

Karyotype analysis of Siberian larch
(*Larix sibirica* Ledb. and
Larix sukaczewii Dyl.)

Karyotypanalys av sibirisk lärk

by

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The natural area of distribution of Siberian larch¹ extends roughly from lake Onega in the west to lake Baikal in the east and from Arctic sea in the north to the Altai mountains in the south (Fig. 1, *cf.* Ostenfeld and Syrach Larsen 1930, Schenck 1939, Timofeev 1961). Russanow (1959) states that larch occupies more than one-third of the whole forest area in the USSR. Under the influence of different ecological conditions in this huge region, larch has differentiated itself morphologically and physiologically into various types (Szafer 1913). Sukaczew (1938) distinguishes four climatical ecotypes in *Larix sibirica*: *rossica*, *obensis*, *jenisensis* and *altaiensis*, which have their areas of distribution in north-eastern part of European Russia, around the rivers Obs and Jeniseis and in Altai mountain ranges respectively. On the basis of the physiological and morphological differences, Dylis (1947) separates from the Siberian larch the species *Larix sukaczewii* in the west (which roughly corresponds to the area of the Sukaczewian ecotypes *rossica* and partly *obensis*) and calls the rest Siberian larch, *Larix sibirica* Ledb. On the eastern borders *Larix sibirica* and the neighbouring species *Larix gmelinii* Rupr. form spontaneous hybrids—*Larix czekanowskii* (Szafer 1913).

This broadly outlined differentiation of Siberian larch, as also its near relationship with *Larix decidua*, raises the question, whether the taxonomic connections can be supported by evidence of karyotypic differences. This problem will be discussed in the present paper.

Material:

In the years 1960—61 seeds of indigenous stocks of *Larix sibirica* and *Larix sukaczewii* were sent to the Department of Forest Genetics, Royal College of Forestry, Stockholm, through the courtesy of the Academy of Sciences and other institutions of the USSR for the purpose of provenance trials. The seed originating from open-pollination during the years 1958—60 represents the entire area of distribution of Siberian larch except for the

¹ In this paper the term "Siberian larch" is used as a collective name for both *Larix sibirica* Ledb. and *Larix sukaczewii* Dyl.

northern regions around the rivers Obs and Jeniseis from which collections could not be obtained.

In the year 1963 a part of the seed material from 10 provenances (nos. 5—14) was used for the present cytological investigation. For comparative karyotype analyses of Siberian and European larch, four provenances of *Larix decidua* (nos. 1—4) were also included in the study (*cf.* Fig. 1).

Methods:

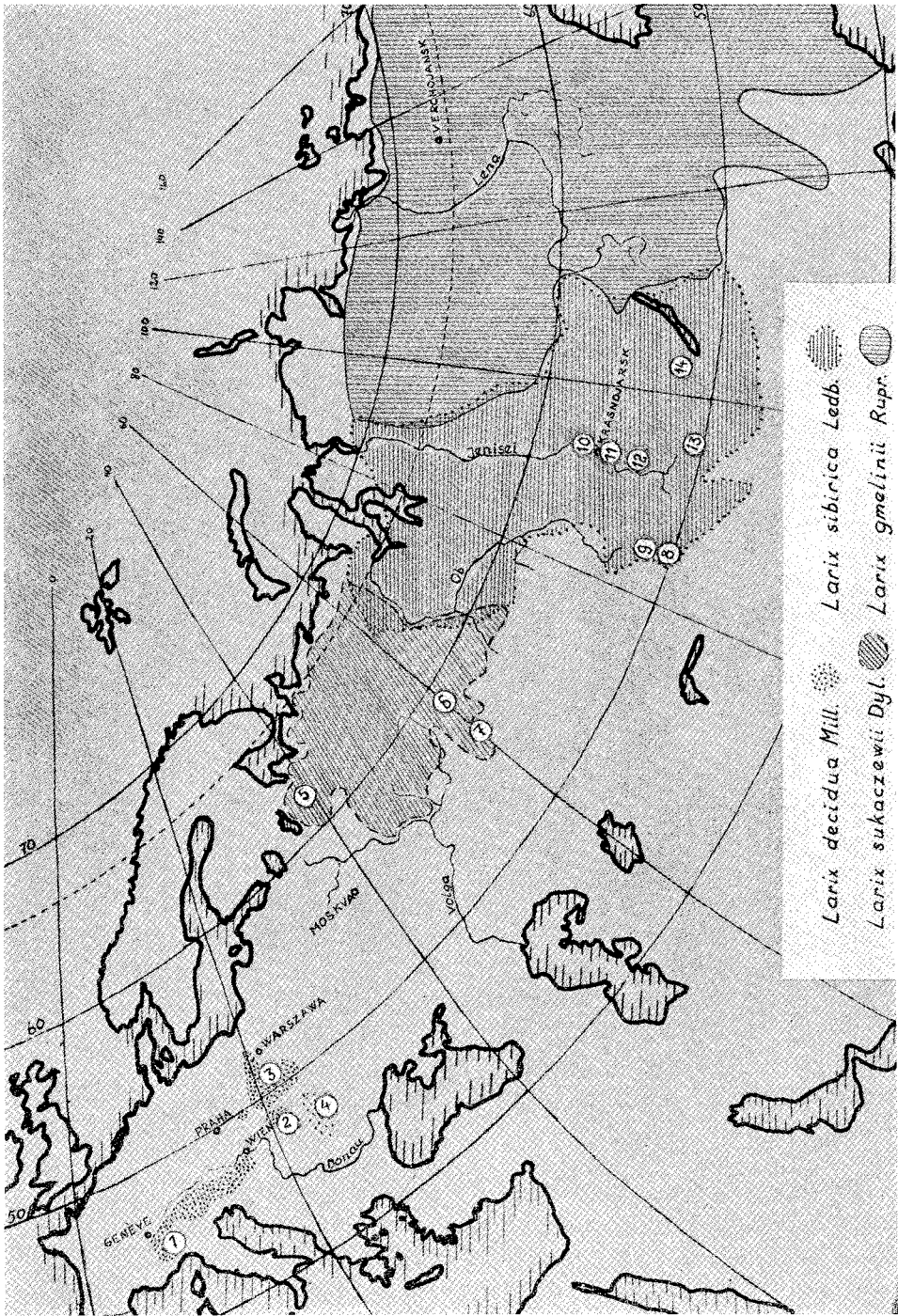
The cytological investigations were carried out on the mitosis of the root tips of germinating seedlings. When the roots were 3—5 mm long, they were treated with 0.1 % colchicine solution for 24 hours, followed by fixing them in Carnoy's fluid (2—4 hours), and finally hydrolysed in a mixture of 2 parts absolute alcohol and 1 part hydrochloric acid for 10—30 minutes. Squash preparations were examined in 2 % acetocarmin and most of them were made permanent. Suitable chromosome plates were microphotographed and measurements of the chromosomes were undertaken on enlarged positives. From each pair in a plate only the better homologous chromosome was measured. The absolute values obtained by measurement of chromosome length were put in relation to the average chromosome (= 100 units) of the plate. In this way it was possible to compare the measurement values of different plates with one another. For each chromosome the centromere-index too was determined as the ratio of the shorter arm to the entire length of the chromosome. Even occurrence of the secondary constrictions was marked.

The cytological technique and the measurement procedures have been described in detail earlier (Simak 1962).

Material from two provenances of *Larix sukaczewii* (nos. 5 and 7) and three provenances of *Larix sibirica* (nos. 10, 12 and 14) was used for the measurement of chromosomes in order to set up an idiogram. The results of this investigation have been confirmed on the basis of special cytological analyses of the rest of the provenance material (e.g. occurrence of satellites, *cf.* Table 1).

An idiogram which is based only on chromosome length and arm relationships should be considered as an "apparent idiogram", because a correct identification and drawing of the individual chromosomes in a plate is not always possible with the help of the above-mentioned criteria. If there are two chromosomes of minor differences in length, the genetically shorter one

Fig. 1 The natural distribution of *Larix decidua*, *Larix sibirica*, *Larix sukaczewii* and *Larix gmelinii*. The locations from which seeds have been used for the karyotype analysis are marked with numbers in circles (No. 6: **Verhotura**, No. 14: **Tjerenchovo**. For the rest see Tables 1 and 2).



may appear "longer" due to preparation and will thus be included as such in the idiogram and vice versa. The same holds good also for the identification of shorter or longer arms of a chromosome. Due to this the identity of the two chromosomes or chromosome arms may be changed by mistake and a *reversal of order* or *arm reversal* would result. This mistake can hardly be avoided by the present-day cytological techniques but in sufficiently large material it is possible to calculate the probability of the frequency with which *reversal of order* or *arm reversal* takes place and within certain limits to correct mathematically the values in the "apparent idiogram".

The nature of *reversal of order* and *arm reversal* has been analysed in detail by Matern and Simak (in preparation).

Results:

The basic number of chromosomes in *Larix sibirica* and *Larix sukaczewii* is 12 (Löve and Löve 1961). Six of them are iso- and the others heterobrachial.

The variations of the chromosome morphology, in spite of chromosome length and centromere-index between the different provenances of *Larix sibirica* and *Larix sukaczewii* as also between these two larch species, lie within the range of error which cannot be eliminated at present while making an idiogram. Statistical analysis of the results is hence meaningless, as has been shown earlier on similar material of *Larix decidua* (Simak 1962). In view of this, a common basic idiogram was prepared for both *Larix sibirica* and *Larix sukaczewii* on the basis of the material investigated.

Keeping in view what has been said above about reversal of order and arm reversal, one can identify the following chromosomes and chromosome groups for the Siberian larch with the help of chromosome length, centromere-index and the occurrence of satellites:

Chromosome I is the longest of the karyotype and is distinguishable from the shorter chromosome II.

Chromosome II can be easily recognized from the chromosomes in group III & IV which possess satellites.

Chromosome group III—IV: Both the chromosomes possess distinct secondary constrictions. One of the chromosomes appears a little shorter than the other, but a definite individual identification is not possible.

Chromosome group V—VI: This group of isobrachial chromosomes can be recognized without difficulty. As stated above, the chromosome group III and IV is marked with satellites and the next group (VII—XII) consists entirely of heterobrachial chromosomes.

Chromosome group VII—XII: As is visible in the idiogram, all the six chromosomes are progressively shorter and one must take into account many reversals of order even between more than two neighbouring chromo-

somes in the idiogram. In a good slide one can possibly separate chromosome VII and VIII as an independent pair from the rest of the group.

Both Siberian and European larch have identical chromosome morphology in spite of chromosome length and centromere-index (*cf.* Fig. 2), but they show distinct qualitative differences in chromosomes IV and VII.

Chromosome IV: In European larch this chromosome has a very weak and not always visible tertiary constriction. This differs from the distinct secondary constriction of chromosome III (Fig. 2). As against this, *Larix sibirica* and *Larix sukaczewii* have in both the chromosomes III and IV very clear secondary constrictions. In view of the fact that in the Siberian larch the secondary constrictions occur only in these two chromosomes (III & IV) and because in the interphase nucleus 3—4 nucleoli are almost always observed, it may be inferred that both these chromosomes are also the nucleolar organizers (Fig. 4).

Tab. 1 *Larix decidua*

Larix decidua: Analysis of satellite occurrence in the heterobrachial chromosome group.

No.	Provenance	No. of heterobrachial chromosomes		No. of slides analysed	Ratio:
		total	with satellites		total no. of chromosomes
1	St. Vincent	686	114	5	6 : 1
2	Strbské Pleso	651	63	5	10 : 1
3	Blizyn	313	24	5	13 : 1
4	Belis	371	42	3	9 : 1
	Total	2021	243	18	8 : 1

Tab. 2 *Larix sibirica* & *Larix sukaczewii*

Larix sibirica & *sukaczewii:* Analysis of satellite occurrence in the heterobrachial chromosome group.

No.	Provenance	No. of heterobrachial chromosomes		No. of slides analysed
		total	with satellites	
5	Sjenkursk	728	—	5
7	Utjalinsk	48	—	1
8	Ust-Kansk	27	—	1
9	Tjarysjsk	355	—	5
10	Kazatjinskoje	1403	—	5
11	Ermakovskoje	552	—	5
12	Askizsk	536	—	5
13	Sjagonar	465	—	5
	Total	4114		32

Chromosome VII: In the European larch the longer arm of this chromosome possesses a satellite (Figs. 2 and 5). The material of *Larix sibirica* and *Larix sukaczewii*, on the other hand, did not show any satellite in chromosome VII.

In Tables 1 and 2 the results of chromosome analysis are presented, which was carried out on slides from different provenances of *Larix decidua*, *Larix sukaczewii* and *Larix sibirica*. All investigations were made at the same time using the same cytological technique. In each slide all free-lying and well identifiable heterobrachial chromosomes were counted (irrespective of the fact whether they lie inside or outside a cell), and the number of chromosomes with satellites, if any, was noted.

For the European larch the ratio of "with" to "without" satellite chromosomes was 1:8. As against this, in *Larix sibirica* and *Larix sukaczewii* no chromosome with satellite could be noted from the 4114 chromosomes analysed.

Discussion:

The idiogram of the Siberian larch was set up on the basis of the karyotype analyses and other observations of different provenances of *Larix sibirica* and *Larix sukaczewii*. In species with a wide geographical distribution—just as larch has—it is necessary to investigate individuals from the entire area before the idiogram of the species is definitely established. The karyotype of a species with a large area of distribution can have much variation. Yamamoto (1933) has shown that *Rumex acetosa* in Japan and East Asia has 8 different karyotypes, each of which is localised in a definite area. *Larix sibirica* and *Larix sukaczewii* have, however, only one basic karyotype common to the whole area of distribution, as far as could be determined by the present investigation.

The group of the Euro-asiatic larch (*cf.* Szafer 1913, Sukaczew 1924) is very difficult to interpret taxonomically. Some taxonomists want to class the whole group as a species, whereas others intend to split the group into many independent species (e.g. *Larix europaea*, *Larix sudetica*, *Larix polonica*, *Larix sibirica*, *Larix sukaczewii*). As criteria for this differentiation of species, morphological characters are often used. In many cases, however, such characteristics are of little diagnostic value, as they are not distinct—they have a more or less gradual character.

Intermediary types are often found between the neighbouring *Larix* species, even if these are geographically isolated. The taxonomic position of these types is very vague. The Polish larch, on the basis of morphological characters, is considered to be an intermediary type between European and Siberian larch, e.g. *Larix sibirica* subsp. *polonica* (according to Sukaczew), *Larix polonica* (according to Szafer), *Larix decidua* var. *polonica* (according

to Ostenfeld and Syrach Larsen). Raciborski (1899), who described this larch for the first time, considers it to be more closely related to the Siberian larch than to the European larch. The sporadically occurring larch in Roumania has been identified as European (Hayek 1906), Polish (Szafer 1913) and Siberian (Pantu and Procopianu-Procopovici 1901).

These few examples show how the morphological criteria alone are insufficient to clarify the taxonomic position of a population. In such cases the taxonomist may often get some help from karyotype analyses (*cf.* Lewitsky 1931, Gregory 1941).

The earlier karyotype analysis of *Larix decidua* (Simak 1962) and the present investigation show that we have to deal with two karyotype groups in the case of Euro-asiatic larch, namely:

1. the group of the European larch
2. the group of the Siberian larch

To the European karyotype group belongs larch which builds isolated areas in the Alps, Sudetic Mountains, Slovakia, Poland and Roumania. The Siberian karyotype includes *Larix sukaczewii* and *Larix sibirica*.

In each karyotype group all the larches have the same chromosome morphology, but as against this, both the groups are clearly distinguishable from one another on the basis of qualitative characteristics of chromosomes IV and VII. In particular the presence of the secondary constriction in chromosome VII of the European karyotype and its absence in the corresponding chromosome of the Siberian karyotype is a distinct mark of identification between these two karyotype groups.

It can hardly be supposed that this difference can be explained as an artifact. In parallel investigations conducted with the same cytological technique the constriction always occurred in the European larch and not in the Siberian larch. Parallel cytological analyses of both species of larch were carried out because the secondary constriction of chromosome VII in *Larix decidua* can sometimes appear different depending on the cytological technique used (Simak 1962).

It may be noted that in the material of *Larix decidua* investigated here, the secondary constriction in chromosome VII appears on an average less often, than it would be expected (1:8 instead of 1:6). Moreover the differences in this respect among the various provenances are great, and the problem will therefore be subjected to a critical study later. Because of the fact that chromosome VII of Siberian larch does not possess a satellite, it is impossible to identify it in the morphologically uniform group VII—XII. Cytological analyses of hybrids between *Larix decidua* and *Larix sibirica* will certainly help to clarify the situation. Both species hybridize easily and natural as well as artificial hybrids have been observed (Eklundh 1943, Saarnijoki 1943).

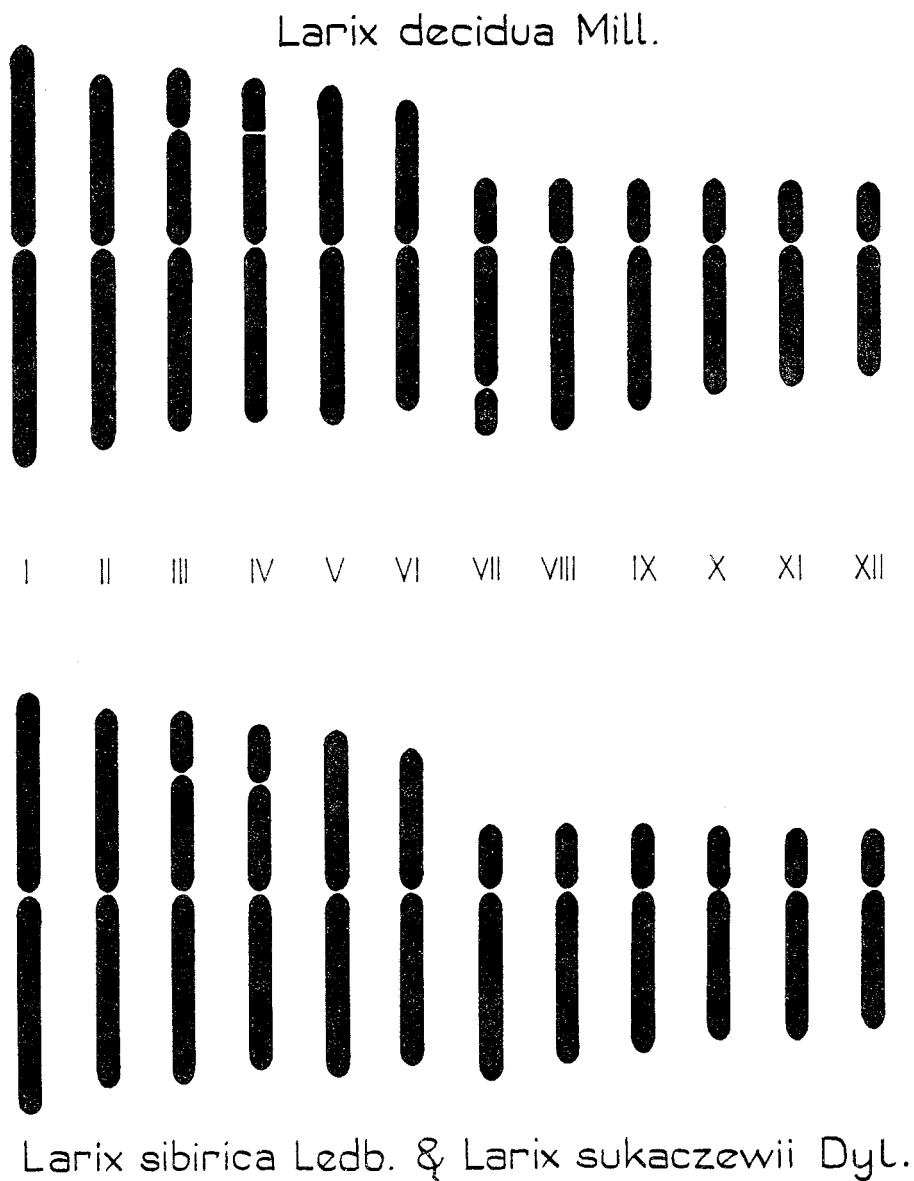


Fig. 2 "Apparent idiograms" (see text) of *Larix decidua* and *Larix sibirica* & *Larix sukaczewii*

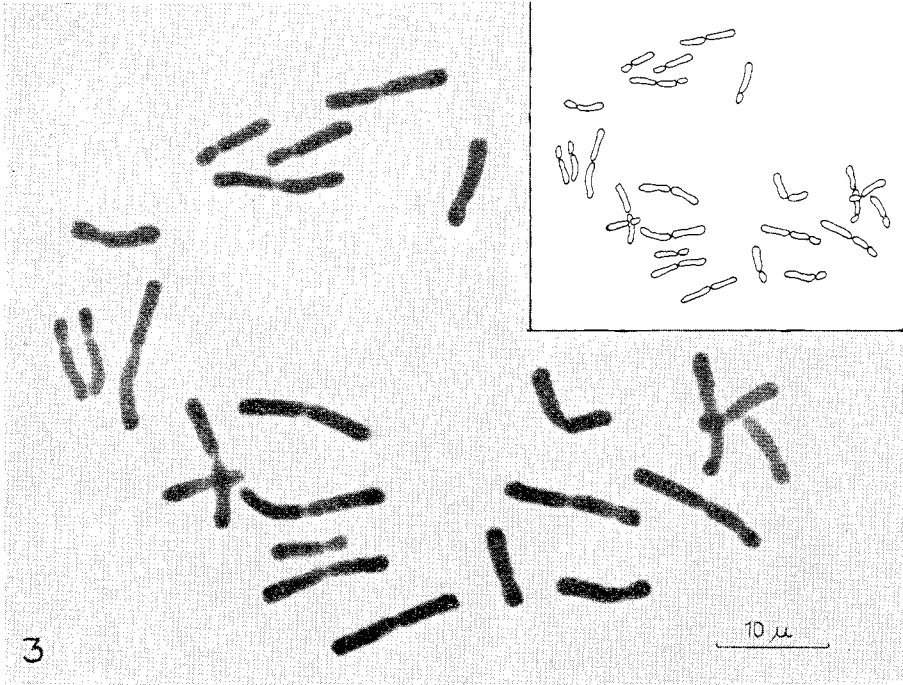


Fig. 3 The 24 chromosomes of *Larix sibirica* (Provenance 13, Askizsk). Picture taken by phase contrast.

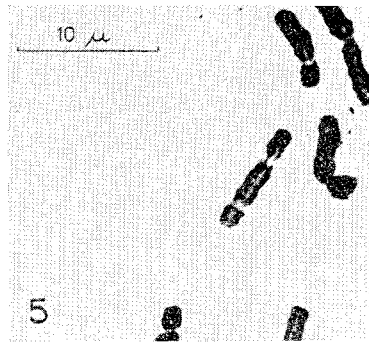
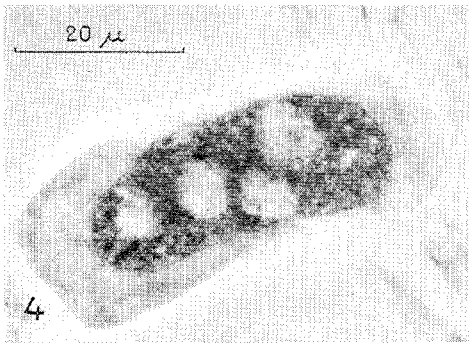


Fig. 4 *Larix sibirica*: An interphase nucleus with four nucleoli.

Fig. 5 Section of a plate with *Larix decidua* chromosomes. Note the heterobrachial chromosome VII with the secondary constriction on the long brachium.

Sukaczew's opinion (1924) that the Siberian larch is phylogenetically younger than the European larch would suggest that the secondary constriction in chromosome VII has been lost during the evolution of the Siberian karyotype group. Also Paczoskij's theory (cit. by Sukaczew), that the Polish larch is the original form of the Euro-asiatic larches implies that the European karyotype group with secondary constriction in chromosome VII is phylogenetically older. However, the reverse is also possible, namely that the secondary constriction in chromosome VII is phylogenetically younger and has arisen from the Siberian karyotype. This supposition would then support Köppen's theory, which states that the Siberian larch during the migration period has preserved the original morphological characteristics better than *Larix decidua* (Köppen 1889).

The present investigation proves that the analysed larch from Roumania belongs to the European karyotype. Because of the sporadic occurrence of larch in Roumania, it would be desirable to conduct cytological analysis on material from a wider area, especially from the areas Ceahlău, Bukovina etc. where the taxonomy of the species is not clearly defined (cf. Grintescu and Antonescu 1924).

The grouping of the Euro-asiatic larches in two karyotype groups, as has been done in this paper, agrees with Dylis' phylogenetical diagram. On the basis of his own investigations and Sukaczew's morphological, physiological and historical studies, he considers the Polish larch as a branch of *Larix decidua* and *Larix sukaczewii* as a branch of *Larix sibirica* (Dylis 1947).

The results of the karyotype analysis, of course, need not always be the single deciding factor for the taxonomic classification of a species. As example of this are the many *Pinus* species, which in spite of their undisputed position as separate species in many cases show the same or very similar morphology of the karyotypes (cf. Saylor 1961). Not all species differences must imply changes in chromosome morphology; the differentiation of species often lies at the gene level.

Summary

On the basis of karyotype analysis of the mitotic chromosomes in root tips of the germinating seedlings, a common basic idiogram was set up for the Siberian larch (*Larix sibirica* Ledeb. and *Larix sukaczewii* Dyl.). No differences in the chromosome morphology were found between these two Siberian larch species. The seed material originated from different autochthonic stocks.

The Siberian larch has the basic number of chromosomes as $n = 12$. Six of them are isobrachial and the others heterobrachial. Chromosomes III and IV possess secondary constrictions, which in both cases separate long satellites.

According to chromosome length and centromere-index, the chromosomes of Siberian larch are identical with those of the European larch. However, qualitative morphological differences are present between both larch species. *Larix decidua* has in chromosome IV a weakly visible tertiary constriction, Siberian larch, on the other hand, has in this chromosome a very distinct secondary constriction which is also the nucleolar organizer. *Larix decidua* has on the longer arm of chromosome VII a secondary constriction which is not present in the Siberian larch. On the basis of cytological analysis the Euro-asiatic larches can be divided into two karyotype groups: 1) group of the European larches (that is, larch in the Alps, the Sudetic Mountains, Slovakia, Poland and Roumania) and 2) group of the Siberian larches (*Larix sibirica* and *Larix sukaczewii*).

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Sammanfattning

Karyotypanalys av sibirisk lärk

I denna karyotypanalys undersöktes olika provenienser av *Larix sibirica* och *Larix sukaczewii*. Frömaterialet härstammade från autoktona bestånd i Sovjetunionen (fig. 1). En jämförande analys mellan de sibiriska lärkarterna (*Larix sibirica* och *Larix sukaczewii*) och den europeiska lärken (*Larix decidua*) har också utförts. Resultatet av undersökningen kan sammanfattas i följande punkter:

1. Inga skillnader i kromosommorfologi mellan *Larix sibirica* och *Larix sukaczewii* har konstaterats. Ett s. k. »apparent idiogram» (se text) gemensamt för båda lärkarterna kunde därför uppställas (fig. 2).

2. Av kromosomerna ($n=12$) hos de sibiriska lärkarterna är sex isobrachiata och sex heterobrachiata. Kromosomerna III och IV har satelliter (fig. 3).

3. Enligt kromosomlängd och centromerindex är idiogrammen för de sibiriska lärkarterna och *Larix decidua* identiska. Det finns dock kvalitativt morfologiska skillnader i kromosommorfologin mellan dessa arter (fig. 2):

Larix decidua har i kromosom IV en svagt synlig tertiär konstriktion, och i kromosom VII på den längre armen en tydlig satellit.

Larix sibirica & *sukaczewii* har i kromosom IV en tydlig sekundär konstriktion och i kromosom VII finns det ingen satellit.

På basis av dessa cytologiska analyser kan den euro-asiatiska lärken indelas i två karyotypgrupper.

I. Europeiska lärkgruppen (lärk från Alperna, Sudetbergen, Slovakien, Polen och Rumänien).

II. Sibiriska lärkgruppen (*Larix sibirica* och *Larix sukaczewii*).

Резюме

Кариотипный анализ лиственницы сибирской

В этом кариотипном анализе исследовались различного происхождения, т. е. из различных географических пунктов лиственница сибирская (*Larix sibirica* Ledeb.) и лиственница Сукачева (*Larix sukaczewii* Dyl.).

Семенной материал, на котором базировались исследования, был собран в аутохтонных насаждениях Советского союза (рис. 1).

Сравнительный анализ был сделан также между сибирскими видами лиственницы, с одной стороны, и европейской лиственницей (*Larix decidua* Mill.), с другой стороны.

Результаты данных исследований могут быть сведены в следующие пункты:

1. Никаких морфологических различий между хромосомами *Larix sibirica*, с одной стороны, и *Larix sukaczewii*, с другой стороны, не было констатировано. А поэтому для обоих этих видов лиственницы может быть построен общий т. н. «apparent idiogram» (см. в тексте и рис. 2).

2. Из $n = 12$ хромосом, присущим сибирским видам лиственницы, 6 хромосом являются изобрахиальными и 6 хромосом гетеробрахиальными. Хромосомы III и IV имеют сателитов, т. е. спутников (рис. 3).

3. На основании длины хромосом и центромериндекса идиограмма является идентичной как для сибирских видов лиственницы, с одной стороны, так и европейской лиственницы, с другой стороны. Но одновременно с этим имеются всетаки морфологические различия между морфологией хромосом этих видов (рис. 2): *Larix decidua* имеет в хромосоме IV одну слабо видимую третичную перетяжку (constriction), а в хромосоме VII имеет на длинном плече отчетливо заметный сателит. *Larix sibirica* и *Larix sukaczewii* имеют в хромосоме IV одну отчетливо видимую вторичную перетяжку, в то время как в хромосоме VII они никакого сателита не имеют.

На основании этих цитологических анализов евро-азиатская лиственница может быть подразделена на две карiotипические группы:

I. *Европейская группа лиственниц* (лиственница Альпийских гор, Судетских гор, Словакии, Польши и Румынии).

II. *Сибирская группа лиственниц* (*Larix sibirica* и *Larix sukaczewii*).

* * *

Резюме перевел на русский язык инж.-лесовод И. Руссанов.

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