Bot. Tidsskr. 39, 57–133 (1925). — HERMANN, F.: Flora von Nord- und Mitteleuropa. Stuttgart (1956).. — IVERSEN. J.: Landnam i Danmarks Stenalder. Danm. Geol. Unders. II, 6. 7–68 (1941). — JENTYS-SZAFEROWA, J.: Biometrical studies on the collective species Betula alba L. I. Inst. Rech. Forets Doman. Pologne. Warszawa (1937). — JENTYS-SZAFEROWA, J.: Biometrical studies on the collective species Betula alba L. II. item (1938). — JENTYS-SZAFEROWA, J.: Analysis of the collective species Betula alba L. Part I. Aim and method of the work on the example of Betula verrucosa EHRH. Bull. Int. Acad. Pol. sc. et lett., Cl. sc. math. et nat., Ser. B (I;). Krakau (1949). — JOHNSSON, H.: Växtförädling av björk — mål och medel. Svensk Papperstidn. 44 (1941). — JOHNSSON, H.: Triploidy in Betula alba L. Bot. Notiser 1944, 85–96. — JOHNSSON, H.: Interspecific hybridization within the genus Betula. Hereditas 31, 163–176 (1945). — JOHNSSON, H.: Progeny of triploid *Betula* verrucosa EHRH. Bot. Notiser 1946, 282–290. — JCHNSSON, H.: Studies on birch species hybrids I. Hereditas 35, 115–135 (1949). — KIRCHNER, O. v., LOEW, E., und SCHRÖTER, C.: Lebensgeschichte der Blütenpflanzen Mitteleuropas. Bd. II, 1. Stuttgart (1911). — KLAEHN, F. U.: Untersuchungen über das Artenproblem des Formerikreises Betula alba L. unter morphologisch-zytogenetischer Betrachtungsweise. Diss. Göttingen (1950). — KLAEIIN, F. U., und RUNDQUIST, E.: Die Birke. Eine Zusammenfassung der neuesten Untersuchungsergebnisse in forstbotanischer und forstbenutzlicher Hinsicht und zur waldbaulichen Behandlungsweise dieser Holzart. Allg. Forst- und Jagdzeitg. 123, (1952). — LINNAEUS, C.: Species plantarum. Holmiae (1753). — LÖVE, A.: A new triploid Betula verrucosa. Svensk bot. Tidsskr. 38, 381—393 (1944). — MANG, F.: Dendrologischer Teilbericht über die Exkursion des Botanischen Vereins zu Hamburg vom 27. 5. 1962 in den Kisdorfer Wohld. Als Mskr. vervielf. Hamburg 1962. — MANSFELD, R.: Verzeichnis der Farn- und Blütenpflanzen des Deutschen Reiches. Ber. dtsch. bot. Ges. 58a, (1940). — MORGENTHALER, H.: Beiträge zur Kenntnis des Formenkreises der Sammelart Betula alba L. Diss. Zürich (1915). — NATHO, G.: Variationsbreite und Bastardbildung bei mitteleuropäischen Birkensippen. Feddes Repert. 61, 211—273 (1959). — OEHLER, E.: Art- und Gattungskreuzungen. In: Roemer-Rudorf, Handb. Pflanz.züchtung Bd. 1. 2. Aufl., 563—611. Berlin (1958). — OVERBECK, F., und SCHMITZ, H.: Zur Geschichte der Moore, Marschen und Wälder Nordwestdeutschlands. I. Mitt. Provinz.st. Naturdenkmalpfl., Heft 3, 1-179. Hannover (1931). — PRIEHÄUSSER, G.: Der Formenkreis von Betula verrucosa und B. pubescens im Bayrischen Wald. Mitt. Staatsforstverw. Bayerns, 27. Heft (1952). — REESE, G.: Euploidie, Aneuploidie und B-Chromosomen bei Caltha *palustris* L. Planta 44, 203–268 (1954). — REGET, E.: Bemerkungen über die Gattungen Betula und Alnus. Bull. Soc. Imp. Nat., Moscou, 38 (1865). — ROTH, A. W.: Tentamen florae germanicae. I. Leipzig (1788). — RUBNER, K.: Die pflanzengeographischen Grundlagen des Waldbaus. Radebeul u. Berlin (1953). — SCHMUCKER, TH.: Die Baumarten der nördlich-gemäßigten Zone und ihre Verbreitung. Silvae Orbis, Bd. 4 (1942). — SCHÜTRUMPF, R.: Pollenanalytische Untersuchungen der Magdalenien- und Lyngby-Kulturschichten der Grabung Stellmoor. Nachr.blatt Dtsch. Vorzeit 11, 231–238 (1935). — SCHÜTRUMPF, R.: Paläobotanisch-pollenanalytische Untersuchung der paläolithischen Rentierjägerfundstätte von Meiendorf bei Hamburg. Ver. Arch. Reichsinst. 1, 1–54 (1936). — SCHÜTRUMPF, R.: Die pollenanalytische Unte-suchung der Rentierjägerfundstätte Stellmoor in Holstein. In: A. RUST, Die alt- und mittelsteinzeitlichen Funde von Stellmoor. Neumünster (1943). — STERN, K.: Populationsgenetische Probleme in der Baumrassenforschung und Forstpflanzenzüchtung. Schweiz. Z. Forstwesen 109, 458–477 (1958). — STERN, K.: Bericht über die 6. Tagung der Arbeitsgemeinschaft für Forstgenetik und Forstpflanzenzüchtung 1958. Silvae Genetica 8, 123–125 (1959). — STERN, K.: Über einen grundsätzlichen Unterschied der forstlichen Saat- und Pflanzgutgesetzgebung in der Schweiz und der Bundesrepublik Deutschland. Schweiz. Z. Forstwesen 111, 145–163 (1960). — TISCHLER, G.: Die Chromosomenzahlen der Gefäßpflanzen Mitteleuropas. 's-Gravenhage (1930). — TÜXEN, R.: Die Pflanzengesellschaften Nordwestdeutschlands. Mitt. flor.-soziol. Arb.gemeinsch. Niedersachs., Heft 3. Hannover (1937). — WEBER, E.: Betrachtungen zur Diskriminanzanalyse. Z. Pflanzenzüchtung 38, 1–36 (1957). — WEBER, E.: Genetische Analysen von Merkmalen mit fluktuierender Variabilität auf mendelistischer Basis. Diss. Stuttgart-Hohenheim (1958). — WETTSTEIN, W. v., und PROPACH, H.: Sichtungsarbeit zur Birkenzüchtung. Züchter 11, 279–280 (1939). — WOODWORTH, R. H.: Cytological studies in the Betulaceae. I. Betula. Bot. Gaz. 87, (1929).

On the Chromosomes of Pseudotsuga macrocarpa and Pseudotsuga menziesii

By H. CHRISTIANSEN

The Genetic Laboratory of the Royal Veterinary and Agricultural College, Copenhagen, and The Danish state Forestry's Tree Improvement Station, Humlebaek, Denmark.

(Received for publication April 18, 1963)

According to GÖHRE (5) the following species of *Pseudotsuga* are known at present: P. *menziesii* (MIRBEL) FRANCO = P. *taxifolia* BRITTON and *P. macrocarpa* MAYR, both from thc western part of USA; P. *japonica* BEISSNER and *P. Wilsoniana* HAYATA, both from Japan; *P. sinensis* DODE and *P. Forestii* CRAIB from China (Yuannan).

As pointed out by BARNER and CHRISTIANSEN (1) only the chromosome number of *P. menziesii* has up to now been published.

A couple of months ago the present author received by courtesy of Amanuensis B. SØGAARD, the Arboretum of the Royal Veterinary and Agricultural College, Hoersholm and The Pacific Southwest Forest and Range Experiment Station, USA Forest Service, Berkeley, California, a small quantity of sceds of *Pseudotsuga macrocarpa* MAYR. Using this material it was possible to determine the chromosome number of this species; it is 2n = 24.

The chromosome number was counted on root tip metaphases after treatment in 0.25% colchicine for 4 hours, fixation in Carnoy, hydrolyzation in 1n HCL at 60° C. 12 min., staining in Feulgen 30—60 min., treatment with Pectozyme 30—60 min. and squash in 45% acetic acid.

As shown in *Figs.* 1 *and* 2 *the* 24 chromosomes of *P. macrocarpa* fall in two groups: 12 chromosomes with median or submedian constrictions and 12 shorter heterobrachial chromosomes. No mitotic irregularities were observed.

If we compare the idiogram of the chromosomes of *P. macrocarpa (Fig. 2)* with the idiograms of the chromosomes of *P. menziesii* var. *viridis* and *Larix* shown in *Figs.* 7, 8 *and 9* of (1), it will be seen that the idiogram of P. *macrocarpa* has the same characteristic appearance as that of *Larix*. It also corresponds closely to the constructed idiogram of the chromosomes of *P. menziesii* in which the two pairs of telocentrics were replaced by 2 chromosomes with median constriclions thereby making the chromosome number 2n = 24 (cf. [1]).

Thus there is a close relationship not only between the ovules and pollination mechanisms of *Larix* and *Pseudotsuga* (cf. DOYLE [3] and [1]), but also as regards the morphology of the chromosome complements.

We have now the chromosome numbers of two species of *Pseudotsuga* viz.: *Pseudotsuga menziesii* 2n = 26 and *P. macrocarpa* 2n = *24.* As some doubt has been expressed as

Fig. 1. — 24 mitotic root tip chromosomes of *Pseudotsuga macrocarpa* (× ca. 1800). — *Fig. 2.* — Idiogram of the chromosomes shown in fig. 1. — *Figs. 3 and 4.* — Camera lucida drawings of the 13 bivalents of *Pseudotsuga menziesii:* T_1 and T_2 are telocentrics; 5 bivalents have median or submedian constrictions. — *Fig. 5.* — 4 telocentric mitotic chromosomes of *Ps. menziesii:* $a\text{-}a_1$, $b\text{-}b_1$ lying together in root tip cell (arrow); no centromeres visible (× ca. 333). — *Fig. 6.* — Enlargement of fig 5 (× ca. 1400). — *Fig. 6 A.* — Drawing showing the 4 telocentrics in situ. — *Fig. 5 A.* — Although the distance between plates of fig. 5 A and figs. 5, 6, 6 A is only ca. 2½ cell length, the 4 telocentrics in fig. 5 A are scattered over the cell, while those in figs. 5, 6, 6 A make the impression as if broken at centromere but moved only slightly apart.

to the correctness of the first mentioned number, the following review may be of interest:

In 1933 Sax and Sax (7) stated the chromosome number of *Pseudotsuga menziesii* (= *P. taxifolia)* as x = **13** (counted on endosperm tissue). In 1951 Seitz (8) in his list of chromosome numbers in woody plants quoted the above numbers but added: "Doubt has repeatedly been expressed as regards these numbers; they need checking". In 1953, Zenke (9) examined Meiosis in *P. menziesii* var. *viridis* and found the haploid number n = 13. — In 1958, Durrieu-Vabre (4) found 2n = 24 in root tip metaphases. She suggested that the thirteenth pair found by Sax and Sax are in reality fragments belonging to one of the subterminal pairs, and suggested as a possible cause of the fracture "la présence d'un élément peut-être très fragile se segmentent facilement au niveau du centromère". Mme D-V cites Langlet (6) for 2n = 24 in root tip metaphases. In 1962 the present author in cooperation with Barner (1) made a survey of the somatic chromosomes of *P. menziesii* var. *viridis* and found 26 units of which 4, the shortest, had telocentric centromeres. We did not at the time know the work of Mme Durrieu-Vabre but suggested that the four telocentric chromosomes might be fragments of two long chromosomes with median or submedian centromeres. This suggestion still holds good and will be commented on below.

In an attempt to find some new evidence to elucidate this unusual situation we reexamined our slides containing meiotic divisions in *Pseudotsuga menziesii* var. *viridis,* and found two metaphase I plates each showing beyond doubt 13 bivalents. Camera lucida drawings of these plates are shown in *Figs 3 and 4.* The bivalents shown in *Fig. 3* are at approximately the same stage as those shown in Zenke's *Fig. 10a—c* (9); The two bivalents marked T_1 and T_2 and Zenke's bivalents Nos, XII and XIII are evidently identical, and they no doubt consist of the two telocentric pairs. The bivalents shown in *Fig. 4* are at a slightly later stage; the bivalents T_1 and T_2 have only one chiasma (terminalized) and seem to be precociously in disjunction. This would seem strong evidence that at least the trees examined in USA (Sax and Sax), in southern Germany (Zenke) and those here in Denmark actually have 26 chromosomes. But the fact that 4 of these chromosomes are teloncentric and much shorter than the others (cf. [1] *Fig. 6c)* nevertheless leaves a certain doubt. This doubt is accentuated by the finding

of 2n = 24 in *P. macrocarpa* and by the circumstance that the 4 telocentric chromosomes in *P. menziesii* can be arranged to fit into the idiogram of the chromosomes of this species in such a manner that the number of chromosomes becomes 24 instead of 26 ([1] *Figs. 7 and 8).*

Now, if the number of chromosomes in *P. menziesii* were originally 24, 2 of the four telocentric chromosomes would be supernumerary, and irregularities in meiosis and mitosis resulting in varying chromosome numbers were to be expected. Certain irregularities have actually been observed (1), but they are relatively few, and no other chromosome numbers than 24 and 26 have been reported. The telocentrics seem therefore not to behave like extra chromosomes in other plants, and according to Darlington (2) no case of supernumerary chromosomes in woody plants is known.

On the present evidence the conclusion must therefore be that even if the telocentrics have once been fragments, they have now attained the level and behaviour of normal chromosomes, and the chromosome number of *P. menziesii* var. *viridis* must until further be considered 2n = 26

There is, however, another and interesting possibility which, if it is confirmed, will add a new and dangerous source of errors in chromosome counting to the multitude already known.

As mentioned above Mme Durrieu-Vabre has suggested that the chromosome number of *P. menziesii* is 2n = 24 and that the two additional chromosomes found by other workers are the result of breakage, during preparation, of one pair of chromosomes with extraordinary fragile centromeres. If we have understood Mme D-V correctly this would in other words mean that the physical properties of the centromeres of one pair of chromosomes must be such that in undisturbed cells meiosis and mitosis are normal with respectively n = 12 and 2n = 24, but when the cells are pretreated, fixed and squashed, the centromeres of this pair usually break both in meiosis and mitosis and simulate chromosome numbers of n = 13 and 2n = 26 respectively, always provided that the chromosome number and chromosomal behavior are the same in the trees examined by Mme D-V as in those examined by other workers.

That chromosomes may break during preparation is of course well known to all chromosome students. The causes of the breakage may be too much pressure, insufficient maceration, or possibly the contraction due to pretreatment and (or) fixation, but the safeguard has always been that the breakage usually occurs in different chromosomes and both in centromeres and chromosome arms.

In order to test Mme D-V's theory we reexamined our slides for possibly overlooked plates with 24 chromosomes. No such plates came to light, but we found one mitotic early metaphase plate (cf. *Figs. 5, 6, 6a)* in which the 4 telocentric chromosomes were lying two and two in continuation of each other (probably in connexion with a nucleolus) with only a gap where the centromeres should have been. This plate was lying at a distance of about two and a half cell lengths from another plate at approximately the same stage of development but with the four telocentrics scattered over the plate *(Fig. 5A).* In view of the small distance between the two cells the differences in treatment must have been very small, but nevertheless they behaved differently. No trace of centromere-connections was found, and it is possible that the position of the telocentrics is accidental, or that it has something to do with the nucleolus.

A search was also made for diakinesis and metaphase I plates with 12 bivalents in which the two bivalents containing the 4 telocentrics should be replaced by one large bivalent containing a pair with median or submedian constriction. The result was negative, but the significance hereof should not be overestimated because as a matter of fact only a small percentage of PM cells in *Pseudotsuga* can ever be analyzed with accuracy; and this reservation also applies to mitotic metaphase plates.

The plates shown in *Figs. 5—6A* give a hint that breakage like that proposed by Mme D-V could perhaps happen in mitotic plates, but the 13 bivalents found by Mme Zenke and by us could not very well be caused by breakage of a bivalent during preparation, at least not as far as our present knowledge goes.

It is, however, a very interesting problem, and it would greatly facilitate its elucidation if Mme D-V would kindly furnish some more details, particularly as regards the variety *(viridis, glauca, caesia)* of *P. menziesii* to which the trees examined by her belong. It would furthermore be of interest to know if only mitotic plates with 2n = 24 were found, or whether also plates with other chromosome numbers occurred. An examination of PMC meiosis of her material would be particularly useful.

On account of the considerable morphological variation even within the varieties of *P. menziesii* the possibility cannot be excluded that races with different chromosome numbers may exist.

The author wishes to thank Professor, Dr. phil. C. A. Jørgensen, The Genetic Laboratory of the Royal Veterinary and Agricultural College, Copenhagen, for help and advice.

Summary

The somatic chromosome number of *Pseudotsuga macrocarpa* is determined as 2n = 24.

It is suggested that there is a close relationship not only between the ovules and pollination mechanisms of *Pseudotsuga* and *Larix* but also as regards the morphology of their chromosome complements.

Although there is considerable evidence that *Pseudotsuga menziesii* may once have had 24 somatic chromosomes, the evidence is as yet not strong enough to justify alteration of the present chromosome number, 2n = 26. A survey is made of previous chromosome counts in *P. menziesii.*

A suggestion made by Mme Durrieu-Vabre that the correct number of somatic chromosomes in *P. menziesii* is 24, and that 2 of the 26 chromosomes now listed are fragments originating from breakage, during preparation, of one chromosome pair with particularly fragile centromeres, is discussed.

Zusammenfassung

Titel der Arbeit: *Über die Chromosomen von Pseudotsuga macrocarpa und Ps. menziesii.*

Die somatische Chromosomenzahl von *Pseudotsuga macrocarpa* wurde mit 2n = 24 festgestellt.

Es wird weiter angedeutet, daß es eine enge Verwandtschaft zwischen Samenanlagen und Bestäubungsmechanismus von *Pseudotsuga* und *Larix* gibt und ebenso auch hinsichtlich der Morphologie ihrer Chromosomen-Komplemente.

Obwohl es einen wesentlichen Hinweis dafür gibt, daß *Pseudotsuga menziesii* einmal 24 somatische Chromosomen gehabt haben konnte, so ist doch bisher dieser Befund noch nicht zwingend genug, um eine Veränderung der gegenwärtig geltenden Chromosomenzahl von 2n = 26 zu recht-

fertigen. Es wird hierzu ein Überblick über vorläufige Chromosomenzählungen bei *Ps. menziesii* gegeben.

Die Vermutung von Frau DURRIEU-VABRE, daß die richtige somatische Chromosomenzahl bei *Ps. menziesii* 24 ist und daß lediglich 2 der 26 jetzt überall verzeichneten Chromosomen Fragmente sind, die durch Bruch von einem Chromosomenpaar mit besonders zerbrechlichem Centromer durch die Präparation entstehen, wird diskutiert.

Résumé

Titre de l'article: *Chromosomes de Pseudotsuga macrocarpa et Pseudotsuga menziesii.*

On a déterminé le nombre chromosomique de *Ps. macrocarpa:* 2 n = 24.

On pense qu'il existe une relation étroite entre *Pseudotsuga* et *Larix,* non seulement pour le mécanisme de la pollinisation et de la fécondation, mais aussi pour la morphologie des garnitures chromosomiques.

Bien qu'il existe une forte présomption pour que *Ps. menziesii* ait pu parfois présenter 24 chromosomes, la preuve n'est pas encore suffisante pour justifier la modification du nombre chromosomique actuel de 2 n = 26. On donne une liste des comptages des chromosomes faits jusqu'ici pour *Ps. menziesii.*

On discute l'hypothèse faite par Madame DURRIEU-VABRE, selon laquelle le nombre exact de chromosomes est 24, et 2 des 26 chromosomes ne sont que des fragments venant de la cassure, au cours de la préparation, d'une paire de chromosomes à centromères particulièrement fragiles.

Literature Cited

(1) BARNER, H., and CHRISTIANSEN, H.: The formation of pollen, the pollination mechanism, and the determination of the most favourable time for controlled pollination in *Pseudotsuga menziesii.* Silvae Genetica **11**, 89—124 (1962). — (2) DARLINGTON, C. D.: Chromosome Botany. London 1956. — (3) DOYLE, J., and O'LEARY, M.: Pollination in *Tsuga, Cedrus, Pseudotsuga,* and *Larix.* Sci. Proc. Roy. Dublin Soc., Vol. **21**, No. 21 (N. S.), 1935. — (4) DURRIEU-VABRE (Mme): Chromosome de *Pseudotsuga douglasii* CARRIÈRE. Comtes-rendus Séances de l'Acad. Sci. **246**, (4) 3660—3633 (1958). — GÖHRE, K.: Die Douglasie und ihr Holz. Berlin, 1953. — (6) LANGLET, O.: Svenska Skogsvårdsfören. Tidskr. **32**, 1—11 (1934). — (7) SAX, K., and SAX, H. J.: Chromosome number and morphology in the conifers. J. Arnold Arboretum **14**, 356—375 (1933). — (8) SEITZ, F. W.: Chromosomenzahlenverhältnisse bei Holzpflanzen. Zeitschr. Forstgenetik **1**, 22—32 (1951). — (9) ZENKE, U.: Untersuchungen über den Ablauf der Meiosis bei *Pseudotsuga taxifolia* BRITTON. Zeitschr. Forstgenetik **2**, 96—102 (1953).

Microsporogenesis in Pinus echinata and Pinus taeda

By F. MERGEN, G. R. STAIRS, and E. B. SNYDER[1])

(Received for publication June 11, 1963)

This paper presents photomicrographs of major stages in pine microsporogenesis that are generally illustrated in forest botanical literature only by drawings. It also includes original data on the phenology of these stages in *Pinus echinata* MILL. (loblolly pine) and *P. taeda* L. (shortleaf pine).

Strobili were collected on January 25 and 27, February 9 and 16, and March 13, 1962, from trees of both species, growing sympatrically in two natural forests near the Institute of Forest Genetics in southern Mississippi. Specimens were fixed in 3:1 propionic acid-alcohol and smeared in acetocarmine. With the exception of *Figure 1 D,* showing diakinesis in *P. taeda,* photographs of *P. echinata* illustrate the various stages.

Microsporangiate strobili of *P. echinata,* collected in late January, contained archesporial cells in the microsporangium *(Figure 1 A).* These cells had angular outlines and large nuclei. On February 9, meiosis had started. A variety of stages were present and, depending on the maturity of the bud, some of the cells were still in the microspore mother cell (MMC) stage while others had progressed to anaphase I. The MMC appeared rounded, as is illustrated by the meiotic prophase at zygonema *(Figure 1 B).*

By pachynema *(1 C),* chromosome pairing was complete, and the chromosomes appeared as dark-stained, bead-like structures. During diakinesis *(1 D)* staining was dark and chiasmata were visible. Contraction of the chromosomes during early and late metaphase *(1 E, 1 F)* and alignment at the metaphase plate were well defined. A late anaphase, a telophase, and a dyad are illustrated in *1 G, 1 H,* and *1 I.* The synchronous condition of metaphase II is shown in *1 J,* anaphase II in *1 K,* and telophase II in *1 L.*

After the latter stage, interphase nuclei are formed in the microspores and the four young pollen grains comprising the tetrad begin to separate *(2 A).* In some of the microspores, rudimentary air sacs were already visible under phase illumination while they were still within the wall of the original MMC (photograph not shown). *Figures 2 B and 2 C* are illustrations during expansion of the air sacs after the pollen grains were freed from the MMC wall. First vegetative divisions of the pollen grains were

Table 1. — Comparative phenology of developmental stages in pine microsporogenesis in southern Mississippi in 1962.

Date	*Pinus echinata*	*Pinus taeda*
January 25	Archesporial cells	Microspore mother cells, meiotic prophase, metaphase I, anaphase I, telophase I
February 9	Microspore mother cells, and in advanced buds up to telophase I	
March 6		Mature pollen (dehiscence)
March 13	Anaphase II, tetrad, free microspores, first vegetative division of microspores, and mature microspores in advance buds	
April 2	Mature pollen (dehiscence)	

[1]) MERGEN and STAIRS are on the staff of the Yale University School of Forestry, New Haven, Connecticut. SNYDER is a member of the Institute of Forest Genetics, which is maintained at Gulfport, Mississippi, by the Southern Forest Experiment Station, Forest Service, U. S. Department of Agriculture. The research was carried out cooperatively.