947). — BINGHAM, R. T.: Breeding blister rust resistant western white pine. III. Comparative performance of clonal and seedling lines from rust-free selections. Silvae Genet. 15: 160—164 (1966). — BINCHAM, R. T., SQUILLACE, A. E., and DUFFIELD, J. W.: Breeding blister rust resistant western white pine. J. Forestry 51: 163—168 (1953). — BINGHAM, R. T., SQUILLACE, A. E., and WRIGHT, J. W.: Breeding blister rust resistant western white pine. II. First results of progeny tests including preliminary estimates of heritability and rate of improvement. Silvae Genet. 9: 33—41 (1960). — FREESE, FRANK: Testing accuracy. Forest Sci. 6: 139—145 (1960). — GODDARD, R. E., PETERS, W. J., and STRICKLAND, R. K.: Cooperative forest genetics research program. Fourth Progress Rep. Univ. Fla. Sch. Forest. Res. Rep. 7, 16 pp. (1962). — SNEDECOR, GEORGE W.: Statistical methods applied to experiments in agriculture and biology. 4th Ed. Iowa State Coll. Press, Ames, Iowa, 485 pp. (1946). — YATES, F.: Contingency tables involving small numbers and the χ^2 test. J. Roy. Statist. Soc. Suppl. 1: 217—235 (1934).

A Comparative Karyotype Analysis of Pseudotsuga menziesii (Mirb.) Franco, and Pseudotsuga wilsoniaiia (Hayata)

By GERALD THOMAS and KIM K. CHING[1])

(Received for publication June 27, 1967)

On a morphological scale, the karyotype of a species can be characterizod according to number, total length of the individual chromasomes comprising the set, and position of primary and secondary constrictions. The ~karyotypeof a species is mostly a fixed character, but over a long time karyotypes undergo gradual evolution. Moreover, different species within a genus frequently have visibly different karyotypes; comparisons of chromosome morphology between related species can yield valuable information on the processes of evolution and serve as valuable tools to the taxonomist (SWANSON 1964).

Six species are currently recognized in the genus Pseudotsuga (DALLIMORE and JACKSON 1948, GÖHRE 1958, LI 1953). These are Douglas-fir (P. menziesii [MIRB.] FRANCO), bigcone Douglas-fir (P. macrocarpa MAYR), Japanese Dovglas-fir (P. *japonica* BEISSNER), Formosan Douglas-fir (P. wilsoniana HAYATA), Chinese Douglas-fir (P. *sinensis* DODE), and Forest's Douglas-fir (P. forrestii CRAIB). Among these species, karyotypes have been reported on only two of them, P. menziesii and P. macrocarpa. The present study is of the karyotypes of P. wilsoniana and P. menziesii and presents a comparative karyotype analysis for all three species.

Review of Previous Work

The first major publication on chromosome morphology among the conifers was by KARL and HALLY SAX in 1933. They found a basic number of n = 12 for most of the specimens they studied, with two exceptions in the Pinaceae, Pseudolarix, with n = 22, and Pseudotsuga, with n = 13. They attributed the extra chromosome in Pseudotsuga, which was apparently telocentric, to segmental interchange and duplication. Besides the telocentric chromosome, they found six metacentric chromosomes and six submetacentric chromosomes.

ZENKE (1953) studied meiosis in F. menziesii var. viridis (= menziesii). She reported 13 ibivalents at diakinesis of meiosis, which confirmed the findings of SAX and SAX (1933). Five pairs of bivalents were large with apparently median to submedian centromeres; six pairs were smaller and apparently had subterminal centromeres. The exact classification of the two smallest pairs could not be accurately discerned.

ANDREE DURRIEU-VABRE (1958) found n = 12 chromosomes in root tip metaphases of *Pseudotsuga douglasii* (= menziesii). She expressed each chromosome in terms of a ratio (R) in which the numerator was the value of the shorter arm and the denominator that of the langer arm. Her results showed that ratios of three chromosome pairs were 1 : 1; of two pairs, 3 : 4; of one pair, 2 : 3; of four pairs, 1 : 2; and of two pairs, 1 : 3.

BARNER and CHRISTIANSEN (1962) published findings on the chromosomes of P. menziesii var. *viridis*. They reported a haploid number 13, which agreed with the findings of SAY and SAX (1933) and ZENKE (1953). The 13 chromosomes consisted of five approximately isobrachial chromosomes, six heterobrachial chromosomes, and two apparently telocentric chromosomes. Their examinations were made on both meiotic and mitotic figures, and, at times, they observed meiotic irregularitios of pairing and of anaphase separatiori. These irregularities did not occur with great frequency, and most cells were normal.

CHRISTIANSEN (1963) studied root-tip mitoses of P. macrocarpa and reported 2n = 24 for that species. The karyotype included six pairs of metacentric chromosomes and six pairs with subterminal centromeres.

Materials and Methods

Two seed lots, one of Douglas-fir and one of Formosan Douglas-fir, were used for all investigations made. Both seed lots were from single-tree collections. Seeds of Douglas-fir came from a tree near Corvallis, Oregon; seeds of

[1]) The authors are, respectively, graduate assistant and Forest Geneticist, Forest Research Laboratory, School of Forestry, Oregon State University, Corvallis.

Formosan Douglas-fir were from a collection made at Ta Chia Chi, Taiwan, and were obtained through the courtesy of the Taiwan Forestry Bureau.

Pretreatment

Radicles of the germinating seedlings were cut and placed in a 0.1 percent solution of Lindane for 4—6 hours to shorten the chromosomes and to break down the spindle apparatus.

Root tips were transferred to FARMER's fixative (3 : 1, absolute alcohol : glacial acetic acid) for about 24 hours then were hydrolyzed in 1 *N* hydrochloric acid for 10—15 minutes at 60° C.

Next steps were to soak the root tips in FEULGEN stain for 30—60 minutes then to put them into 45 percent acetic acid for 10 minutes to further soften the tissue and to set the stain. The material was then transferred to a microscope slide, and, after a drop of acetocarmine had been added, was squashed.

Measurement of Chromosomes

The slides were examined for cells that had mitotic chromosomes well spread and, as nearly as possible, in one plane. Appropriate cells were photographed at 1,450 ×magnification *(Figures 1 and 2)*, then printed so that the longest chromosomes were about 5 centimeters long. The enlarged print was closely compared with the original cell viewed through the microscope so that each chromosome on the print could be outlined in ink to show such details as the position of the centromeres, location of secondary constrictions, and location of ends that might be hidden through overlapping.

The actual measurements of the chromosomes were made as follows:

The chromosomes on a given print were numbered (1—24 for Formosan Douglas-fir and 1—26 for Douglas-fir).

The measurements were made from ends of the chromosome arms using an engineer's scale (60 units to the inch), but the centromere regions were not included in the measurements. Furthermore, any curved chromosome arms were measured along a series of straight lines tangent to the arc described by the curve. The longer of the two arms of a given chromosome was designated as the "a" arm; the shorter, the "b" arm (SAYLOR 1961). After all chromosomes of a print had been measured, homologous pairs of chromosomes were chosen from examination of the original print and matching of similar arm lengths. Secondary constrictions were used as guides to pairing whenever possible.

Average values for total length and for lengths of the arms were obtained for each homologous pair.

After an average length had been computed for each pair of chromosomes, a relative value was estimated to permit comparisons between cells. Total relative lengths were obtained by computing average chromosome length for a given cell and relating absolute lengths to this standard.

$$\text{Average chromosome length} = \frac{L1 + L2 + \ldots + L12 + L13}{13}$$

$$\text{Relative length} = \frac{\text{Absolute length}}{\text{Average length}}$$

The relative lengths of long and short arms of each chromosome pair were obtained by computing percentage of total absolute length that the long and short arms represented and multiplying this percentage by total relative length previously computed.

The chromosomes were arranged in order of descending values of relative length with their centromeres on a horizontal line and their short arms directed upward.

With the criteria of SIMAK (1962), it was possible to classify the chromosomes of both Douglas-fir and Formosan Douglas-fir by location of centromeres in median, submedian, or subterminal positions. Ratios of short to long arms for chromosomes with median centromeres ranged from 0.75 to 1.00. Ratios for chromosomes with submedian centromeres ranged from 0.50 to 0.75, and ratios for those with subterminal centromeres were 0.50 or less.

Figures 1 and 2. — Microphotographs of the chromosomes. — *On the left:* Douglas-fir. — *On the right:* Formosan Douglas-fir.

Results of Karyotype Analysis

Diploid number. — Literature reviews on the chromosome morphology of Douglas-fir show that a controversy exists regarding the haploid number of chromosomes possessed. Data from the present study of squashes of root tips from *P. menziesii* var. *menziesii* support the findings of BARNER and CHRISTIANSEN (1962). Chromosome counts made on 50 cells distributed over 40 plates revealed a somatic number of 2n = 26 chromosomes. No other numbers were observed, except in a few instances when chromosome arms were broken during preparation of the material. Furthermore, measurements of chromosomes from nine cells distributed over seven slides revealed five chromosome pairs with approximately median centromeres, six pairs with definite subterminal centromeres, and four chromosomes that appeared to be telocentric.

Chromosome counts made on 30 cells distributed over 18 slides gave a somatic number of 2n = 24 chromosomes for Formosan Douglas-fir. Again, no exceptions to this number were observed. The karyotype of this species, as determined from 19 cells distributed over seven slides, was similar to that observed in bigcone Douglas-fir by CHRISTIANSEN (1963). There were six chromosome pairs with median to submedian centromeres and six pairs with subterminal centromeres.

Relative unit lengths of chromosomes. — *Table 1* gives the mean relative unit lengths of the paired chromosomes of Douglas-fir and Formosan Douglas-fir respectively. The relative lengths of the various chromosomes were nearly constant from cell to cell for both species as shown by analysis of variance.

For Douglas-fir, the largest differences in relative lengths between neighboring chromosomes occurred between chromosomes 5 and 6 (21 units) and chromosomes 12 and 13 (12 units). Differences between other neighboring chromosomes could not be readily discerned on the basis of relative length alone. As pointed out by SIMAK (1962), when differences in length between neighboring chromosomes are small, sometimes the longer of two chromosomes will appear to be shorter than its neighbor because of differential contraction and bending. A chromosome reversal is said to have occurred; the result is erroneous classification by length. Unless the probability of this event is considered, small differences in relative lengths cannot be used as accurate criteria for identification. In instances where there is no chance of a chromosome reversal taking place, differences in relative lengths may be used for identification. For example, one can readily differentiate between chromosomes 1 and 3 on the basis of relative lengths because of gross difference in length between the two chromosomes (16 units) and little chance of a reversal occurring.

Differences are largest between chromosomes 1 and 2 (8 units) and 6 and 7 (24 units) in Formosan Douglas-fir. Again, differences between neighboring chromosomes are not large enough to be of value for identification.

Position of centromere. — Douglas-fir has five chromosome pairs with median centromeres, six pairs with subterminal centromeres, and two pairs with centromeres that appear to be located terminally. Formosan Douglas-fir, on the other hand, has six chromosome pairs with median centromeres and six pairs with subterminal centromeres.

Occurrence of constrictions. — If secondary constrictions occur with regularity in a given chromosome, they may be used for diagnostic purposes. Secondary constrictions were found in certain chromosomes of both Douglas-fir and Formosan Douglas-fir. Some of these constrictions appeared in every cell examined and can be considered to be characteristic features of the chromosomes on which they occur.

In Douglas-fir, chromosome 3 of the haploid set had a secondary constriction in the distal end of one of its arms. Since the constriction appeared to be in the longer arm half the time and in the shorter arm half the time, an arm reversal was taking place because differential contraction and bending may cause one arm to appear shorter than the other arm. It was, therefore, impossible to determine in which arm the constriction occurred. The satellite formed by the constriction made up 19 percent of the total length of the chromosome *(Figure 3)*.

In addition to the constriction found on chromosome 3, a secondary constriction was found on chromosome 10 of the haploid set of Douglas-fir. This constriction did not appear in all cells examined but occurred about 70 percent of the time and, therefore, may be taken as real and not artifact. The constriction may be the same one seen by BARNER and CHRISTIANSEN (1962) in their work on Douglas-fir. It is very close to the centromere, and a small piece of chromatin, comprising about 6 percent of the total length of the chromosome, is isolated between the constriction and the centromere.

A diffuse constriction was seen infrequently in chromosome 11 of Douglas-fir in the long arm approximately 22 units from the centromere. It was, however, seen in only five cells out of 50 examined, and, if it is not an artifact it does not occur with enough regularity to be of diagnostic use.

Table 1. — Comparisons of the relative unit lengths of the chromosomes of Douglas-fir as determined by several investigators.

Author	Chromosome number in haploid set												
	1	2	3	4	5	6	7	8	9	10	11	12	13
						Douglas-fir							
SAX and SAX (1933)	148	135	123	121	111	108	96	91	81	81	79	69	54
DURRIEU-VABRE (1958)	126	121	120	114	111	108	99	98	88	87	84	45	—
BARNER and CHRISTIANSEN (1962)	139	135	128	123	122	95	90	89	86	86	76	76	56
THOMAS and CHING (1966)[1])	144	136	128	128	120	99	92	87	83	80	77	69	57
						Formosan Douglas-fir							
THOMAS and CHING (1966)[1])	136	128	124	121	119	112	88	82	78	74	71	66	—
						Bigcone Douglas-fir							
CHRISTIANSEN (1963)	128	124	122	120	114	110	89	85	85	78	75	74	—

[1]) Present study.

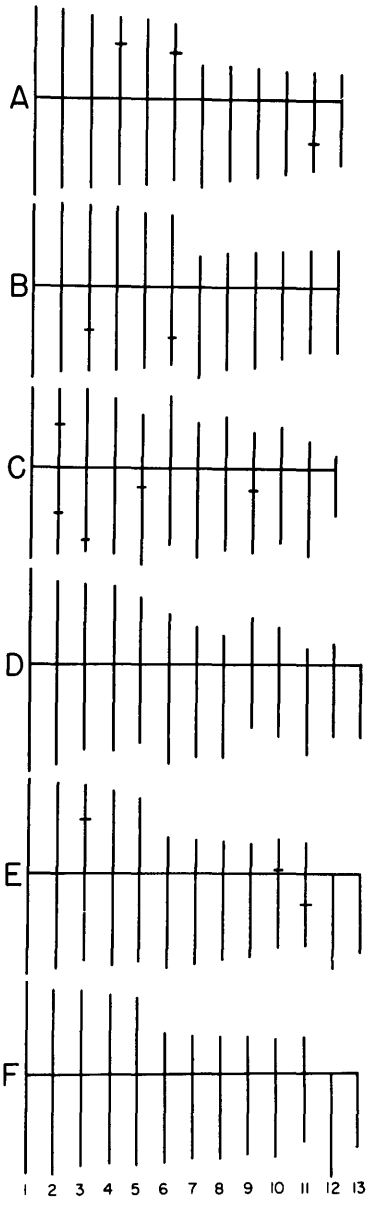

Figure 3. — Haploid idiograms of A, Formosan Douglas-fir by THOMAS and CHING (1966) (present study); B, bigcone Douglas-fir constructed from the diploid idiogram of CHRISTIANSEN (1963); C, Douglas-fir by DURRIEU-VABRE (1958) rearranged according to decreasing relative lengths of chromosomes; D, Douglas-fir by SAX and SAX (1933) rearranged according to decreasing relative lengths; E, Douglas-fir by THOMAS and CHING (1966) (present study); and F, Douglas-fir constructed from the diploid idiogram of BARNER and CHRISTIANSEN (1962).

Formosan Douglas-fir also had three chromosomes in its haploid set with secondary constrictions *(Figure 3)*. The first occurred in the distal end of the short arm of chromosome 4 and the satellite formed by the constriction comprised 18 percent of the total length of the chromosome. Chromosome 6 had a secondary constriction in the distal end of its short arm, and again the satellite made up 18 percent of the total length of the chromosome. While, however, the constriction in chromosome 4 appeared in every cell examined, the constriction in chromosome 6 was evident only about half the time. Chromosome 11, which is heterobrachial, very infrequently showed a diffuse constriction in the distal end of its long arm. This constriction could be clearly seen in only three cells examined.

Comparisons With Results of Other Investigators

Tables 1 and 2 show that results for Douglas-fir of the present study and those of SAX and SAX (1933) and BARNER and CHRISTIANSEN (1962) agree closely. The differences are small and can probably be attributed to errors in measurement. On the other hand, data for Douglas-fir presented by DURRIEU-VABRE (1958) differ significantly from those of the other investigators in two respects: She found 12 chromosomes in the haploid set; her measurements for chromosomes 1, 2, and 12 are much smaller than those reported by other investigators. Moreover, the near agreement between DURRIEU-VABRE's results and those of CHRISTIANSEN (1963) for bigcone Douglas-fir and of the present authors for Formosan Douglas-fir should be noted. The major discrepancy seems to be in values reported for chromosome 12.

Secondary constrictions observed in the present study on some of the chromosomes of Douglas-fir were not mentioned by SAX and SAX (1933) *(Figure 3)*. BARNER and CHRISTIANSEN (1962) reported finding a "peculiar" secondary constriction close to the centromere in chromosomes 14 and 15 in the diploid set of Douglas-fir, but did not mention others. In the present study, a constriction was found that was identical to the one described by BARNER and CHRISTIANSEN, but it occurred in chromosomes 19 and 20, which as a pair comprise chromosome 10 of the haploid set. In addition, there was a secondary constriction in the distal portion of one of the arms of chromosome 3. Finally, in a very few instances, a rather diffuse constriction in chromosome 11 was observed.

DURRIEU-VABRE (1958), who presented a haploid idiogram of Douglas-fir, reported that there were two secondary constrictions in chromosome 1 and one each in chromosomes 2, 6, and 7. The occurrence of secondary constrictions, as reported by DURRIEU-VABRE, was not, however, observed in either species in the present study.

CHRISTIANSEN (1963) showed secondary constrictions in the long arms of chromosomes 5 and 12 of the diploid set (chromosomes 3 and 6 in haploid set, respectively) of bigcone Douglas-fir *(Figure 3)*. This finding was somewhat analogous to the results of the present study for Formosan Douglas-fir, which revealed secondary constrictions in the short arms of chromosomes 4 and 6 of the haploid set. The fact that in the former species the secondary constrictions were found in the long arms of the chromosomes but in the second species they occurred in the short arms may be an artifact caused by the phenomenon of arm reversals.

Discussion

A suggestion to account for the origin of the difference in chromosome numbers between Douglas-fir, bigcone, and Formosan Douglas-fir is that the extra chromosome is derived from a break in a metacentric chromosome, presumably across the region of the centromere. This suggestion is simple and accounts for the two apparently telocentric chromosomes found in Douglas-fir but does not account for instability found in metacentric chromosomes that were broken across their centromeres to give telocentrics (MCCLINTOCK 1932, RHOADES 1940). Their instability is thought to be one reason why telocentrics do not occur naturally. Furthermore, chromosomes formerly thought to be telocentric in a number of species have been shown to be acrocentric (WHITE 1945). That is, they are rod-shaped and possess a tiny arm that usually cannot be seen.

SAX and SAX (1933) speculated that segmental interchange and duplication through nondisjunction would account for

Table 2. — Comparisons of the relative unit lengths of the short arms of the chromosomes of Douglas-fir as determined by several investigators.

Author	Chromosome number in haploid set												
	1	2	3	4	5	6	7	8	9	10	11	12	13
						Douglas-fir							
Sax and Sax (1933)	69	61	59	57	37	49	27	22	34	27	12	15	—
Durrieu-Vabre (1958)	58	58	58	51	40	53	34	38	27	32	21	11	—
Barner and Christiansen (1962)	67	62	61	57	55	29	27	26	26	24	25	—	—
Thomas and Ching (1966)[1])	69	66	65	61	56	27	25	24	22	26	23	—	—
						Formosan Douglas-fir							
Thomas and Ching (1966)[1])	65	61	59	57	56	54	24	24	21	21	19	19	—
						Bigcone Douglas-fir							
Christiansen (1963)	60	61	60	59	55	53	23	25	26	26	28	28	—

[1]) Present study.

the increase in chromosome numbers in Douglas-fir. They believed that newly formed interchange individuals would be effectively isolated from the original population through gametic sterility of any heterozygotes. This belief was based on their earlier findings that conifers exhibited a prevalence of interstitial chiasmata at meiosis. Thus, meiosis of individuals heterozygous for a translocation would be hampered by the rigidity of the interchange rings because of the interstitial chiasmata. Nondisjunction would result and a consequent high degree of gametic sterility because of duplications and deficiencies.

With the ideas of Sax and Sax (1933) and those of Darlington (1937), who theorized on the increase or decrease of chromosome numbers through reciprocal translocations, a hypothesis was set up to explain the possible derivation of the present karyotype of Douglas-fir.

The hypothesis is outlined in *Figure 4*, starting in the upper left corner with a 2n idiogram of a theoretical species of Douglas-fir with 24 chromosomes (step 1 in the figure).

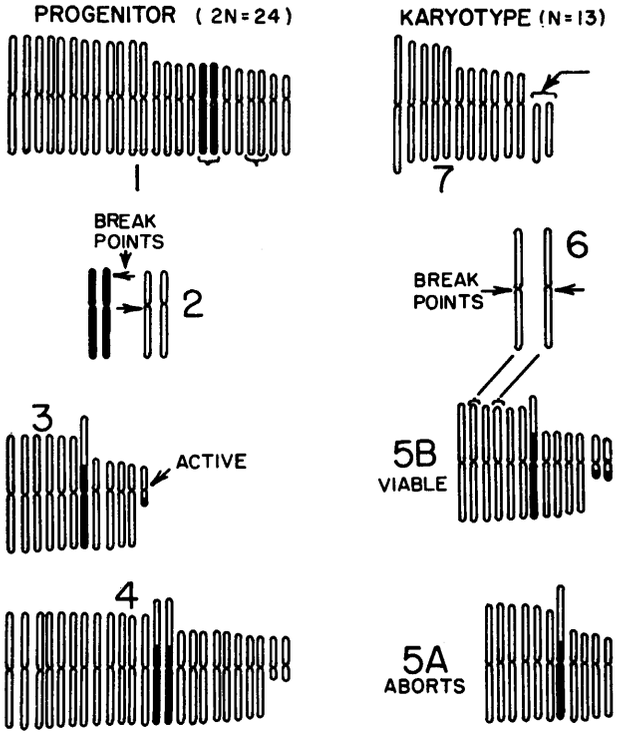

Figure 4. — Hypothesis for the derivation of the karyotype of Douglas-fir with chromosomes from a progenitor with 24 chromosomes (see text).

Suppose that a reciprocal translocation between two nonhomologous chromosomes, both with subterminal centromeres, occurs (step 2). The translocation is uneven, involving the long arm of one of the chromosomes and the short arm of the other. The result is a long chromosome with an approximately median centromere and an extremely short chromosome (step 3). One must assume that the translocation cannot persist in the heterozygous state because nondisjunction results in deficiencies and duplications. If, however, the individual with the translocation should happen to produce some viable gametes carrying the interchange types, selfing can occur, and the resulting individuals are homozygous for the translocation (step 4). As Stebbins (1957) pointed out, at meiotic metaphase the homozygous interchange type will form one very large bivalent and one extremely small bivalent. The small bivalent will very likely fail to disjoin at meiosis and both small chromosomes will pass to the same pole. This latter phenomenon was actually observed in an artificially produced chromosome race in *Crepis tectorum* by Gerassimova (1939), who was cited by Stebbins (1957). Thus, two kinds of gametes are produced, one with no small chromosomes and one with a duplication for the small chromosome. The deficient gametes can be expected to be nonfunctional if the two fragment chromosomes are genetically active (step 5 A). The gametes carrying the duplication should, however, be viable (step 5 B). In effect, a trisomic gamete has been produced. If two further translocations occur (step 6), such that one of the arms of each of the two chromosomes with median centromeres are transferred back to the two small chromosomes, the present karyotype of Douglas-fir is derived (step 7). In the latter translocations, one must assume that the breaks in the isobrachial chromosomes occur near the centromeres. This assumption is necessary to account for the two telocentric, or very nearly telocentric, chromosomes found in Douglas-fir.

The derivation of Douglas-fir outlined is only an hypothesis. Nevertheless, it seems to be a probable explanation from what is known about increase and decrease in chromosome numbers. A test that might shed more light on the evolutionary sequence of events leading to the establishment of Douglas-fir as we now know it would be to examine meiosis in crosses obtained between Douglas-fir and bigcone Douglas-fir for homologies in pairing (Ching 1959).

Abstract

Karyotype analyses have been made on only two of the six recognized species in the genus *Pseudotsuga*, Douglas-fir *(P. menziesii)* and bigcone Douglas-fir *(P. macrocarpa)*.

In the present study, a comparison was made between the karyotypes of Douglas-fir and Formosan Douglas-fir *(P. wilsoniana)*. The basic chromosome number of the Formosan Douglas-fir was found to be 12, unlike Douglas-fir, which has a number of 13. (The basic number for bigcone Douglas-fir is 12, as reported by CHRISTIANSEN in 1962.) The study confirms work of BARNER and CHRISTIANSEN in 1962 on the chromosome morphology of Douglas-fir. Of the 13 chromosomes of Douglas-fir, five have been found to have median centromeres; six, subterminal centromeres; and two, terminal or nearly terminal centromeres. Formosan Douglas-fir was found to have six chromosomes with median centromeres and six with subterminal centromeres.

Literature Cited

BARNER, H., and CHRISTIANSEN, H.: The formation of pollen, the pollination mechanism, and the determination of the most favorable time for controlled pollination in *Pseudotsuga menziesii*. Silvae Genetica **11**: 89—102 (1962). — CHING, K. K.: Hybridization between Douglas-fir and bigcone Douglas-fir. Forest Sci. 5: 246—254 (1959). — CHRISTIANSEN, H.: On the chromosomes of *Pseudotsuga macrocarpa* and *Pseudotsuga menziesii*. Silvae Genetica **12**: 124—127 (1963). — DALLIMORE, W., and JACKSON, A. B.: A handbook of *Coniferae* including *Ginkgoaceae*. 3d ed. London, Edward Arnold and Company, 570 pp. (1948). — DARLINGTON, C. D.: Recent advances in cytology. 2d ed. Philadelphia, P. Blakiston's Son and Co. 671 pp. (1937). — DURRIEU-VABRE, ANDREE: Chromosomes de *Pseudotsuga douglasii* CARRIÉRE. Comptes Rendus de l'Académie des Sciences (Paris) **246**: 3660—3663 (1958). — GERASSIMOVA, HELEN: Chromosome alterations as a factor of divergence of forms. I. New experimentally produced strains of *C. tectorum* which are physiologically isolated from the original forms owing to reciprocal translocation. Comptes Rendus (Doklady) de l'Académie des Sciences de l'URSS 25: 148—154 (1939). — GÖHRE, K. (Ed.): Die Douglasie und ihr Holz. Berlin, Akademie-Verlag, xiii+ 595 pp. (1958). — LI, HUILIN: Present distribution and habitats of the conifers and taxads. Evolution 7: 245—261 (1953). — MCCLINTOCK, BARBARA: A correlation of ringshaped chromosomes with variegation in *Zea mays*. Proc. Nat. Acad. Sci. **18**: 677—681 (1932). — RHOADES, M. M.: Studies of a telocentric chromosome in maize with reference to the stability of its centromere. Genetics 25: 483—520 (1940). — SAX, K., and SAX, HALLY: Chromosome number and morphology in the conifers. Jour Arnold Arbor. **14**: 356—375 (1933). — SAYLOR, L. C.: A karyotypic analysis of selected species of *Pinus*. Silvae Genetica **10**: 77—84 (1961). — SIMAK, M.: Karyotype analysis of *Larix decidua* MILL. from different provenances. Medd. SkogsforsknInst., Stockh. 51: 1—21 (1962). — STEBBINS, G. L.: Variation and evolution in plants. 3d ed. New York, Columbia University Press, xix + 643 pp. (1957). — SWANSON, C. P.: Cytology and cytogenetics. Englewood Cliffs, N. J., Prentice-Hall, Inc., x + 596 pp. (1964). — WHITE, M. J. D.: Animal cytology and evolution. Cambridge, Cambridge University Press, 375 pp. (1945). — ZENKE, URSULA: Untersuchungen über den Ablauf der Meiosis bei *Pseudotsuga taxifolia* Britton. Zeitschr. Forstgenet. 2: 96—102 (1953).

Early Growth of Douglas-Fir from Various Altitudes and Aspects in Southern Oregon

By RICHARD K. HERMANN and DENIS P. LAVENDER[1])

(Received for publication May 22, 1967)

High mortality of planted Douglas-fir in the southern Cascade Mountains of Oregon is of great concern to public and private forest owners. To provide a basis for production of planting stock with increased survival potential, a study was initiated in 1961 to determine whether or not topography and altitude had affected genetic differentiation of Douglas-fir in these mountains.

In 1964, plants raised from 14 lots of seed collected along an altitudinal transect were used to establish reciprocal out-plantings in the vicinity of the transect for future study. The present paper reports on investigations of growth of these trees in the nursery and in growth chambers.

Studies of genetic variation in Douglas-fir *(Pseudotsuga menziesii* [MIRB.] FRANCO), recent reviews of which have been made by WRIGHT (11), SCHOBER (7), and SWEET (10), have included in the Pacific Northwest both local differences (4, 6, 8) and differences between widely separated provenances (2, 3).

None of these studies was done in the southern Cascade Mountains of Oregon, where Douglas-fir appears to change markedly with altitude and there are pronounced differences between adjacent north- and south-facing slopes.

Seeds

Collection

Cones were collected from August 20 to September 10, 1961, on a 15- by 10-mile transect on the western slope of the Cascades in southern Oregon. The transect covered an area between 42° 40' and 42° 52' N latitude, and 122° 37' and 122° 49' W longitude. Collections were made at intervals of 500 ft in elevation, from altitudes of 1,500 to 5,000 ft, on north- and south-facing slopes *(Figure 1)*. Distances between the two slopes at each elevation varied from 2 to 6 miles. With exception of the three lowest elevations, points of collection were on opposite sides of the same ridge. Open-pollinated cones were obtained from 2 to 4 trees growing in stands at each site of collection. Distance between sampled trees ranged from 200 to 400 ft. Ages of trees from which cones were taken ranged from 30 to 40 years.

Cones from all trees harvested at each site of collection were treated as a single lot and processed together. To have equal representation of parents in each lot, 5 fresh cones were cut and full seeds counted. The quantity of cones from each tree for the bulked sample was then adjusted to yield about the same amount of seeds. After extracting and cleaning, about 95 percent of each lot was full seeds. Cleaned seeds were stored at 2° C until sowing.

Enough seeds for the study were obtained from each site except at the 2,000 ft elevation, where cones were heavily infested by insects and yielded so few seeds that they were omitted from the investigation.

Weight

Ten samples of 100 seeds from each lot were weighed after they had been stored over 40 percent H_2SO_4 at 20° C for 4 days.

Weight of seeds decreased from low to medium elevations and increased slightly again at high elevations *(Figure 2)*. Up to 3,000 ft, seed from north-facing aspects was heavier than seed from south-facing exposures; above 3,000 ft the trend reversed.

[1]) Forest Ecologist and Forest Physiologist, respectively, Forest Research Laboratory, School of Forestry, Oregon State University, Corvallis, Oregon.