Résumé

Titre de l'article: Evaluation statistique des essais *comparatifs*. —

L'estimation de l'erreur dans une experience sur la variation de hauteurs individuelles porte seulement sur la variation a l'intérieur des parcelles. Elle ne convient donc pas pour tester les differences entre les moyennes des parcelles, même si elles sont adjacentes. On prend comme exemple une plantation comparative d'epicea de MÜNCH (1949). Le plan de cette experience ne permet aucune estimation exacte, car il ne comporte pas de répétitions.

Dans une experience rationnellement disposee, la preuve de la différence entre les moyennes des variétés est donnee par le test de F. Si F n'est pas significatif, toute nouvelle tentative de mettre en évidence des différences est inadmissible. Même si F est significatif, il est inadmissible, par exemple, d'arranger les differences pour toutes les paires possibles en une «table des differences» et de tester l'erreur par le test de t. Les tables de t conviennent pour tester, a des niveaux donnes de probabilité, la difference entre deux valeurs donnees, mais non La serie des arrangements de plus de deux valeurs. En testant $\frac{n\,(n-1)}{2}$ différences, la valeur supposée du degré de signification ne sera pas la valeur reelle. I1 faut employer a la place les tests des series de NEWMAN (1939), KEULS (1952) et DUNCAN (1955) bien que toutes les propriétés de ces tests n'aient pas encore été completement étudiées sur le plan theorique.

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lnvestigations on the Intermediate Type between the Austrian and the Scots Pine

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Introduction

The present investigations were made at the Arboretum of the Royal Veterinary and Agricultural College, Copenhagen, where I studied from the autumn of 1955 until the summer of 1956 as a fellowship holder of the Danish Government. I am much indebted to the Director of the Arboretum, Dr. C. SYRACH LARSEN, as well as to his collaborators, for the help afiorded to me during our discussions. The Arboretum gave me all facilities to perform the necessary trips in the field to collect experimental material: for this I am also grateful. The cytological part of the investigations was made at the Department of Genetics, the Royal Veterinary and Agricultural College, Copenhagen. 1 am much indebted to Professor Dr. C. **A.** JØRGENSEN, head of the Department, and to his assistant Mr. H. CHRISTIANSEN, for the technical help given to me in my work.

I made the statistical interpretation of the experimental material at the Institute for Forest Mensuration, Agricultural and Forestry Faculty, Zagreb, under the guidance of Docent Dr. B. EMROVIĆ, to whom I herewith express my sincere thanks.

In the autumn of 1955 in the company of Mr. K. BRANDT I visited some pine plantations in the area of Djursland at Emmedsbo near Grenaa, East Jutland, Denmark. Mr. A. CRISTENSEN, chief forest officer of that district, drew our attention to three pine trees of the same age growing in a

Fig. 1. — Investigated tree from Emmedsbo. Photo. SØEGAARD.

young planted stand of Austrian pine, which differed from the other trees, and in certain characters seemed to be intermediate between Austrian pine and Scots pine, suggesting a hybrid origin. I collected from these trees some material for investigation. Later I revisited the same locality obtaining additional data. In my work Mr. A. CHRISTENSEN was most helpful, for which I wish to express my thanks.

The colour of the bark and the length and colour of the needles of the trees from which the material was taken diviate from the normal exterior characteristics of the Austrian pine. In these characteristics, the trees in question resemble a type intermediate between the Austrian and the Scots pine. These trees also differed from their neighbours in two other characteristics: height and diameter. They are higher and have a larger diameter than the other trees, despite their being of the same age. The stand in which the trees investigated are growing is 20 years old. It was raised by artificial regeneration. Seeds form the regeneration of this stand had been collected by Mr. J. A. HANSEN from an old mixed stand of Austrian and Scots pine in the same forest district.

Investigations

Some morphological characters

It is common knowledge that the colour of the needles of the Austrian pine is blue-green. The three trees investigated are of an intermediate colour, midway between the Austrian and the Scots pine. There are also differences between the needle lenght of the investigated trees and that of the Austrian and Scots pines. The needles of these trees are shorter than the needles of the Austrian pine, and they are longer than the needles of the Scots pine. This is readily seen from Table I and from Figs. 2, 3 and 4.

Table I. — Arithmetical mean of the length of needles in cm.

Austrian pine			Scots pine			Investigated 3 specimens		
Locality								
Rugaard	Skjersø	Emmedsbo	Scotland	Emmedsbo	Origin unknown	No. 1	No. 2	No. 3
11.36	13.16	11.14	4.26	4.39	6.34	7.16	9.18	7.78

The needles of the Austrian pine are stiff and straight, but the needles of the Scots pine are much more flexible and slightly twisted. The needles of the investigated trees are slightly more flexible than those of the Austrian pine, and they too are slightly twisted.

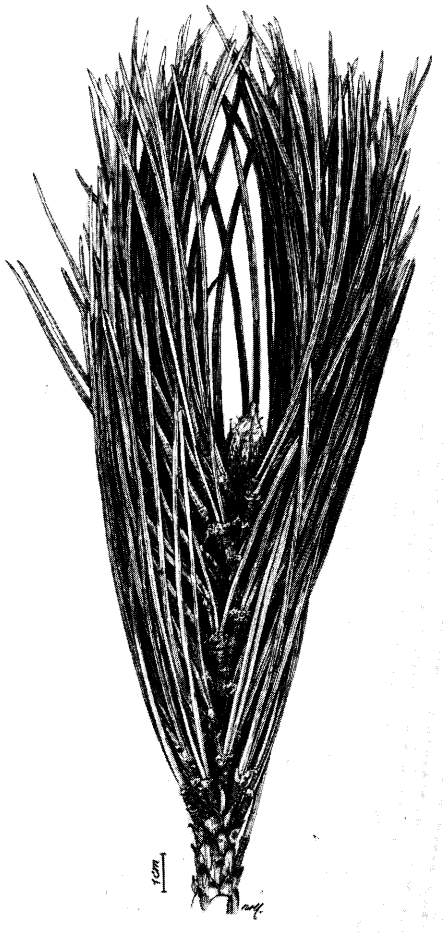

Fig. 2. — Shoot of the Austrian pine.

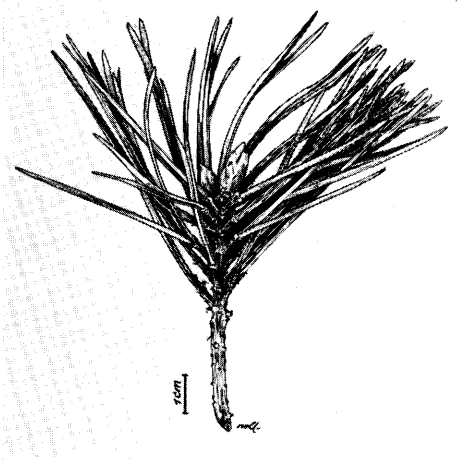

Fig. 3. — Shoot of the Scots pine.

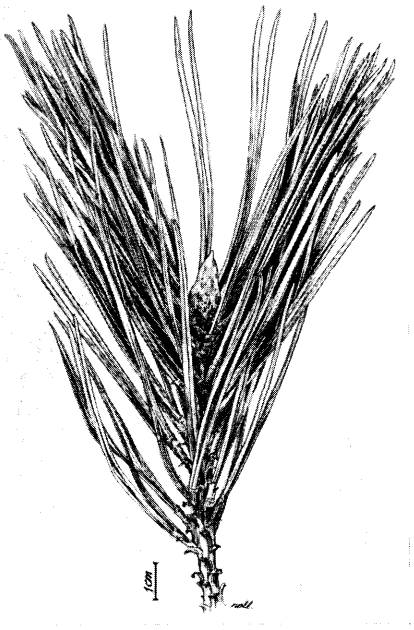

Fig. 4. — Shoot of the investigated specimen from Emmedsbo.

Another important characteristic is the buds. The buds of the Austrian pine are rather large and thick, while those of the Scots pine are shorter and thinner. The buds of the investigated trees are between the two types mentioned (Fig. 2, 3 and 4).

In general the cones of the Austrian pine are 7—8 cm. long, and the apophysis of the cone scales is transversally keeled. The cones of the Scots pine are 2.5—7 cm. long, and the apophysis is either flat or pyramidal. The cones of the trees from Emmedsbo are 5.5 cm. long, while the shape of the apophysis and of the whole cones is intermediate between the Austrian and the Scots pine (Fig. 5, 6 and 7). The colour of the bark of the trees investigated is slightly reddish in the upper part of the stem, which also is in conformity with an intermediate position between the two principal types.

The breast height diameter of the trees investigated is 14, 15, and 20 cm. respectively, while the diameters b. h. of the other trees in the stand range from 7—13 cm. The height of the investigated trees is from 1—1.5 m. greater than that of the other trees in the stand. Furthermore one other and important characteristic was noticed in the trees

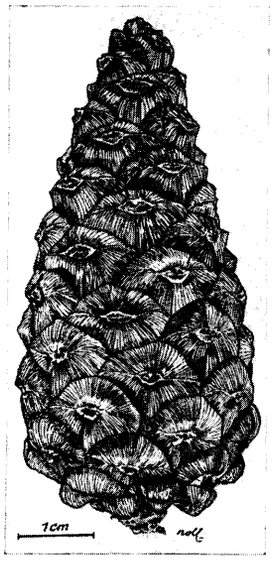

Fig. 5.
Cone of the Austrian pine.

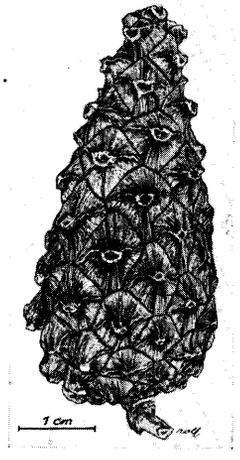

Fig. 6.
Cone of the Scots pine.

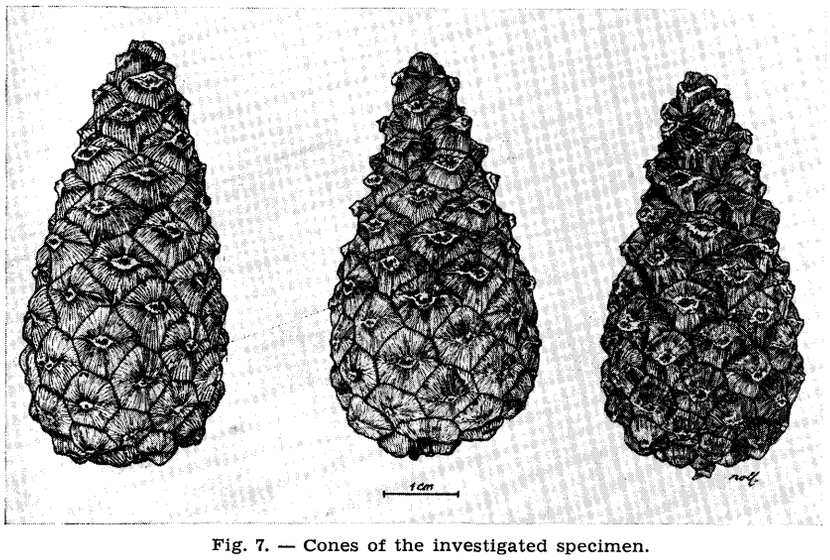

Fig. 7. — Cones of the investigated specimen.

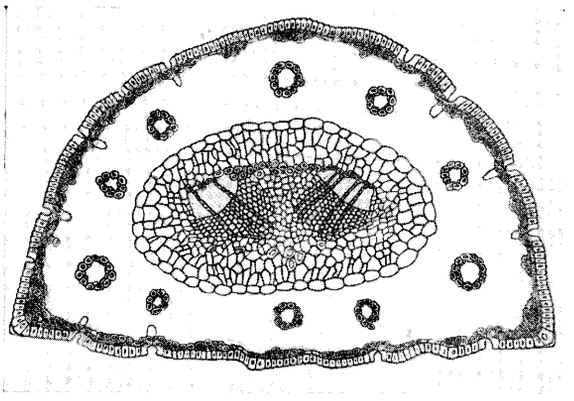

Fig. 8. — Transverse section of the needle of the Austrian pine (enl. 44 ×).

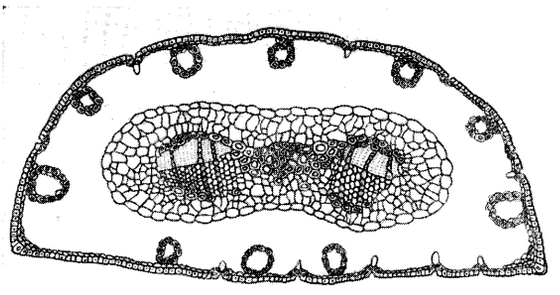

Fig. 9. — Transverse section of the needle of the Scots pine (enl. 43 ×).

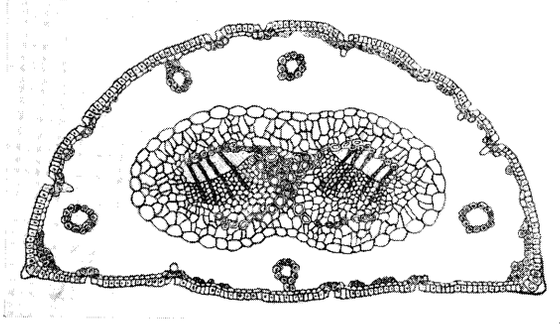

Fig. 10. — Transverse section of the needle of the investigated specimen from Emmedsbo (enl. 44 ×).

investigated, i. e. they bear seeds. An appreciable number of cones were found, whereas there were no cones on the other trees in the same stand.

Structure of the needles

In Table II are shown the structural characteristics of the transversal section of the needles of the Austrian pine, the Scots pine, and the trees investigated. The structure of the needles of the Austrian pine differs considerably from that of the Scots pine, as appears Table II and Fig. 8 and 9. The differences are found in almost all the characters. If we observe the needle structure of the investigated specimens, we shall see that their characters too are intermediate between those of the Austrian and the Scots pine (Fig. 10).

Table II. — Structure of the needles

Species or type	Epidermic cells, Side, convex: Height in μ	Epidermic cells, Side, convex: Width in μ	Epidermic cells, Side, flat: Height in μ	Epidermic cells, Side, flat: Width in μ	Epidermic cells, convex: Shape of lumen	Epidermic cells: Shape	No of stomata	No. of Resin ducts: median	No. of Resin ducts: marginal	No. of Resin ducts: Total	Wall thickness of scler. cells-convex side	Hypodermic cells, No. of layers, Side: convex	Hypodermic cells, No. of layers, Side: flat	Hypodermic cells, No. of layers: in angle	Hypodermic cells, convex side: Wall thickness	Hypodermic cells, convex side: lumen size	Shape of endodermis (pericycle)	Transfusion parenchyma No. of layers: above phloem	Transfusion parenchyma No. of layers: below xylem	No. of layers of scler. cells: between bundles	No. of layers of scler. cells: above phloem	Needle: Width in μ	Needle: Height in μ
Austrian pine	29—36 (40)	(14) 18 (22)	(22) 25—29 (36)	14—19	lengthened	rectangular	(6) 9—15 (21)	(4) 7—12 (15)	—	(4) 7—12 (15)	thick, very thick	2—3 (4)	(1) 2 (3)	(2) 3—4	thick	narrow	oval	(2) 3	(4) 5 (6)	—	1	1450—1700 (1960)	880—1050 (1350)
Scots pine	(15) 17—19	(11) 15 (19)	15—17 (19)	15—19	roundish	quadrangular	11—20	—	6—12	6—12	thick	1	1	(1) 2	thin	wide	lengthened	2	(2) 3	6—10	1—2	1330—1700	645—825
Investigated trees from Emmedsbo	19—23	13—19	(15) 19 (23)	15—19	roundish, lengthened	quadrangular (rectangular)	7—20	2—7	1—3	2—9	thick (very thick)	1—2 (3)	1—2	2 (3)	moderately thick	narrow, wide	oval, lengthened	2—3	3—4 (5)	(1) 3—5	1—2	1365—1575 (1750)	800—975

The epidermal cells in a transversal section of the needles in the Austrian pine are rather rectangular; those in the Scots pine are rather quadratic in shape; the shape of the epidermal cells of the investigated specimens is intermediate between the two. The size of the cells is also intermediate between the two types mentioned. The shape of the stomata, too, differs in the two above-mentioned types, while the stomata of the investigated specimens is intermediate in shape (Figs. 11, 12 and 13). In the Austrian pine there are 2—3 layers of hypodermal cells on the convex side of the needle, while in the Scots pine there is only one layer of these cells. The investigated specimens usually have 1—2 layers of hypodermal cells.

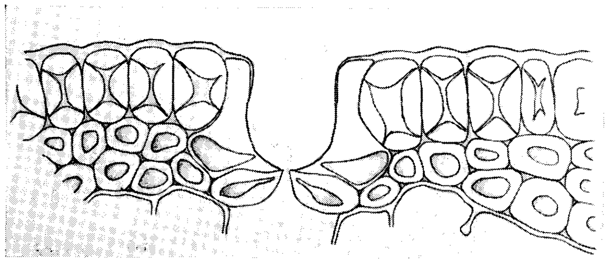

Fig. 11. — Austrian pine. — Transverse section of the needle. — Detail: epiderma, stomata, and hypoderma (enl. 345 ×).

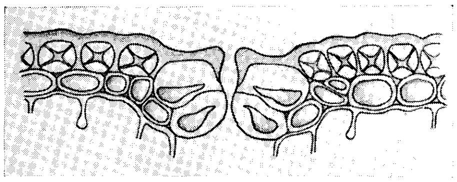

Fig. 12. — Scots pine. — Transverse section of the needle. — Detail: epiderma, stomata, and hypoderma (enl. 345 ×).

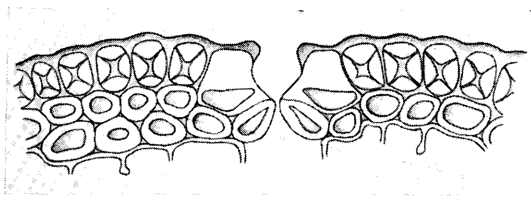

Fig. 13. — Investigated specimen from Emmedsbo. — Transverse section of the needle. — Detail: epiderma, stomata, and hypoderma (enl. 345 ×).

There is a marked difference between the Austrian and the Scots pine in the position of resin ducts. In the Austrian pine they are always embedded in the parenchyma, while in the Scots pine they are closely adjacent to the hypoderma. In the investigated specimens, however, some of the individual resin ducts are subjacent to the hypoderma, while the others are to be found in the parenchyma. The pericycle in the Austrian pine is rather oval; in the Scots pine it is more lengthened and in the middle flattened from both sides. The pericycle in the investigated specimens is intermediate in shape, as shown in the figures.

In the Austrian pine there are no sclerenchymatous cells between the two bundles within the stele, in the Scots pine there are 6—10 rows. The investigated specimens have only a few layers of these cells. The position of bundles in the needle in the investigated specimens was intermediate between the two types. In the Austrian pine the bundles lie rather close to each other; in the Scots pine they are more distant from each other.

Cytological investigations

In morphological and structural characteristics, the three trees investigated seem to represent a type, intermediate between the Austrian and the Scots pine. Therefore the hypothesis that they are hybrids of the two species might be verified by cytological investigations. For this purpose twigs with flower buds were collected, and the reduction division of the microspore pollen mother cells was studied in carmine acetic acid squashes. The reduction division was found to be normal. Later investigations established that also the pollen was normal.

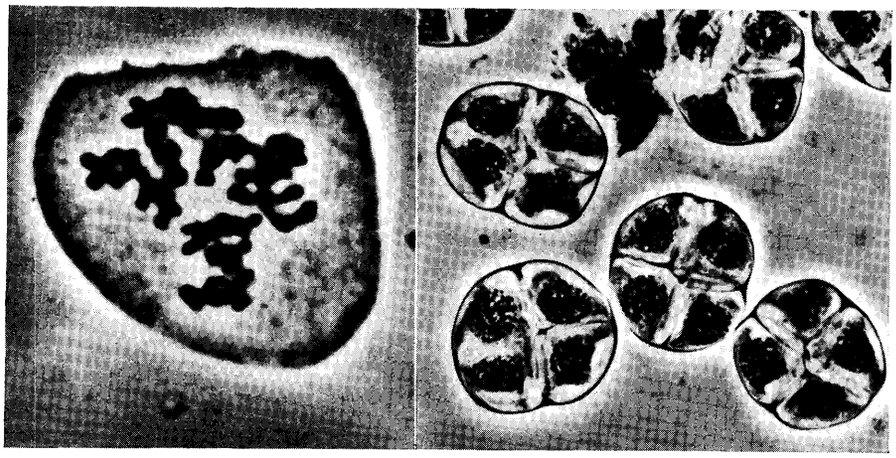

Fig. 14. — Microspore pollen mother cell: — (a) Early metaphase I (enl. appr. 680 ×). — (b) Tetrads (enl. 270 ×). — Photo CHRISTIANSEN.

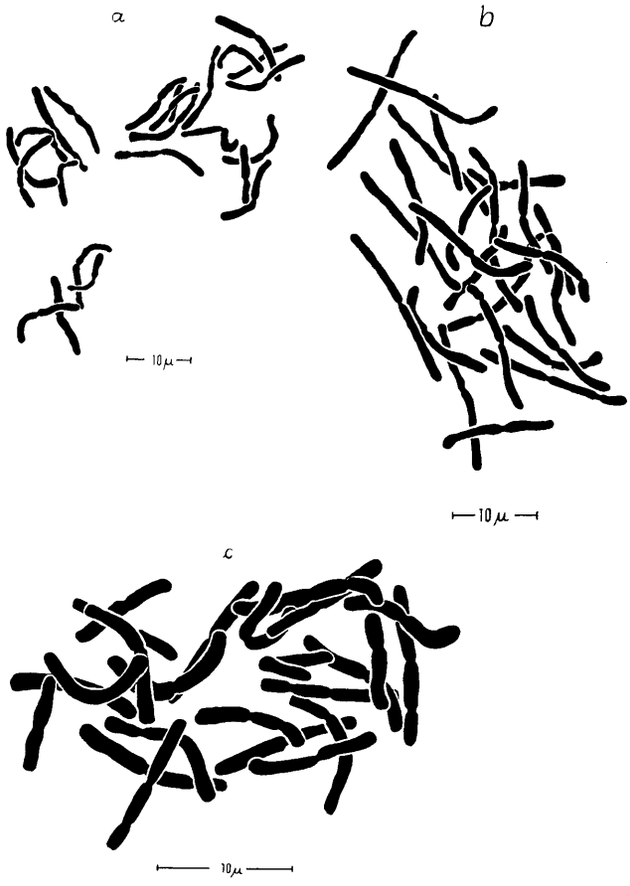

Fig. 15. — Chromosomes of the somatic cells in metaphase: — (a) Austrian pine. — (b) Scots pine. — (c) Investigated pine from Emmedsbo.

The somatic cells were examined in order to find out whether there existed any difference in the morphology of the chromosomes between the Austrian pine, the Scots pine, and the specimens of the three trees investigated. The seedling root tip cells in metaphase were investigated.

The material used comprised 5 cells of the Scots pine, 5 cells of the three specimens from Emmedsbo, and 6 cells of the Austrian pine. The chromosomes were drawn, using a drawing apparatus, and then measured.

Investigating the morphology of the chromosomes, secondary constrictions were also taken under consideration. No reliable differences could be found.

Investigation of the length of the chromosomes was as follows:

1. The chromosomes were arranged according to size. The smallest was given number 1, and the largest number 24.

2. As the cells in the outer and inner layers of the root tip are variable in size, the chromosomes are also variable in length. For this reason it is not possible to compare the absolute length of the chromosomes of the Austrian pine, the Scots pine, and the three specimens from Emmedsbo, but the relative length of the chromosomes must be used for comparison.

3. The graphical representations show that the inclinations of the graphs are characteristic of the species, while the levels or the positions of these lines are dependent on the position of the cells in the root tip, as stated above.

4. Statistical interpretation: in the first place, for each chromosome-curve, transformed into a straight line, as calculated the regression coefficients and the variances were determined. The data obtained were analysed in the manner shown in HALD's manual (9). It was then found that the straight lines of a given species could not be considered as parallel, it being most probable that the inclination is influenced by the position of the cell in the root tip. However, if we consider the regression coefficients as given quantities (see Table III), and if we calculate the arithmetical mean of these regression coefficients, then, according to FISHER's t-test, the arithmetical means between the Scots pine and the Austrian pine differ significantly from one another, while the arithmetical means are not significant between the three trees from Emmedsbo on the one hand, and each of the two pine species on the other.

Table III. — Regression coefficients of the chromosome sizes and the serial number

No.	Austrian pine	Scots pine	Investigated specimen
	b	b	b
1	1.725326	2.846521	3.259891
2	1.540869	2.523260	2.249021
3	1.412065	2.201304	1.679130
4	2.380760	2.658478	2.178260
5	1.466739	2.800326	1.314456
6	1.391413		
b	1.652862	2.605978	2.136152

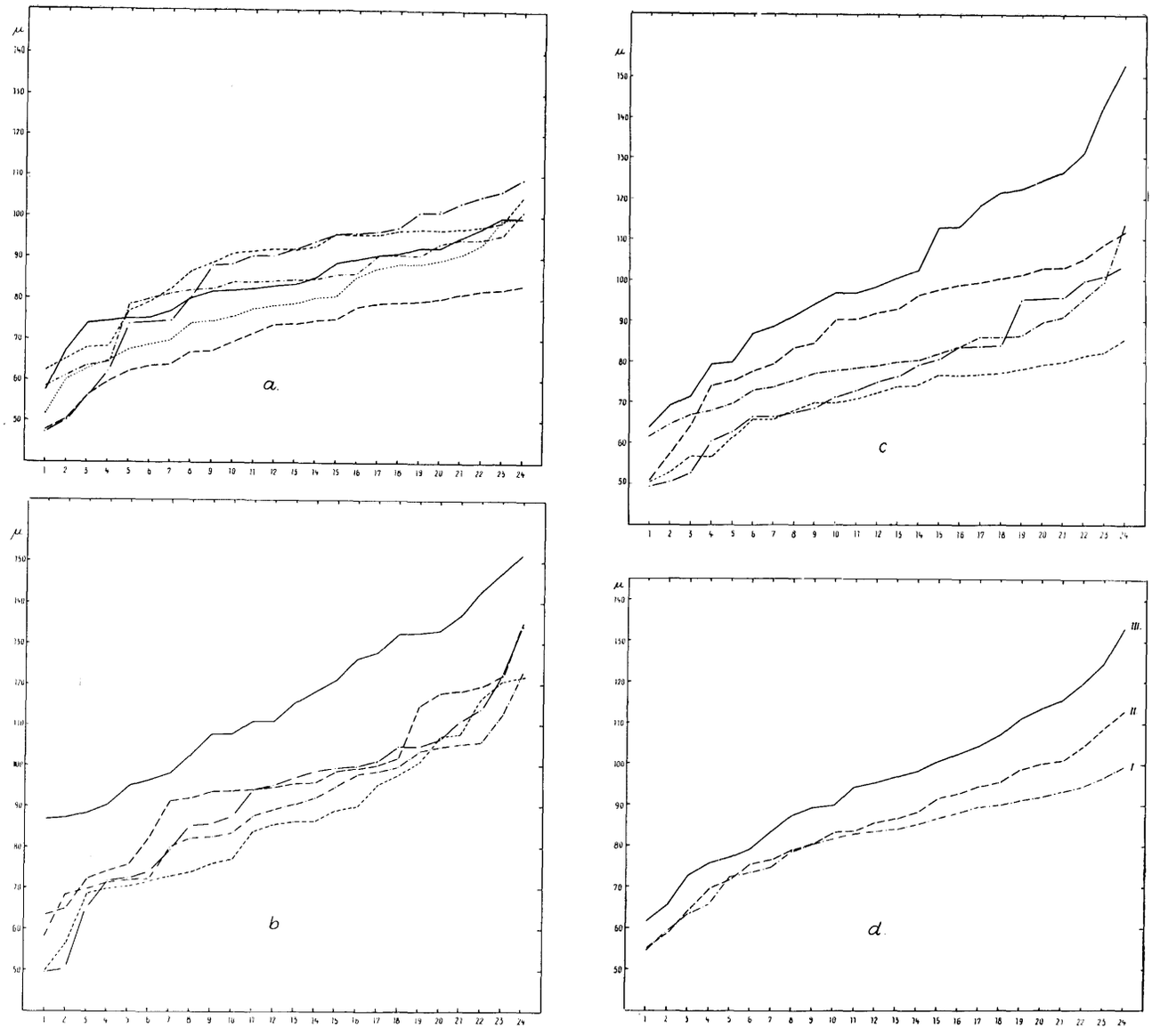

Fig. 16. — Chromosome size graphs: — (a) Austrian pine. — (b) Scots pine. — (c) Emmedsbo specimens. — (d) Mean values of chromosome size: — I. Austrian pine. — II. Emmedsbo specimens. — III. Scots pine.

The mean length of the chromosomes within one cell was treated in the same manner, i. e. by considering it a given quantity. The arithmetical means computed from these quantities do not differ significantly between the Scots and the Austrian pine.

Discussion

On the basis of the external morphology of the trees and of the needle structure, the Austrian pine is clearly distinguishable from the Scots pine. According to investigations carried out thus far, hybrids of these two pines are very rare in nature. In the literature two hybrids are described, i. e. *Pinus Neilreichiana* Reichardt (11) and *Pinus permixta* Beck (1). That such hybrids exist in nature is made probable by the investigations of Duffield (7) and A. de Jamblinne (6), who succeeded in producing them artificially. The specimens described here are in their external morphological characters of the intermediate type, which corresponds to the hybrids described by Reichardt and Beck.

Very important characteristics of needle structure of the Austrian and the Scots pine are: epidermis, stomata, hypodermis, and position of the resin ducts. Those two species are clearly distinguishable in these characteristics, but the three specimens here investigated, show, among other things, intermediateness in these characteristics.

The cytological investigations revealed that in the reduction division of the pollen mother cells of the three specimens investigated no irregularities existed. It was likewise found that the pollen was fertile. However, Duffield established that in 10 artificial hybrids among the *Pinus* species, the pollen showed in top cases a sterility of only 40—50%; for the reason stated, we can suppose that sterility is not always a reliable indication in determining hybrids.

It is interesting to note that on examining the chromosome length of the somatic cells significant differences were found between the Austrian and the Scots pine. The three specimens investigated were intermediate in regard to chromosome length.

These significant differences were obtained when the inclinations of regression lines were considered (under

the assumption that the size of the chromosomes is a linear function of the serial number). When the absolute quantities are taken into consideration no significant differences were obtained because the position of the cell within the root affects their size (see p. 16 under 2).

The cytological data may be summarized as follows:

1. The differences in size between the small and the large chromosomes in the somatic cells is greater in the Scots pine than in the Austrian pine.

2. With respect to the chromosome length of the somatic cells the investigated three specimens are intermediate between the Austrian and the Scots pine.

The cytological investigations neither confirm nor refute the assumption that the three specimens investigated are hybrids. Since they are intermediate with respect to the external morphological characteristics and the structure of the needles, the conclusion may be drawn that these three specimens — in all probability — are hybrids between the Austrian and the Scots pine. This conclusion is also supported by the fact that such hybrids are described in the literature, and that they have been obtained artificially. As the investigated trees are taller and have a larger diameter than the Austrian pines of the same age in the stand, they seem to be growing faster than the pure species, i. e. they possess hybrid vigour.

Summary

In the region of Emmedsbo, near Grenaa, East Jutland, Denmark, in a stand of Austrian pine, there were noticed three trees, which, according to external morphological characteristics, differed from the other trees. These trees looked like hybrids between the Austrian and the Scots pine, and these investigations were carried out with the purpose of establishing evidence of this hypothesis. On the basis of these investigations the following conclusions can be drawn:

1. The external morphological characteristics of the tree and the structure of the needles of the investigated three specimens are intermediate between the Austrian and the Scots pine (Figs. 4, 7, and 10, Table II).

2. In the reduction division of the pollen mother cells no irregularities exist (Fig. 14).

3. The pollen in the investigated trees is fertile.

4. There exists a difference in the length of the chromosomes between the Austrian and the Scots pine. Statistical interpretation of the data presented here shows the inclination of the regression lines (the length of the chromosome being a linear function of the serial number) to be significantly different in the Austrian pine and the Scots pine, whereas there was no significant difference between the three specimens from Emmedsbo on the one hand, and each of the two species on the other.

5. On the basis of the external morphological investigations, and to some extent also on the basis of the cytological investigations it is possible to conclude, that the three specimens investigated are in all probability hybrids between the Austrian and the Scots pine.

6. These hybrids display some hybrid vigour.

Zusammenfassung

Titel der Arbeit: *Untersuchungen über eine Intermediärform zwischen Pinus nigra ssp. austriaca und Pinus silvestris.* —

In der Gegend von Emmedsbo, nahe Grenaa in Ostjütland, Dänemark, fielen in einem Schwarzkiefernbestand 3 Bäume auf, die sich hinsichtlich ihrer morphologischen Merkmale von den anderen unterschieden. Diese drei Stämme erweckten den Eindruck von Bastarden zwischen Schwarzkiefer und Silvestris. Die vorliegenden Untersuchungen hatten das Ziel, diese Hypothese zu prüfen, und führten zu folgenden Ergebnissen:

1. Äußere morphologische Merkmale und Nadelstruktur der drei untersuchten Individuen stehen zwischen den entsprechenden Daten der *P. nigra, austriaca* und der *P. silvestris* (Abb. 4, 7, 10, Tab. II).

2. Die Reduktionsteilung der Pollenmutterzellen zeigt keine Unregelmäßigkeiten (Abb. 14).

3. Der Pollen der untersuchten Bäume ist fertil.

4. Zwischen Schwarzkiefer und *P. silvestris* bestehen Unterschiede in der Chromosomenlänge. Die statistische Auswertung der mitgeteilten Größen zeigt signifikante Unterschiede in der Neigung der Regressionslinien (die Chromosomenlänge ist eine lineare Funktion der Probeserien-Nummer) zwischen *P. nigra* und *P. silvestris,* wohingegen keine gesicherten Differenzen zwischen diesen beiden Arten einerseits und den drei Emmedsbo-Kiefern andererseits festgestellt werden konnten.

5. Sowohl die morphologischen als auch bis zu einem gewissen Grade die zytologischen Untersuchungen machen es möglich, die drei fraglichen Einzelkiefern mit hoher Wahrscheinlichkeit als Bastarde zwischen *P. nigra* ssp. *austriaca* und *P. silvestris* zu bezeichnen.

6. Eine gewisse Bastardwüchsigkeit ist an diesen Hybriden zu erkennen.

Résumé

Titre de l'article: *Recherches sur un type intermédiaire entre le pin noir d'Autriche et le pin sylvestre.* —

Il existe, dans la région d'Emmedsbo, près de Grenaa, dans l'Est du Jutland (Danemark), un peuplement de pins noirs d'Autriche où ont été remarqués trois arbres, qui, d'après leurs caractères morphologiques externes, sont différents des autres. Ils ressemblent à des hybrides entre le pin noir d'Autriche et le pin sylvestre et les recherches ont eu pour but de vérifier cette hypothèse. Les conclusions suivantes ont pu être apportées:

1. Les caractères morphologiques externes et la structure des aiguilles des trois individus étudiés sont intermédiaires entre le pin noir d'Autriche et le pin sylvestre (fig. 4, 7 et 10, tableau II).

2. Il n'existe aucune irrégularité dans la méiose des cellules mères des graines de pollen (fig. 14).

3. Le pollen des arbres étudiés est fertile.

4. Il existe une différence dans la longueur des chromosomes du pin noir d'Autriche et du pin sylvestre. L'interprétation statistique des données montre que les pentes des droites de régression (la longueur du chromosome étant une fonction linéaire par rapport au numéro dans la serie) sont significativement différentes pour le pin noir d'Autriche et le pin sylvestre, alors qu'il n'existe aucune différence significative d'une part entre les trois arbres d'Emmedsbo, d'autre part entre chacun de ces arbres et les deux espèces.

5. Sur la base des études de morphologie externe et aussi dans une certaine mesure d'après les recherches cytologiques, on peut conclure que les trois arbres sont probablement des hybrides entre le pin noir d'Autriche et le pin sylvestre.

6. Ces hybrides manifestent un certain hétérosis.

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On the Extraction of Forest-Tree Pollen from Inflorescences forced in a specially designed house

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When large series of controlled crossings are to be made, there is frequently risk of mixing the pollen from different trees, the catkins of which have been brought indoors at the same time for forcing.

To eliminate this source of error as completely as possible, and to increase the pollen yield, the Plant Breeding Station of the State Forestry, located at Humlebaek near Copenhagen, had in the year 1949 a house built and specially adapted for extraction of pollen.

Description of the Pollen-House

In this pollen-house (cf. *figs. 1, 2)* catkin-bearing twigs from each male are isolated in small, pollentight, glass-walled compartments, which need not be opened until the pollen is ready for collection.

Special arrangements are made for ventilation and renewal of water.

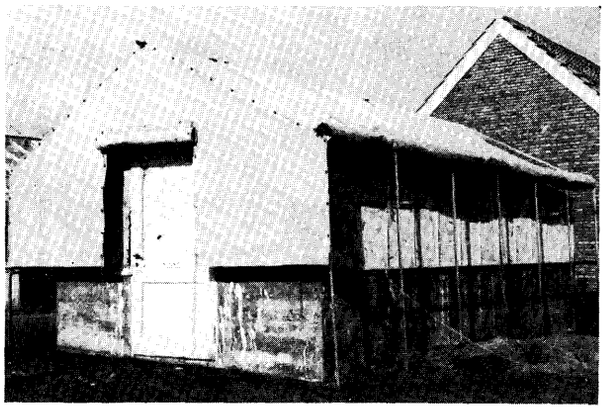

Fig. 1. — Pollen-house. Awnings for roof and gable ends. Side open.

The temperature in the pollen-house, or sections thereof, is regulated by awnings, supported by an iron framework and placed at a suitable distance from the glassroof and -walls to allow air to circulate freely between the awnings and the pollen-house.

The temperature inside each compartment is, within certain limits, controlled by means of infrared lamps.

The house is constructed on the principle of a greenhouse. Its outer base measurements are 3×6 m.

Within, on each side of a 1.5 m. wide central corridor, are 6 small glass-walled compartments, i. e. 12 seperate rooms for forcing and extraction of pollen.

Fig. 2 shows the corridor with compartments on either side. The floor of each compartment measures 103×80 cms. The height of the compartment towards the corridor is 126 cms., towards the outer wall 84 cms. All rabbets of the windows, etc., are sealed with foam-rubber strips.

Each compartment has two glass-doors opening on to the corridor. The lower glass pane of each door may be opened seperately *(fig. 3)*. In the outer wall of each compartment is a small glass window for ventilation, cf. *fig. 1*, where the ventilation windows are just discernible.

To ensure ventilation without risk of contamination by foreign pollen, the openings of the ventilation windows, as well as those of the single lower panes of the glass doors, may be covered by pollen-proof material (aerotex), stretched on special metal frames.

Two water containers for catkin-bearing twigs are placed in holes in the floor of each compartment. These containers are actually irrigators, each one with an outlet under the floor of the compartment. Through plastic tubes connected to the outlets the containers may be filled or emptied from the corridor without opening the compartments *(fig. 2)*.

To facilitate collection of pollen the floors of the compartments are covered with cellophane in which cuts are made for the containers. Foam-rubber strips are pressed around the edges of the cuts and the upper part of the containers to prevent entrance of extraneous pollen.

Use of the Pollen-House

Branchlets with male catkins are cut off at suitable lenghts, the end of the stems slightly pounded and put into the containers. These are then filled with water, the doors are closed and the forcing of the catkins may begin.

When the catkins are fully stretched and the anthers yellow, the forcing is finished, and the next step is to make the anthers discharge their pollen. This is done by a special extraction process.

The extraction process is best accomplished when the air-humidity is slightly reduced, but not so much that the anthers may shrink and their opening mechanism be destroyed. In order to reduce air-humidity the water in