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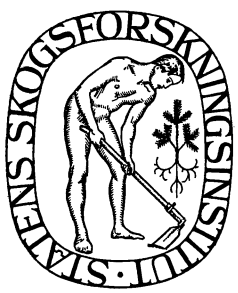

Karyotype Analysis of *Larix decidua* Mill. from Different Provenances

Karyotypanalys av olika provenienser hos europeisk lärk (Larix decidua Mill.)

by

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Introduction

European larch (*Larix decidua* Mill.) occurs naturally in five separate areas, the names of which are frequently used by foresters to distinguish geographical races of this species.

I. The *alpine* larch occurs within a range covering the largest area. It is found at altitudes between 400 metres and 2,000 metres in an area arching from the Maritime Alps over Switzerland and Italy to the Vienna Forest and northern Yugoslavia (TSCHERMAK 1935; v. WETTSTEIN 1946; MORANDINI 1956).

II. The *Sudetic* larch is found in altitudes between 400 metres and 800 metres in a very small area in northern Moravia and Silesia in the Jeseniky Mountains (HERRMANN 1933; RUBNER 1943; ŠIMAN 1943).

III. The *Slovakian* larch occurs within a range extending south of the highest Carpathian Mountains (High Tatra) within the boundaries of Slovakia at altitudes between 400 metres and 1,600 metres (ŠIMAN 1943; SIMAK 1958; BLATTNÝ & ŠŤASTNÝ 1959).

IV. The *Polish* larch is found in scattered stands within an area delineated in Fig. 1. This variety of larch is a lowland type occurring at altitudes between 200 metres and 600 metres (SZAFFER 1913; MAUVE 1932; MACIEJOWSKI 1956).

V. The *Romanian* larch occurs sporadically in the Romanian Carpathians. Larch growing in the Russian part of the Carpathians may also be considered as belonging to this group (GRINTESCU 1931; ŠIMAN *cit.*).

In these geographically and altitudinally separate areas, larch has differentiated itself into several morphologically and ecologically specific types which are often considered to represent different races or even separate species. This great variation in the characteristics of larch poses the question whether there are any morphological differences between the chromosomes of the various larch types or whether the species *Larix decidua* Mill. has a constant karyotype throughout its range. This question will be considered in detail in the present paper.

Material

Seed-lots used for the karyotype investigations were collected from eight provenances largely representing the natural area of distribution of the European larch (Fig. 1). The seed of each provenance was gathered from several trees under strict scientific control to make sure the origin and autochtony of the seed.

Table 1. Material.

Provenance	Long. E	Lat. N	Altitude m	1 000-seed weight, g.
St. Vincent	6°50′	44°45′	1 550	10,1843
St. Maria	10°25′	46°35′	1 600	7,6194
Cavedine	11°00′	46°00′	650	6,4521
Semmering	15°45′	47°35′	1 200	5,6383
Karlovice	17°27′	50°07′	650	3,7286
Štrbské Pleso	20°05′	49°07′	1 370	4,9645
Skarzysko	20°45′	51°15′	380	3,8550
Coltul Rosu	23°20′	46°20′	1 100	4,4557

In addition to the general data on origin, table 1 also presents the 1,000-seed weight of filled seeds in the material. Filled seeds were selected by X-ray photography (SIMAK & GUSTAFSSON 1954). The variation in the 1,000-seed weight is an example of the numerous morphological features differentiating the various larch types from each other (*cf.* BOUVAREL & LEMOINE 1958; GENYS 1960).

Methods

The present investigations were made on material obtained by squashing tips of roots developed from germinating seeds. The chromosomes were measured on five plates for each provenance; each plate originating from one root tip only. The mean values obtained from the measurements represent the karyotype of the provenance concerned. However, for confirmation of the results, several more plates were examined, although these data are not included in the statistical analysis.

A. *Techniques used for the cytological investigation*

1. Germination: The seeds were placed for germination on a moist filter paper in a Petri dish at room temperature.
2. Colchicine treatment: When the root, developed from the germinating seed, reached a length of 5 mm, the seed was transferred to another Petri dish containing filter paper moistened with an 0.1 per cent colchicine solution. Germination then continued for another 20—40 hrs at room temperature.

3. Fixing: The germinating seeds were transferred from the colchicine treatment to Carnoy's fixative (3 parts absolute alcohol, 1 part acetic acid and 1 part chloroform). Length of fixing time was 30 min.—16 hrs.
4. Storage of the material: If the fixed material could not be processed immediately, it was effectively stored in 70 per cent alcohol, even for two months.
5. Hydrolysis: After fixing or storage in alcohol the seeds were placed in a mixture of 2 parts alcohol and 1 part hydrochloric acid. Length of treatment at room temperature was 15—20 min.
6. Staining and squashing: Squashing directly in 2 per cent acetic-orcein solution usually sufficed to effect a deep staining of the chromosomes. Alternatively the material should be stained in orcein solution for 20—30 minutes before being squashed. If staining is still light, microscopic examination can be carried out in the phase contrast microscope (*cf.* BATTAGLIA 1957 and Fig. 2).
7. Semi-permanent slides: Slides of this kind may be produced easily by coating the edges of the cover glasses with nail polish. Air is thus prevented from entering between the cover glass and the slide.
8. Permanent slides: The semi-permanent slides are placed in acetone. When the cover glass is removed, the material is transferred to absolute alcohol for one minute before being permanented in euparol.

Expedient and simple, this method produced good results compared with other methods.

During the development of the method described above, several other procedures were tried, *e.g.* fixing according to LEVITSKY, hydrolysis in HCl (1 N), staining in various agents (acid carmine, Feulgen). However, none of these methods appeared superior to the one described.

B. Chromosome measurements

The plates considered most suitable for analysis were photographed at about 1,000 × magnification. In printing, the film was further enlarged to make the longest chromosome (I) on the plate about 5 cm long. If the chromosomes were placed at different levels on a plate satisfactory otherwise, pictures were taken at the various levels. The enlarged prints were again compared with the material under the microscope and all diffuse but important details of the picture of artifact nature (*e.g.* chromosome overlapping, chromosome breakages, vertical rises in the chromosomes etc.) were drawn on the photograph. After the chromosome contours had been outlined, the picture was set for measurements (Fig. 3).

Before the measurements, all the chromosomes were numbered from 1—24 (preferably 1—12 for the isobrachial chromosomes and 13—24 for the heterobrachial ones, Fig. 3). Numbering facilitates the subsequent search for the homologous chromosomes. Of each pair of homologous chromosomes, only the better one was measured by means of compasses, *i.e.* a free, straight chromosome was preferred to a bent one or to a chromosome overlapping its homologous partner. In questionable cases both the homologous chromosomes were measured to produce a mean value. Thus a haploid set of chromosomes was obtained showing the length of both the brachia and the satellite, if the latter was present. The regions of centromeres and constrictions were omitted from measurements.

C. Processing of data and results of measurements

Since the enlargements of the chromosome plates varied strongly for technical reasons, the absolute values obtained by measurements have been reduced to relative values to permit comparisons between the plates. The average chromosome of each plate (K_{a+b}) was chosen as standard (= 100) on the basis of the twelve chromosomes on each plate concerned. If I e, II e, III e, . . . are used to denote the length of chromosomes I, II, III, . . . , the average chromosome of this plate may be computed as follows

$$K_{a+b} = \frac{\text{I e} + \text{II e} + \text{III e} \ldots\ldots\ldots + \text{XII e}}{12}$$

The mean values of the short brachia (K_a) and the long brachia (K_b) were also computed in the same way for the average chromosome. All the twelve chromosomes of the plate were then related to this standard.

The reduced chromosomes in each plate were classified according to the following principles.

1. The chromosomes were placed in the order of their total relative length, the longest chromosome (I) first and the shortest chromosome (XII) last.
2. The chromosomes were arranged in an idiogram by placing their centromeres on a horizontal line (Fig. 8), always with the short brachium directed upward.
3. These principles could not be applied to chromosomes with secondary constrictions which were placed in one part of the idiogram (III, IV, VII) regardless of chromosome length. The chromosomes III and IV were always placed with the satellite upward; chromosome VII with the satellite downward.

Results

The FLORY graph (1936) of the phylogenesis of the gymnosperms showed a certain relationship between no. chromosomes and the taxonomic units of this subdivision. With some exceptions three basic chromosome numbers can be distinguished in the gymnosperms, *viz.* $n = 8$ or 9 for Cycadales, $n = 7$ for Gnetales, and $n = 12$ for Ginkgoales and Coniferales (chromosome numbers in the gymnosperms *cf.* DARLINGTON & JANAKI AMMAL 1945; SEITZ 1951; MEHRA & KHOSHOO 1956 a and b; LÖVE & LÖVE 1961). The two most important Swedish species, Scots pine and Norway spruce, and larch, which is introduced, belong to the class last mentioned.

The first information on the number of chromosomes in larch (*Larix decidua*) was presented by STRASBURGER 1892; BELAJEFF 1894; NĚMEC 1910, in all the cases with $n = 12$. SAX & SAX 1933 in their classical work on the chromosome morphology of the conifers, where 53 species were investigated, also presented an idiogram for the karyotype of *Larix decidua*. However, they mentioned nothing of the occurrence of secondary constrictions. In the same year HRUBÝ (1933) published a comparative study of the chromosomes of three larch races occurring in Czecho-Slovakia (*Larix decidua* Mill., *sensu stricto, Larix sudetica,* Dom., and *Larix polonica,* Rac.). HRUBÝ found no differences in the no. chromosomes between these three races, although it had been said previously that *Larix sudetica* is possibly tetraploid (*cit.* HRUBÝ). However, the morphological features of the chromosomes were not investigated. Making his observations on mitotic anaphase, HRUBÝ noticed clear secondary constrictions in one pair of the chromosomes. KNABEN (1953) presented a chromosome graph for *Larix decidua* principally agreeing with the idiogram of SAX & SAX. In both cases six of the twelve haploid chromosomes are heterobrachial and six are isobrachial. Similar conditions are also visible in a photograph of the somatic chromosomes of larch published by BARNER & CHRISTIANSEN (1960). KNABEN (*cit.*) also presented secondary constrictions in the two second biggest isobrachial chromosomes (as estimated from an idiogram and a drafted plate of somatic chromosomes) in her investigation of a triploid hybrid of *Larix decidua* and *Larix occidentalis*. The hybrid was produced artificially and described by SYRACH LARSEN & WESTERGAARD (1938). Polyploidy is otherwise rare among the conifers. In larch, however, a spontaneous autoploid has been found in addition to the alloploid mentioned above, *viz.* the tetraploid larch (*Larix decidua* Mill.) from Denmark analyzed cytologically by CHRISTIANSEN (1950).

Primarily, none of the authors cited here, except SAX & SAX, intended to investigate the chromosome morphology of larch. Moreover, the investigations were often based on rather scanty data.

Summarizing, we may conclude from the information mentioned above:
a. the basic number of chromsomes in *Larix decidua* Mill. is 12;
b. six of the chromosomes are isobrachial and six are heterobrachial (no data on the length of the chromosomes, however, are available);
c. the number of chromosomes with constrictions is reported to be 1—2.

Concerning the twelve chromosomes the following morphological features have been specially investigated in the present paper, keeping in view the specificity of the eight provenances:
1. the relative length of the chromosomes;
2. the position of the centromeres;
3. the occurrence of constrictions.

Relative length of the chromosomes

Table 2 shows the average length of each individual chromosome in relation to the mean chromosome length of the karyotype ($K_{a+b} = 100$). This relative length of a certain chromosome varies greatly between the provenances, *e.g.* the length of chromosome I measures 140 units and 148 units for the provenances of Skarzysko and Karlovice, respectively. Yet these differences between the provenances are non-significant for any one of the twelve chromosomes as shown by the analyses of variance conducted separately for each chromosome of the karyotype (*cf.* F-values in table 2).

Since no definite differences in the karyotype of larch have been stated between the eight provenances with respect to the relative length of the twelve chromosomes, the statistical data have been processed uniformly. The largest chromosome (I) of a karyotype representing the entire material is thus 144 units and the shortest chromosome (XII) is 67 units.

Differences in length between two neighbouring chromosomes in the karyotype are shown in the following series: I-16-II-4-III-6-IV-2-V-10-VI-18-VII-2-VIII-7-IX-5-X-3-XI-4-XII. Major differences occur only between the chromosomes I and II (16 units) and between the chromosomes VI and VII (18 units). Elsewhere, it will be difficult to identify the various chromosomes of a plate on the basis of the relative length only.

As an illustration consider the two chromosomes II and III. Chromosome III shows a characteristic constriction not appearing in II. It is therefore possible to distinguish clearly between these two chromosomes.[1] However, 40 measurements of the lengths of the chromosomes showed that II was longer than III in 26 cases, and smaller than III in 10 cases, whereas the two chromosomes appeared to be equal in 4 cases. Thus, if chromosome III had not displayed

[1] The chromosomes II and III have here been tacitly assumed not to be mistaken for any one of the remaining ten chromosomes.

any distinguishing constriction and if the relative length had been the only criterion available, an erroneous classification would have been made in 10—14 cases. This would also effect the mean length values of the chromosomes concerned. Differences in length between the chromosomes would appear greater than those obtained by correct identification.

Assuming that the true lengths (population averages) of the chromosomes I—XII form a decreasing sequence, the appearance of a case where a chromosome with higher number is longer than a chromosome with lower number will here be called a *reversal of order*. These reversals of order may be due to the biological variation and/or the contraction effect, inaccuracy in the length measurements of bent or broken chromosomes etc. It is hardly possible with techniques now used in cytological studies to eliminate those variations in chromosome length which cause these reversals. This is particularly the case with the long chromosomes of the conifers.

On the basis of a statistical assumption, however, it is possible to compute the probable frequency of reversals of order between two chromosomes of certain mean lengths and to judge on the basis of this frequency to what extent the difference in the length of the chromosomes is influenced by such reversals.

An estimate of this kind established that the length differences between chromosomes I and II (16 units) and between chromosomes VI and VII (18 units) are significant. KNABEN (1953), however, stated that the chromosomes VI, VII, and VIII are equally long. This being the case, there would still be no difficulty in distinguishing these chromosomes from each other since they are separable on the basis of other morphological features reported later. The second greatest length differences occur between the chromosomes V and VI (10 units) and between the chomosomes VIII and IX (7 units). Considering the variation of observed differences, only the last one is significant. The chance of reversal of order between the chromosomes V and VI was calculated at 21 per cent and at 5 per cent between the chromosomes VIII and IX. The chromosomes IX—XII being quite equal in length, it is impossible to distinguish the chromsomes from each other on the basis of length only.

Calculations of reversals of order in karyotype investigations of somatic tissues are complicated since a comparison between two chromosomes must always take into consideration the occurrence of homologous chromosomes, *i.e.* a total of four chromosomes may be involved in a reversal of order. It may therefore be preferable in gymnosperm analysis to make haploid tissues, *e.g.* endosperm, the object of karyotype investigations. The statistical theory supporting the computation of reversals of order will be published in a separate paper (MATÉRN & SIMAK).

On the basis of the present investigation, it may be pointed out that special attention should not be paid to the minor differences in length between neighbouring chromosomes unless the potential occurrence of reversals of order is also simultaneously considered.

Position of the centromere in the chromosomes of larch

The twelve chromosomes of larch from all the eight provenances may be divided into two groups on the basis of the position of the centromere; the longest six isobrachial chromosomes have median centromeres and the six heterobrachial chromosomes have subterminal centromeres. Chromosomes with subterminal, submedian and median centromeres are differentiated by means of the following values of their brachial ratios (short/long): < 0.50, 0.50—0.75, and 0.75—1.00 respectively, or, as in table 3, by the corresponding length of the short brachium expressed in per cent of the total length of the chromosome: < 33.3 per cent, 33.3—42.8 per cent, and 42.8—50.0 per cent respectively. To compute the position of a centromere of an isobrachial chromosome correctly, it is necessary to identify exactly both the chromosome brachia. In the chromosomes III and IV an identification is possible due to their having secondary constrictions in one brachium. The chromosomes I, II, V, and VI are distinguishable by measurements of one long brachium and one short brachium. However, the identity of the brachia is not therefore ascertained. Since the length of the brachia in a specimen varies on account of uneven contraction and brachial bends, chromosome breakages etc., the chromosome brachium which is actually longer, may appear shorter in the specimen and vice versa (brachial reversal). The more median a centromere, the more frequent a brachial reversal. An identification of the brachia of an isobrachial chromosome is therefore quite uncertain if the brachial length only is used as a criterion of differentiation. The following example further elucidates the point.

The average position of the centromere in chromosome III may be computed in two ways ($n = 40$).

1. with reference to constriction:

$$\frac{\text{the brachium with constriction} \times 100}{\text{total length of the chromosome}} = 48.5\ \%$$

2. without reference to constriction:

$$\frac{\text{the "shorter" brachium} \times 100}{\text{total length of the chromosome}} = 47.6\ \%$$

The position of the centromere in chromosome III correctly computed (example 1) indicates that the brachium with constriction averages 48.5 per cent of the total length of the chromosomes. In nine cases of 40 this brachium

Table 2. Relative length of the chromosomes.

Provenance	Chromosome Nos.											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
St. Vincent	147	126	119	119	112	102	94	87	81	74	72	67
St. Maria	144	128	125	120	116	106	89	85	77	73	69	67
Cavedine	141	126	121	116	115	109	90	86	79	74	72	68
Semmering	144	127	125	120	115	104	89	86	78	75	71	66
Karlovice	148	126	125	117	119	107	84	86	79	74	69	66
Štrbské Pleso	143	131	126	115	117	109	86	84	77	74	70	68
Skarzysko	140	126	124	119	119	106	89	85	80	77	73	65
Coltul Rosu	146	130	126	117	115	103	86	88	77	74	70	67
Mean value	144	128	124	118	116	106	88	86	79	74	71	67
F-values	0.59	0.29	0.75	0.50	0.92	1.68	2.21	0.48	0.92	0.71	0.76	0.38

The F-values pertain to the analysis of variance for the length of each chromosome, *between provenances*.

Levels of significance ($v_1 = 7$; $v_2 = 32$)

* 5 per cent 2.31
** 1 » » 3.25
*** 0.1 » » 4.72

Table 3. The short brachium* expressed in per cent of the total length of the chromosome.

Provenance	Chromosome Nos.											
	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
St. Vincent	49	47	48	49	44	46	25	24	27	29	31	31
St. Maria	48	47	52	48	45	47	25	25	27	29	30	31
Cavedine	47	46	48	49	47	48	25	27	29	31	32	31
Semmering	49	45	51	49	45	45	26	26	29	30	30	32
Karlovice	49	49	50	48	49	45	26	26	28	30	31	32
Štrbské Pleso	47	46	48	48	46	48	25	25	27	29	30	31
Skarzysko	49	45	47	49	48	48	26	25	28	28	30	33
Coltul Rosu	46	45	45	48	48	47	27	26	28	30	30	32
Mean value	48	46	49	49	47	47	26	26	28	30	31	32

* Exc. the chromosomes III och IV where the brachium with satellite has always been related to the total length of the chromosome.

was longer than the other. Thus, if the constriction would not appear, this brachium would be mistaken for the longer chromosome brachium (brachial reversal) in nine cases and the position of the centromere would then have been 47.6 per cent according to the second example. If the brachia cannot be identified exactly, a consistent and erroneous reduction of the short brachium and an elongation of the long brachium occurs at the computation of the relationship between the "short" brachium and the total length of the chromosome in all cases with brachial reversals. The centromere is consequently "moved" from median to submedian position. This condition also pertains to the chromosomes I, II, V, and VI. Their centromere position would actually have been more median than that represented by the value computed.

Theoretically, the computation of the frequency of brachial reversals is based on the same assumptions as those which were applied to reversals of order in the previous discussion. If details are omitted (*cf.* MATÉRN & SIMAK), the following requirements may be established in this karyotype analysis to secure a significant difference in length between the long brachium (m_1) and the short brachium (m_2) of a chromosome, on the basis of 40 measurements:

$m_1 - m_2 > 12$ per cent of the total length of the chromosome, *i.e.* the short brachium should not exceed 44 per cent of the total length of the chromosome.

None of the isobrachial chromosomes fulfilled these requirements as shown by the compilation below

Chromosome	I =	10.2	per cent
,,	II =	7.5	,, ,,
,,	V =	6.8	,, ,,
,,	VI =	6.4	,, ,,

Not even the great difference in the brachial length of chromosome V, provenance St. Vincent (table 3), is significant. The average difference (12.4 units) between the short brachium (49.7 units) and the long brachium (62.1 units) is only 11.2 per cent of the total length of the chromosome (111.8 units). Since these values are based on five observations only, the requirements of significance must be higher than for 40 observations.

The example shows that it is practically impossible to calculate exactly the centromere position of median and some submedian chromosomes.

The chromosomes VII—XII are heterobrachial. The brachia of each chromosome differ clearly from each other with respect to length and no brachial reversal occurs at the measurements. An analysis of variance was made for each chromosome in this group to investigate whether there are any differences between the provenances with respect to brachial relations (short brachium in per cent of the total length of the chromosome). This was not

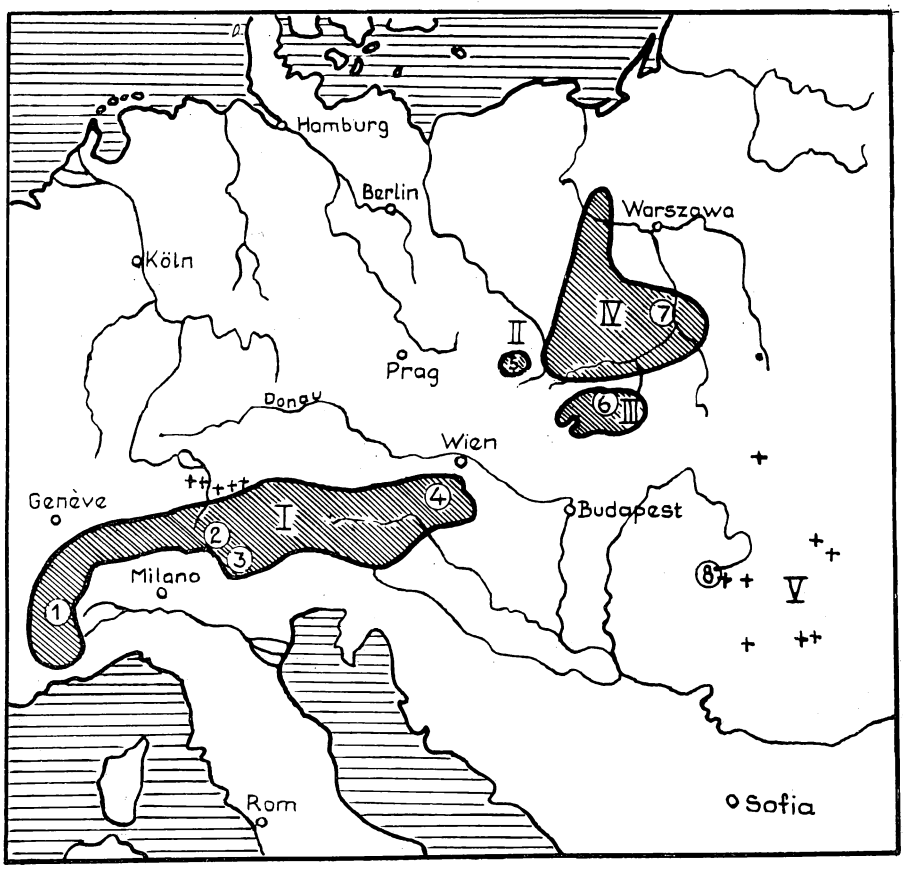

Fig. 1. The internal occurrence of European larch delineated by heavy lines (after RUBNER). I. Alpine larch, + sporadic occurrence. II. Sudetic larch. III. Slovakian larch. IV. Polish larch. V. Romanian larch, + sporadic occurrences. 1—8 mark the locality of the provenances investigated. 1. St. Vincent, 2. St. Maria, 3. Cavedine, 4. Semmering, 5. Karlovice, 6. Štrbské Pleso, 7. Skarzysko, 8. Coltul Rosu.

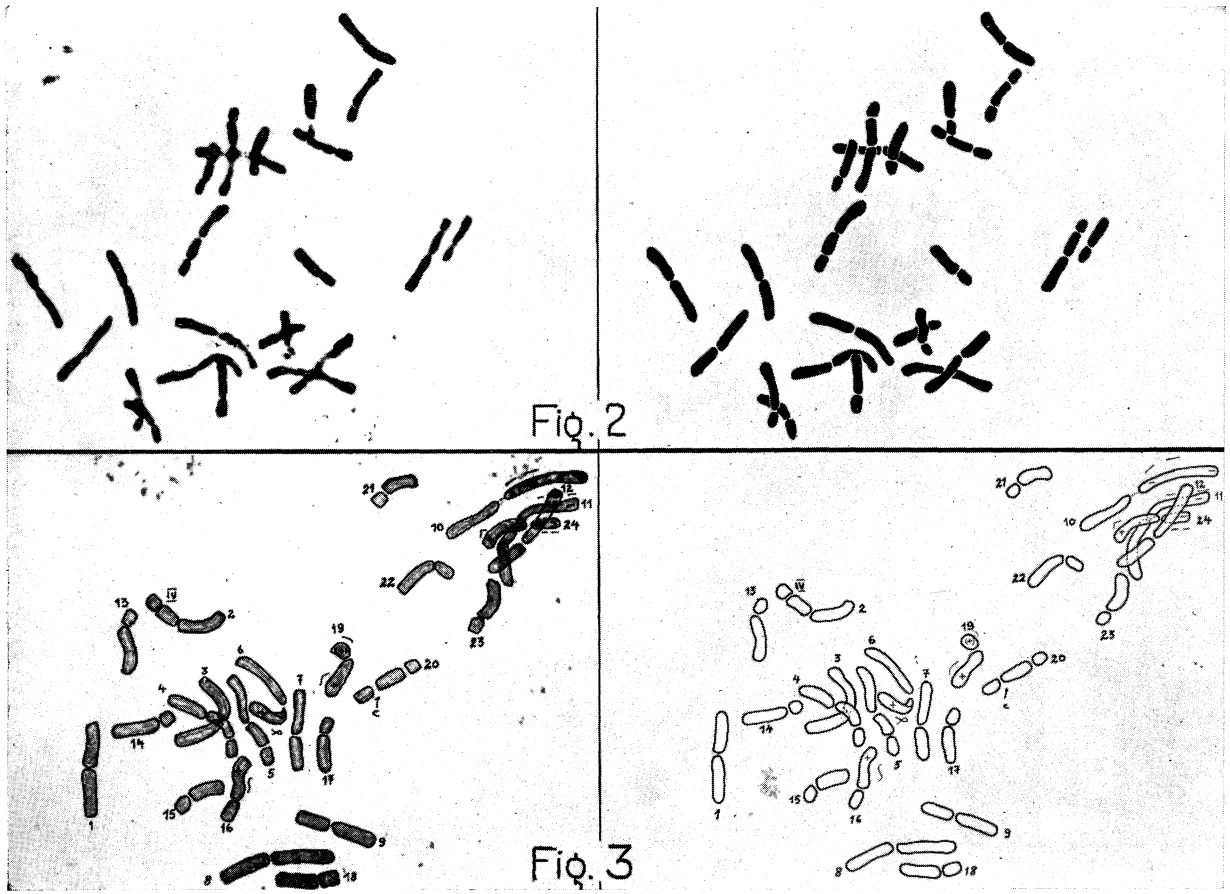

Fig. 2. Provenance Cavedine. Picture taken by phase contrast since orcein staining of the chromosomes appeared very light.

Fig. 3. Provenance Semmering. Example showing the method of drafting on a photographic underlay. All the overlappings, breakages, stretchings and other details as well as chromosome contours featured on the outlined picture (right) were drawn directly on to the photograph (left) when compared with the slides under the microscope.

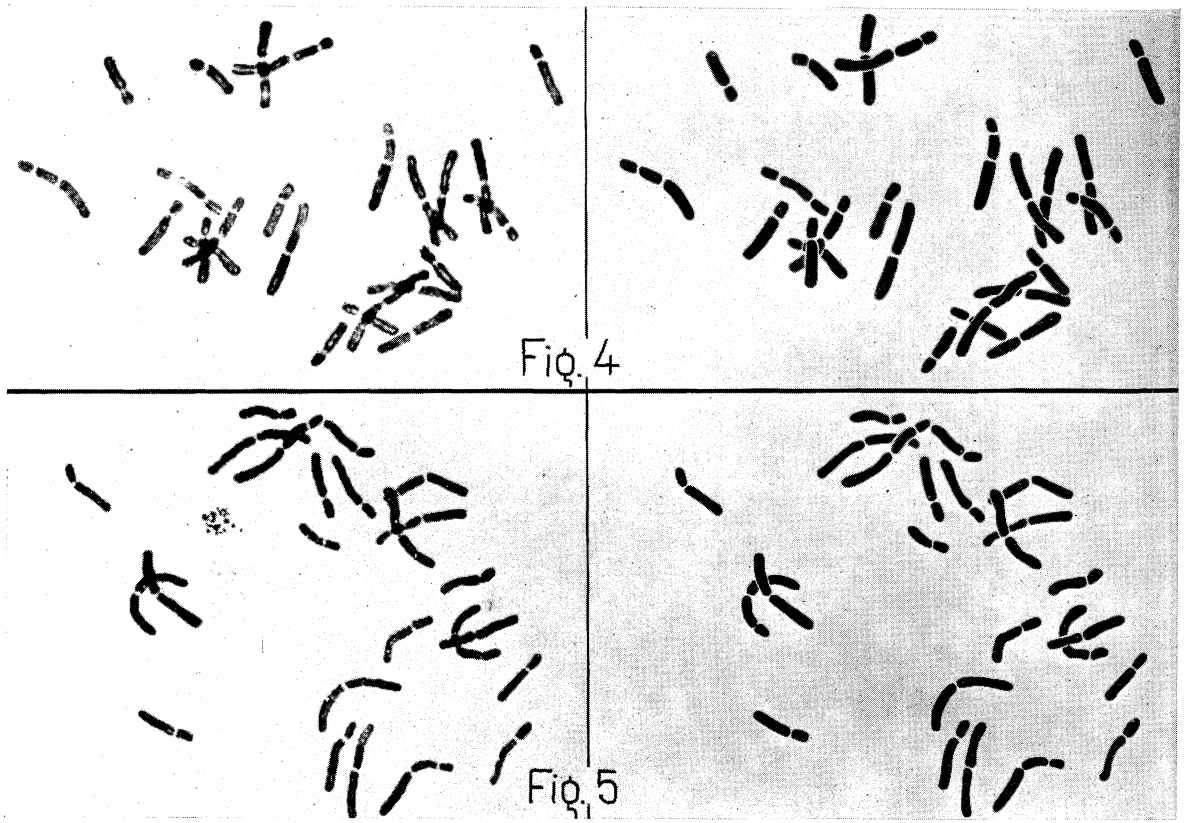

Fig. 4. Provenance St. Vincent. A plate where all three pairs of constrictions are clearly visible.
Fig. 5. Provenance Štrbské Pleso. Slide fixed in the Östergren fixative and stained with Feulgen. The constriction in chromosome VII did not appear. If method described in this paper is used, the constriction shows clearly in this material.

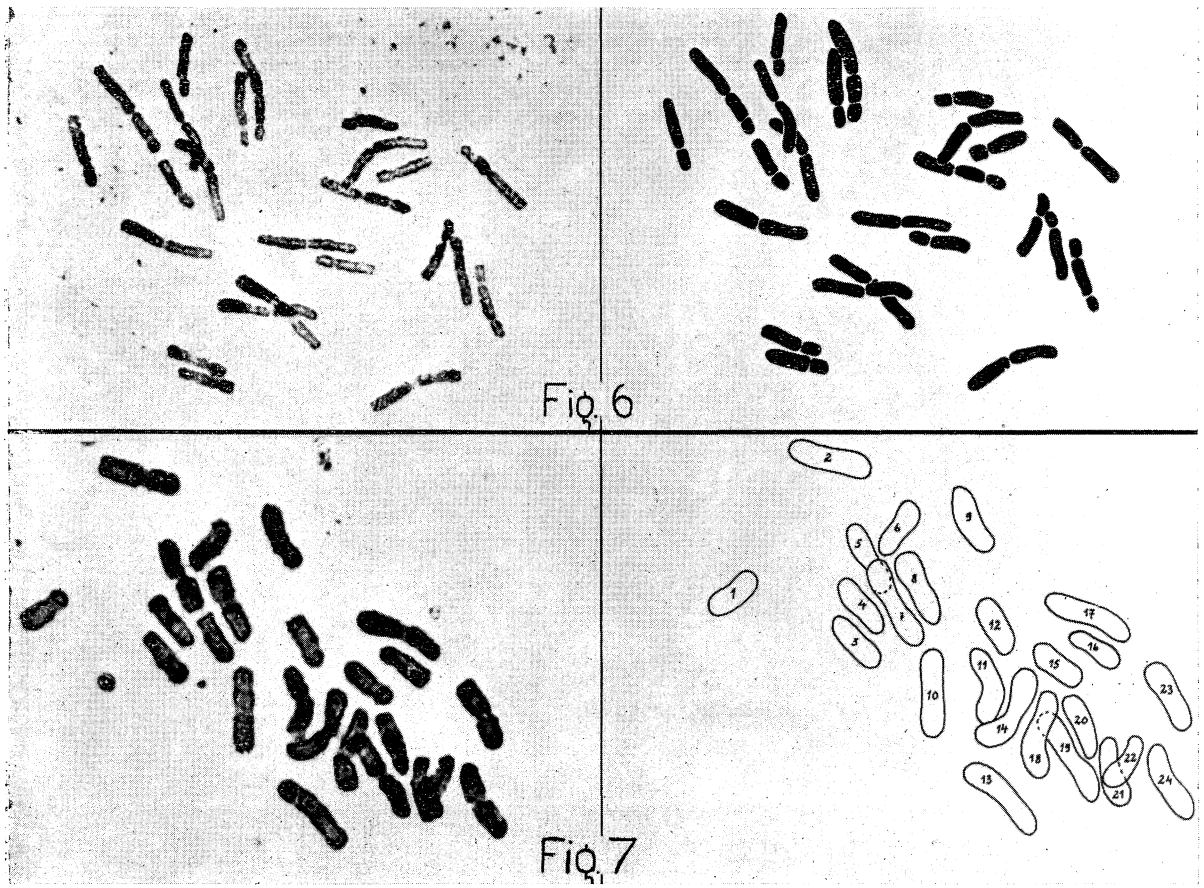

Fig. 6. Provenance Coltul Rosu. In this plate a constriction could be detected only in each of the homologes IV and VII.
Fig. 7. Provenance Skarzysko. After long treatment with colchicine the chromosomes contract quite considerably. This condition is suitable for investigations of the numbers of chromosomes.

the case and the entire material was therefore processed further without attention to the variation between provenances.

The short brachium is approximately equally long in all the six chromosomes, *i.e.* this brachium expressed in per cent of the total length of the chromosome becomes larger in the short chromosomes and vice versa (VIII: 25 per cent; XII: 32 per cent, *cf.* table 3). However, these morphological differences between the chromosomes VII—XII cannot be used for diagnostic purposes when the differences in brachial relations are small and uncertain on account of the high frequency of reversals of order among the chromosomes.

Occurrence of constrictions

Secondary constrictions and others may be of great diagnostic value if they occur consistently in certain chromosomes of the karyotype. These constrictions, however, need not always appear clearly presumably on account of *i.a.* technique used for specimen preparation (LEVAN 1946). Prechilling of the material may sometimes induce heterochromatic chromosomal constrictions that would otherwise remain invisible with the normal technique of preparation (*cf.* DARLINGTON & LA COUR 1940). Limited experiments with prechilled (+ 2° C) germinating seeds of larch, however, did not produced similar results in the present investigation.

Constriction-like cross stripes which occur irregularly in the chromosomes are artefacts and cannot be used for diagnostic purposes (*cf.* SAYLOR 1961). In cases of this kind the real constrictions, too, lose their diagnostic value since they cannot with certainty be distinguished from the artificial constrictions.

Constrictions have been observed in the chromosomes III, IV, and VII of larch from all the eight provenances with such a consistency that they may serve as a specific feature of the chromosomes concerned. Each constriction separates a rather long satellite. The satellites discussed here are not to be confused with the classic, small satellites first described by NAVAŠIN (1912) with the Russian name "sputnik" but they are oblong, distal parts of chromosomes separated by constrictions that may be rather extended if the chromosomes are not contracted in some way (*cf.* HRUBÝ *cit.*). The satellites of larch rather correspond to the type that was called "linear satellites" by BATTAGLIA (1955). TISCHLER (1942), however, made no distinction between the two types of satellites. Likewise, NATARAJAN *et al.* (1961) denoted the long satellites of *Pinus silvestris*, which are quite similar to those of larch, as "satellites". In agreement with TISCHLER and NATARAJAN *et al.* and for the purpose of avoiding confusion of terminology with new terms, the constricted distal part of the larch chromosomes is here called "satellite".

Chromosome III.—The constriction in this chromosome may be identified

without difficulty in each plate of both the homologues. Since the number of nucleoli is mostly 3—5 in the interphase stage, this most clearly appearing constriction in larch chromosomes may quite probably be a nucleolus-organizer as well. The length of the satellite varies between the provenances from 32 per cent to 38 per cent of the length of the satellite brachium, difference in no case being significant. The average length of the satellite is thus 35 per cent for all the provenances. This is probably the satellite that HRUBÝ (1933) observed in his anaphase plates and which he recorded to be a fifth to a fourth of the total length of the chromosome. The length of the constricted part was reported to be one third of the length of the satellite. It may be pointed out in this context that HRUBÝ made all his observations on chromosomes not contracted artificially. KNABEN (*cit.*) made a drawing of the two satellites from the two second longest chromosomes in the larch karyotype. In both cases satellites were placed on the long brachium of the chromosomes. KNABEN's results cannot be discussed further since information mentioned is obtained only from a chromosome drawing (KNABEN, fig. 3) and no satellite occurrence at all is mentioned in the text. It is probable that the satellited chromosomes presented by KNABEN are identical with the satellited chromosomes III and IV described in this paper.

Chromosome IV.—This chromosome is only six units shorter than chromosome III. Both the chromosomes also have a constriction in the short brachium. The constriction of chromosome IV, however, is much more diffuse. Being more narrow and not identifiable on each plate, it is mostly difficult to process in both the homologues (it is clearly visible, however, in both the homologues shown *e.g.* in fig. 4). This constriction is probably such a heterochromatic region the appearance of which is often dependent on the technique of preparation used. Chromosome IV is not narrower in the neck of constriction as is the case in the chromosomes III and VII. These differences in the constrictions provide possibilities to distinguish chromosome IV from chromosome III. The type of this constriction may be called a tertiary constriction (*cf.* BURNHAM & HAGBERG 1956). The length of the satellite of chromosome IV is about 32 per cent. No further statistical investigations have been made since the satellite is visible only in 70 per cent of all the plates investigated and then mostly in one of the homologues only.

Chromosome VII.—While the chromosomes III and IV have their constrictions in the average short brachium, the heterobrachial chromosome VII has its constriction in the long brachium. The constriction is quite clear and it has been identified on each plate though not always in both the homologues. In this case, too, technique used in the cytological investigation will decide whether the constriction is to appear clearly or not (*cf.* fig. 5). This may be an explanation why HRUBÝ and KNABEN did not observe this characteristic

constriction of chromosome VII. The length of the satellite averages 33 per cent of the long brachium for all the provenances. The differences in satellite length between the provenances (31 per cent—36 per cent) are not significant. The length of the satellite and that of the short chromosome brachium is 25 per cent and 26 per cent of the total length of the chromosome, respectively. This symmetrical position of the centromere and the secondary constriction may lead to mistaking the satellite for the short brachium of the chromosome. The satellite, however, is tapering more towards the constriction than does the chromosome brachium towards the centromere. Yet, potential reversals play no major rôle at a purely morphological analysis. Chromosome VII always has the same appearance whichever way it is turned, the satellite and the short brachium being equally long (*cf.* fig. 8).

Description of the karyotype of larch

The investigation has not revealed any morphological differences between the karyotypes of the eight provenances. It is therefore possible on the basis of the entire material of investigation to establish a karyotype fundamental for the entire species *Larix decidua* (fig. 8).

The indivual chromosomes of the karyotype may be classified into three groups according to identification difficulties:

1. easily identified chromosomes: I, II, III, (IV), and VII;
2. less easily identified chromosomes: V, VI, and VIII;
3. not easily identified chromosomes: IX, X, XI, and XII.

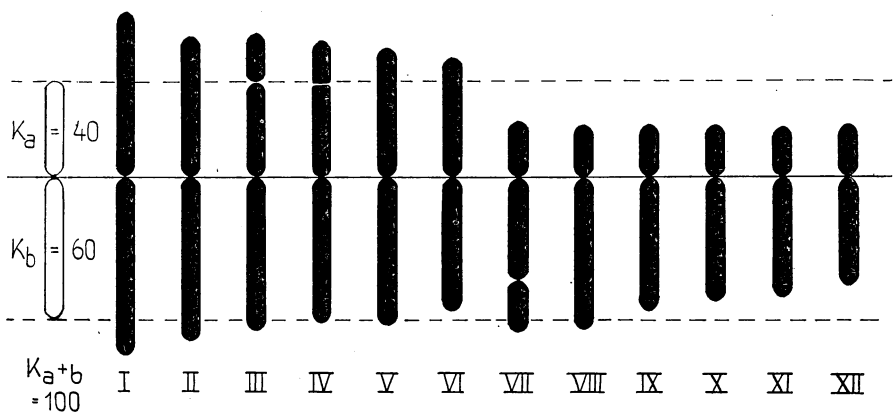

Fig. 8. Karyotype of *Larix decidua*, Mill. Each chromosome length in the idiogram is calculated on the basis of 40 measurement from 8 provenances. The chromosomes are arranged according to the total length, the longest chromosome (I) first and the shortest one (XII) last. The outlined chromosome on the left side of the idiogram is the average chromosome of the karyotype (K_{a+b}), vide text p. 6.

All the chromosomes are put in relation to this standard. The difference in constriction between the chromosomes III and IV is exaggerated.

1. *Chromosome I* is the longest chromosome of the karyotype. Due to its length, 144 units, it differs clearly from the second biggest chromosome. The centromere is median.

Chromosome II has a relative length of 128 units and displays a median centromere.

Chromosome III has a relative length of 124 units and displays a median centromere and an easily visible, rather long, secondary constriction. The long satellite (35 per cent of the short brachium) readily distinguishes this chromosome from the previous one.

Chromosome IV has a relative length of 118 units and it displays a median centromere. Chromosome IV has a constriction in the short brachium showing as a narrow, light cross stripe over the entire width of the chromosome. This chromosome is consequently not narrower in the constriction neck in contrast to chromosome III which displays a clear tapering in the region of constriction. The length of the satellite is 32 per cent of that of the short brachium. It is impossible to distinguish chromosome IV from chromosome V if, for some reason, the constriction cannot be detected in the former one.

Chromosome VII has a relative length of 88 units. The chromosome is heterobrachial and it displays a subterminal centromere. There is a constriction in the long brachium which separates a satellite measuring 25 per cent of the entire length of the brachium. Since this is the only chromosome in the heterobrachial group that has a satellite, it is easily identified.

2. *Chromosomes V and VI* have a relative length of 116 units and 106 units respectively. Both the chromosomes display a median centromere. On plates where chromosome IV can be identified due to the presence of a constriction, it is possible with some certainty to distinguish these two chromosomes from each other.

Chromosome VIII has a relative length of 86 units. Except for chromosome VII, which displays a secondary constriction, chromosome VIII is the longest chromosome in the heterobrachial group. Difference in length from the second longest chromosome in the group, IX, is only 8 units but this difference is sufficient in a good specimen for a distinction between chromosome VIII and the others in the group IX—XII. The identification of these chromosomes is also facilitated by the fact that the variation in the length of the short chromosomes is less than that of the long ones. Of course, the possibility of reversal of order between chromosome VIII and the chromosomes IX—XII cannot be neglected.

3. *Chromosomes IX, X, XI, and XII.* These four heterobrachial chromosomes are difficult to distinguish from each other. Differences in length between the chromosomes are quite slight and there are no other morphological features that can be used as distinguishing marks.

Comparison with results of other investigators

The literature cited above with respect to the karyotype of larch contains no direct data on the length of the chromosomes. Data of this kind can only be procured by measurements on the published pictures of chromosomes. This procedure, however, is rather incorrect since little is known of the accuracy with which the pictures concerned are drawn. Moreover, the measurements are very difficult, particularly in small idiograms.

Of investigations concerning the karyotype of larch, the idiogram (fig. 8) published by SAX & SAX (*cit.*) seems to be the most suitable one for comparison with results obtained from the present study. To this end the values of the length of the chromosomes in both the karyotypes must be transformed to a common denominator. This has been done in two ways:

a. the total length of each chromosome is related to the longest chromosome of the plate;
b. the total length of each chromosome is related to the average chromosome of the plate.

a. The length of the individual chromosomes of the plate is usually related to a certain standard chromosome (= 100), mostly the longest chromosome. This procedure is disadvantageous if the length of this chromosome cannot be determined correctly for some reason *e.g.* because the chromosome is bent, stretched or interlaced with other chromosomes. The longest chromosome being the one which is mostly apt to such unwanted variations in length, this chromosome is least suited as a standard. TJIO & HAGBERG (1951) as well as GELIN & BLIXT (1956) have therefore computed the length of the chromosomes in per cent of the total length of all chromosomes in the karyotype.

b. The average chromosome (K_{a+b}) of the karyotype introduced in this investigation is more suited as a standard than the longest chromosome of the plate. This is apparent from the following comparison between the two procedures.

The longest chromosome was set at 100:

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
SAX & SAX.....	100	95	95	92	92	78	76	70	60	58	58	56
SIMAK.....	100	89	86	82	81	74	61	60	54	51	49	47
Difference.....	±0	+6	+9	+10	+11	+4	+15	+10	+6	+7	+9	+9

According to this comparison, the chromosomes in the karyotype published by SAX & SAX seemed to be longer than the values presented by SIMAK. These differences have occurred because the standard chromosome (I) used by SAX & SAX is quite short in comparison with the one used by SIMAK.

With this procedure the difference in the length of chromosome no. I will affect the entire karyotype.

If the average chromosome of the karyotype is chosen as a standard, the following comparison is obtained:

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
SAX & SAX	128	121	121	118	118	100	97	90	77	74	74	72
SIMAK	144	128	124	118	116	106	88	86	78	74	71	67
Difference	—16	— 7	— 3	± 0	+ 2	— 6	+ 9	+ 4	— 1	± 0	+3	+5

It is only the longest chromosome (I) that has clearly different length in both the karyotype analyses (difference 16). The other chromosomes (II—XII) generally agree in the two investigations and the differences are hardly significant considering the great variation of each mean value.

The unusually good agreement (except chromosome I) is remarkable since SAX & SAX made their observations on chromosomes not contracted artificially.

Additional conclusions cannot be drawn from the comparison since the number of plates investigated by SAX & SAX is unknown. Moreover, no considerations have been taken in the analysis of SAX & SAX to the occurrence of constrictions and potential reversals of order.

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Summary

This karyotype analysis concerns eight provenances of *Larix decidua* which are often considered as different races on account of their rather great differences in ecological and morphological features. The seed material was collected from the West Alps, Central Alps, East Alps, South-East Alps region, Sudetic mountains, Slovakia, Poland, and Romania (table 1). Root tips from germinating seedlings were used for cytological investigations. The following morphological features of the chromosomes of larch were studied:

a. chromosome length,
b. centromere position, and
c. occurrence of constrictions.

The results in short are presented below:

1. Since no morphological differences in the karyotype between the eight provenances have been stated, a fundamental karyotype of *Larix decidua* can be established (fig. 8).
2. Of the twelve chromosomes of larch, haploid set, six are isobrachial and six are heterobrachial. The total length and the brachial relationship (the centromere position) have been determined for each chromosome (tables 2 and 3). Three of the chromosomes have satellites.
3. By means of the morphological criteria mentioned above, a—c, five chromosomes can be identified exactly (chromosomes I, II, III, (IV), and VII), three of the chromosomes are more difficult to recognize (V, VI, and VIII), whereas the remaining chromosomes IX—XII cannot be distinguished from each other.
4. Special attention has been paid to the so-called reversals of order and brachial reversals. The reversals of order occur when attempts are made at distinguishing two chromosomes from one another, which are nearly equal in length, only on the basis of their total length measurements. Due to the variability of length, it may then occur that the chromosome which is really longer, may appear shorter on the plate and vice versa. The identity of the two chromosomes is then confused if based only on the length measurements. A reversal of order occurs.

Similar conditions occur in the case of chromosomes with median centromeres if attempts are made at identifying the rather equally long brachia only by means of differences in length. Such a determination may easily lead to brachial reversals.

Since reversals of order of entire chromosomes and of brachia are of principal importance in karyotype investigations, they deserve careful attention. This topic will be discussed further from a theoretical aspect in a following paper.

Literature

BARNER, H. & CHRISTIANSEN, H. 1960. The Formation of Pollen, the Pollination Mechanism, and the Determination of the Most Favourable Time for Controlled Pollinations in *Larix*.—Silvae Genet. 9: 1: 1—11.

BLATTNÝ, T. & ŠŤASTNÝ, T. 1959. Prirodzené rozšírenie lesných drevín na Slovensku.—Slovenské vydavatelstvo pôdohospodárskej literatúry.—Bratislava 1959.

BURNHAM, C. R. & HAGBERG, A. 1956. Cytogenetic Notes on Chromosomal Interchanges in Barley.—Hereditas 42: 467—482.

BATTAGLIA, E. 1955. Chromosome Morphology and Terminology.—Caryologia 8: 1: 179—187.

— 1957. La microfotografia al contrasto de fase nello studio della morfologia cromosomica.—Caryologia 10: 1: 46—54.

BELAJEFF, W. 1894. Zur Kenntnis der Karyokinese bei den Pflanzen.—Flora 79.

BOUVAREL, P. & LEMOINE, M. 1958. Races du mélèze et grosseur des graines.—Rev. Forest. Franç. 5: 348—350.

CHRISTIANSEN, H. 1950. A Tetraploid *Larix decidua* Mill.—Kgl. Danske Videnskab. Selskab. Biol. Medd. 18: 9.

DARLINGTON, C. D. & JANAKI AMMAL, E. K. 1945. Chromosome Atlas of Cultivated Plants.—George Allen & Unwin Ltd, London 1945.

DARLINGTON, C. D. & LA COUR, L. 1940. Nucleic Acid Starvation of Chromosomes in *Trillium*.—J. Genet. 11: 1 & 2: 185—213.

FLORY, W. S. 1936. Chromosome Numbers and Phylogeny in the Gymnosperms.—J. Arnold Arboretum 17: 83—89.

GELIN, O. E. V. & BLIXT, S. 1956. The Karyotypes of Two X-Ray Mutants in Peas and Their Ancestors.—Agri. Hort. Genet. 14: 148—160.

GENYS, J. B. 1960. Geographic Variation in European Larch.—New Hampshire Forestry and Recreation Comm., Concord, New Hampshire 1960.

GRINTESCU, J. 1931. Le problème du Mélèze dans les Carpathes roumaines.—Guide 6me Excurs. Phytogeograph. Intern. Roumaine 1931, pp. 82—89.

HERRMANN, E. 1933. Die Sudetenlärche.—Tharandter forstl. Jahrb. 84.

HRUBÝ, K. 1933. Rozdíly československých modřínú.—Věstník Královské České Společnosti. Nauk. Třída Matemat.—Přírodovědecká 1933.

KNABEN, G. 1953. En cytologisk analyse av diploid og triploid *Larix*.—Blyttia 11: 105—115.

LEVAN, A. 1946. Heterochromaty in Chromosomes during Their Contraction Phase.—Hereditas 32: 449—468.

LÖVE, Á. & LÖVE, D. 1961. Chromosome Numbers of Central and Northwest European Plant Species.—Opera Botan., Societ. Botan. Lundensi. Suppl. Bot. Not. 5.

MACIEJOWSKI, K. 1956. Modrzew w lasach polskich.—Sylwan 11: A:6—50.

MAUVE, D. 1932. Die polnische Lärche.—Mitt. deut. Dendrol. Ges. 44: 359—363.

MEHRA, P. N. & KHOSHOO, T. N. 1956 *a*. Cytology of Conifers. I.—J. Genet. 54: 165—180.

— 1956 b. Cytology of Conifers. II.—J. Genet. 54: 181—185.

MORANDINI, R. 1956. Il Larice nella Venezia Tridentina.—Pubbl. staz. sperimentale selvicoltura, Firenze 1956.

NATARAJAN, A. T., OHBA, K., & SIMAK, M. 1961. Karyotype Analysis of *Pinus silvestris*. Hereditas 47: 379—382.

NAVAŠIN, S. G. 1912. O dimorfizme jader v somatičeskich kletkach u *Galtonia candicans*. Izvest. Imperatorskoj Akad. Nauk 6: 6: 4: 373—385.

NĚMEC, B. 1910. Das Problem der Befruchtungsvorgänge und andere zytologische Fragen. Berlin.

RUBNER, K. 1943. Das Areal der Sudetenlärche.—Tharandter Forstl. Jahrb. 94: 1/2: 1—99.

SAX, K. & SAX, H. J. 1933. Chromosome Number and Morphology in the Conifers.—J. Arnold Arboretum 14: 356—375.

SAYLOR, L. C. 1961. A Karyotypic Analysis of Selected Species of *Pinus*.—Silvae Genet. 10: 3: 77—84.
SEITZ, F. W. 1951. Chromosomenzahlenverhältnisse bei Holzpflanzen.—Z. Forstgenet. u. Forstpflanzenzücht. I: 1: 22—32.
SIMAK, M. 1958. Den slovakiska lärken.—Sv. Skogsvårdsfören. Tidskr. 1: 123—136. (transl. in Eng. by Prof. M. L. Anderson: The Slovak Larch.—J. Roy. Scot. Forestry Soc. 15: 3: 130—142.)
SIMAK, M. & GUSTAFSSON, Å. 1954. Fröbeskaffenheten hos moderträd och ympar av tall.—Medd. Stat. skogsforskn.inst. 44.
SIMAN, K. 1943. Přirozené rozšíření a proměnlivost modřínu obecného—*Larix decidua* Mill. — Sborník České Akad. zemědelské 18: 79—98.
STRASBURGER, E. 1892. Histologische Beiträge. 4.
SZAFER, W. 1913. Przyczynek do znajomości modrzewi eur-azyatyckich ze szczególnem uwzglednieniem modrzewia w Polsce.—Kosmos 38. Lwow 1913.
SYRACH LARSEN, C. & WESTERGAARD, M. 1938. Contributions to the Cytogenetics of Forest Trees. I. A. Triploid Hybrid between *Larix decidua* Mill. and *Larix occidentalis* Nutt.—J. Genet. 36: 3: 523—530.
TISCHLER, G. 1942. Allgemeine Pflanzenkaryologie. II: 2. Kernteilung und Kernverschmelzung.—Verl. Gebr. Borntraeger. Berlin-Zehlendorf. 1942.
TJIO, J. H. & HAGBERG, A. 1951. Cytological Studies on Some X-Ray Mutants of Barley.—Annales estac. exp. Aula Dei 2: 2: 149—167.
TSCHERMAK, L. 1935. Die natürliche Verbreitung der Lärche in den Ostalpen.—Verl. Julius Springer, Wien 1935.
VON WETTSTEIN, W. 1946. Alpenlärchenrassen.—Züchter. 17/18 Jahrg. 2: 40—44.

Sammanfattning

Karyotypanalys av olika provenienser hos europeisk lärk (*Larix decidua* Mill.)

I denna karyotypanalys undersöktes åtta provenienser av *Larix decidua*. Några av dessa provenienser uppvisar så pass skilda ekologiska och morfologiska särdrag, att de ofta betecknas som särskilda raser eller t.o.m. arter. Frömaterialet insamlades i västalperna, centralalperna, östalperna, sydöstra alpregionen, Sudetbergen, Slovakien, Polen och Rumänien (tab. 1). Rotspetsar från groende plantor användes för cytologiska undersökningar. Följande morfologiska egenskaper hos lärkens kromosomer studerades:

a. kromosomlängd,
b. centromerens läge,
c. förekomst av konstriktioner.

Resultaten kan sammanfattas på följande sätt:

1. Inga morfologiska skillnader i karyotypen mellan de åtta provenienserna har konstaterats. En grundkaryotyp för *Larix decidua* kan följaktligen uppställas (fig. 8).

2. Av lärkens tolv kromosomer — i haploidsats — är sex isobrachiala och sex heterobrachiala. Den totala längden samt relationen mellan armarna (centromerens läge) har bestämts för varje kromosom (tab. 2 och 3). Tre av kromosomerna har satelliter.

3. Med hjälp av ovannämnda egenskaper (a—c), kan fem kromosomer exakt identifieras (kromosom I, II, III, IV och VII). Tre av kromosomerna är svåra att bestämma (V, VI och VIII), samt resten av kromosomerna, IX—XII, går ej att skilja från varandra.

4. I arbetet har speciell uppmärksamhet ägnats åt vad man benämnt »reversal of order» (förväxling av kromosomerna) liksom »brachial reversal» (förväxling av armarna på en kromosom). Det förstnämnda kan inträffa om man försöker skilja två nästan lika långa kromosomer från varandra uteslutande med tillhjälp av deras totala längd. På grund av längdvariationen kan det förekomma, att den i realiteten längsta av två ungefär likstora kromosomer i kromosomplattan framträder som den kortare. De två kromosomernas identitet kan då bli förväxlad och en »reversal of order» sker.

Liknande förhållande inträffar hos kromosomer med median centromer, om identifieringen av de nästan lika långa armarna av en kromosom uteslutande skett med tillhjälp av föreliggande längddifferenser (»brachial reversal»).

»Reversal of order» och »brachial reversal» är av principiell betydelse vid karyotypundersökningar och måste därför noga beaktas. Frågan kommer att behandlas mera detaljerat i ett särskilt arbete.