Karyological analysis of European and Siberian spruce and their hybrids in the USSR

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The European spruce (Picea *abies*/L./KARST.) and Siberian spruce) (Picea obovata LEDEB.) occupy a vast territory in the USSR from the Baltic sea in the West to the sea of Okhotsk in the East, and from the southern border of the tundra to the southern state frontier. The natural distribution of the two species was separated in the glacial period, but formed a broad belt of spontaneous hybrids during post-glacial adaptation. Picea abies (L.) KARST. and Picea obovata LEDEB. possess uniform distinguishing morphological features however, their transitional hybrids possess great variability. This morphological variation in spruce from the USSR and other western countries has been studied extensively for 150 years: (LEDEBOUR, 1833; NYLANDER, 1863; REGEL, 1863; KAUFFMANN, 1866; TEPLOUCHOFF, 1869; SYLVEN, 1909; 1914; WITTROCK, 1914; SUKACHEV, 1928; HEIKINHEIMO, 1937; MEZERA, 1939; DANILOV, 1943; LINDQUIST, 1948; SAARNIJOKI, 1954; PRIEHÄUSSER, 1956; 1958; 1959; RUBNER, 1960; ANDERSSON, 1965; STASZKIEWICZ, 1966; GOLUBETS, 1968; PRAVDIN, KOROPACHINSKY, 1969; BOBROV, 1971; 1972; PRAVDIN, 1972; 1975; ROUDNA, 1972; SCHMIDT-VOGT, 1972). The above authors reported on the variation of cone size, seed scale shape, anatomical morphological features and ecology. This article deals with the karyotype analysis of the initial parental species and their spontaneous hybrids.

Material and Methods

The diversity of spruce cone and seed scale morphology was classified into five groups according to the scheme in Fig. 1. The extreme groups are the representatives of species:

1 — Siberian spruce; 4 — European spruce; 5 — European spruce f. acuminata BECK. 2 and 3 are spontaneous hybrids between group 1 and groups 4 and 5 (the 2nd group is the hybrid with Siberian spruce features dominating, while the 3rd group is the hybrid with European features dominating). According to the classification, proposed by MEZERA (1939) the initial species are attributed to the varieties and within the limits of each variety the following forms are distinguished:

A — var. obovata with forms: 1 — f. transversa; 2 — f. typica; 3 — f. fennica;

B — var. europea with forms: 4 — f. *cunneata;* 5 — f. *typica*; 6 — biloba; 7 — f. triloba;

C — var. acuminata with forms: 8 — f. apiculata; 9 — f. ligulata; 10 — f. typica; 11 — f. squarrosa.

Classification of Picea abies (L.) KARST. *sensulato* manifests all the diversity of forms and varieties (more than 100) as described by systematists. The variability of transitional forms between P. obovata and P. abies is the result of introgressive hybridization and a map of the geographical distribution of spruce diversity by seed scale shape throughout the USSR is illustrated in Fig. 2. Western spruce populations are different from Siberian populations by many morphological and physiological parameters demonstrating that the taxon "species" for P. abies and P. obovata declared by systematists is correct. The respective allopatric populations of these two species are spatially separated by 100° longitude, and are reproductively isolated through the lack of direct cross pollination.

The populations found between P. abies and P. obovata demonstrate gradual morphological changes and are widely

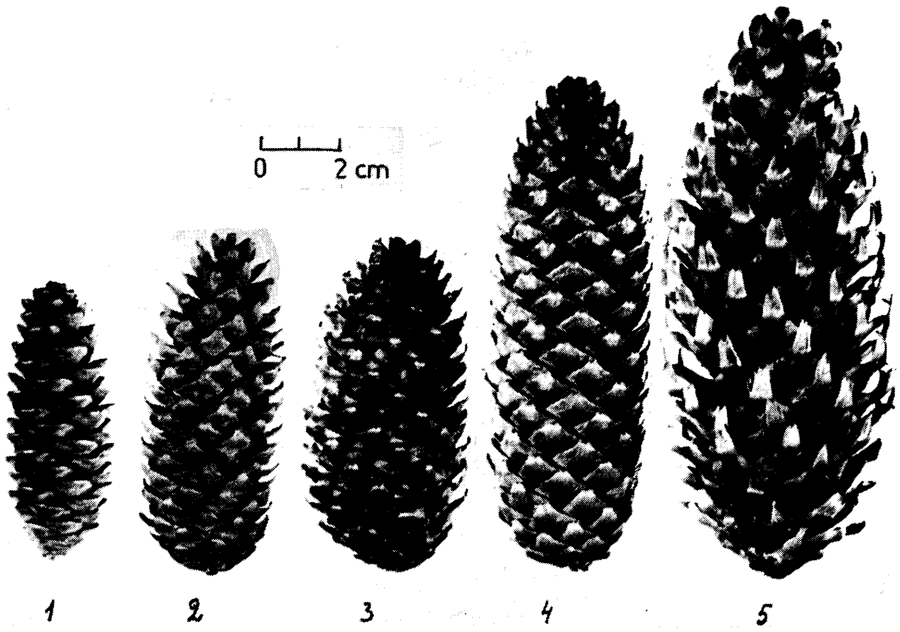

Fig. 1. — Cone types of spruce growing in the USSR by form of seed scales: 1 — ***var. obovata;*** 2 — ***var. obovata × europae;*** 3 — ***var. europae × obovata;*** 4 — ***var. europae;*** 5 — ***var. acuminata.***

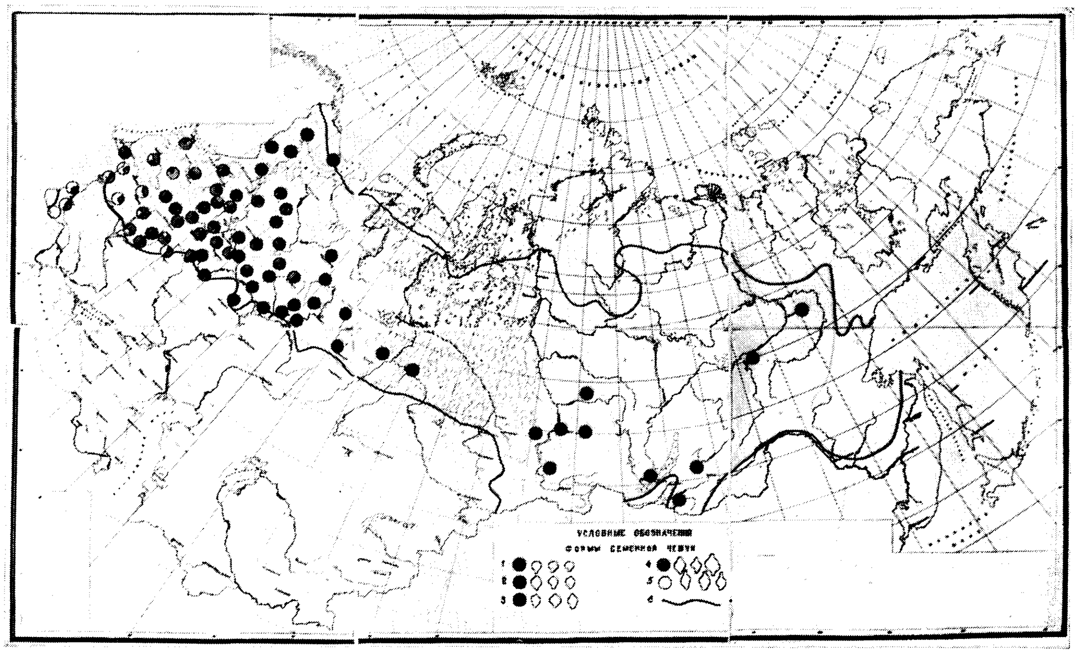

Fig. 2. — A map of geographical distribution of the siberian spruce, european spruce and their hybrids in the USSR; 1 — siberian spruce *(Picea obovata* LEDEB.); 2 — hybrids with dominating characteristics of siberian spruce; 3 — hybrids with dominating european spruce characteristics; 4 — european spruce *(Picea abies* (L.) KARST.); 5 — *Picea abies* (L.) KARST. f. *acuminata* BECK; 6 — the border of spruce distribution area.

distributed on the Russion Plain. The introgressive hybridization spruce populations of the intermediate zones between the extreme species has been given species status *Picea* × *fennica* (REGEL) KOMAROV, and comprise several varieties: *var. fennica* REGEL *var. uralensis* TEPLOUCHOFF, *var. medioxima* NYLANDER, *var. uwarowii* KAUFFMANN.

The seeds used were taken from four taxonomically classified spruce populations. Two sympatric populations from the zone of introgressive hybridization: Karelian ASSR (62° N. lat., 34° E. long.) and Perm region (58° N. lat., 59° E. long.) and two allopatric populations from the Lithuanian SSR (55° N. lat., 21° E. long) and the Altai (49° N. lat., 87° E. long.). The karyological analysis of populations was carried by GABRILAVICHUS (1972, 1973) in the Lithuanian SSR, by SHERSHUKOVA in the Karelian ASSR and Perm region with the help of ABATUROVA (PRAVDIN, SHERSHUKOVA, ABATUROVA, 1974) and by SHERSHUKOVA in the Altai Territory. About 80 samples were taken from each population.

The following parameters were compared: 1. chromosome number; 2. relative chromosome length (= the ratio of chromosome length to the sum of lengths of a haploid or diploid chromosome set of a metaphase plate, given in %); 3. arm ratio; and 4. secondary constriction frequency.

Photomicrographs of metaphase plates are given in *Fig. 3* and illustrate the characteristics of each of the above parameters for allopatric and sympatric populations.

The same phenomena occurs on the continent of North America, where the introgressive hybridization is expressed in the zone of contacts *Picea glauca* (MOENCH) VOSS. *subsp. glauca* and *P. glauca* (MOENCH) VOSS. *subsp. engelmanii* (ROCHE, 1969).

The same cytological methods of PRAVDIN, BUDORAGIN, KRUKLIS, SHERSHUKOVA (1972) were used facilitating the possibility of comparing results. The root tips were treated with 1% colchicine solution for 4 hours at a temperature of 25° C, and fixed with alcohol acetic acid (3 : 1), stained with acetocarmine and the temporary preparations were made. The karyological investigation was carried out with a microscope and drawing apparatus copies were made of the best and clearest metaphase figures. Chromosome identification and determination of differences between the comparable karyotypes were performed by the polykaryogram method (PAVULSONE *et al.* 1970). A polykaryogram or a chromosome dispersion graph allows graphically to express the chromosome distribution in a karyotype based on the chromosome length and centromere index. Using these chromosome parameters the chromosomes were classified into morphological homogenous groups and the mean values of parameters were calculated.

The absolute chromosome length was measured to determine the main morphometric parameter. The spiral index (I_s) (SASAKI, 1961) characterises the variation rate of absolute length of colchicine treated chromosomes by comparing the combined length of the two smallest chromosomes against the combined length of two the largest chromosomes in a chromosome complement. The method displays wide limits of variation, but can demonstrate variation among different populations. The metaphase plates with spiral chromosome index of limits from 52% to 58% were chosen for analysis.

Results and Discussion

1. Chromosome number. (Fig. 3) The chromosome number of European spruce population and the two hybrid populations (the Karelian and Perm) consists of 2n = 24. Additional chromosomes were found in the Siberian spruce populations and this is a very important character of the Siberian spruce karyotype. Tachtadjan considered it to be the character in progress (TACHTADJAN, 1960). KRUKLIS (1971) found in two populations of Siberian spruce the additional chromosomes 2n = 24 1B, 2B, 3B; the frequency of karyotypes with a single additional chromosomes is 20.8% and 25.9%; with two additional ones — 3.8% and 2.0% and with three additional ones 1.8% respectively. At the same time the additional chromosomes were found in *Picea sitchensis* (BONG.) CARR. (MOIR and FOX, 1972). Ac-

Fig. 3. — Somatic chromosome karyogramms: a — Lithuania; b — Karelia; c — Perm; d — Altai; B — chromosomes are shown with arrows.

cording to our data (unpublished) the karyotype from the Altai population of Siberian spruce has chromosomes with 1B — 11% and 2B — 6%.

2. The relative chromosome length. There is no statistical difference in relative chromosome length between the sympatric and allopatric population (see *Table 1).*

Four chromosome pairs with secondary constrictions were observed in the Lithuanian karyotype populations. The chromosome pairs II and III generally demonstrated secondary constrictions as did the IV—VIII and the X chromosome pair.

The individual chromosomes pairs I, II, III were distinguished according to their relative chromosome length in the karyotype of Lithuanian populations. We could not

Tab. 1. — Relative length mean values of individual chromosomes and chromosome groups in karyotypes of European, Siberian and hybrid spruce populations with standard error of means. About 80—100 metaphase plates of each population were used to calculate mean values. Chromosome pairs I—VIII of Karelia, Perm, Altai populations and chromosome pairs V—VIII of Lithuanian population are morphological homogenous, thus they are grouped into a group for calculating mean values.

Chromosome pairs	Relative chromosome length, % Lithuania (allopatric)	Karelia (sympatric)	Perm (sympatric)	Altai (allopatric)
I	5.20 ± 0.01			
II	4.70 ± 0.01			
III	4.68 ± 0.01			
IV	4.37 ± 0.01			
V		4.54 ± 0.04 (I—VIII)	4.53 ± 0.01 (I—VIII)	4.52 ± 0.02 (I—VIII)
VI	4.33 ± 0.01 (V—VIII)			
VII				
VIII				
IX	3.76 ± 0.01	3.70 ± 0.01	3.73 ± 0.01	3.79 ± 0.06 (IX—X)
X	3.68 ± 0.01	3.54 ± 0.01 (X—XI)	3.70 ± 0.04	
XI	3.34 ± 0.02		3.24 ± 0.08	3.21 ± 0.01
XII	2.95 ± 0.01	3.00 ± 0.01	2.89 ± 0.04	2.92 ± 0.008

separate the following ten chromosomes into five individual homologous pairs (IV—VIII), because their relative lengths were very similar. The distribution of the points corresponding to these chromosome pairs are situated unsimilarly on the polykaryogram, and therefore, suggesting differences in relative length between these five chromosome pairs. One pair of this chromosome group possessed a high frequency of secondary constrictions which can serve as the additional morphological trait of it (Mashkin *et al.*, 1973).

The chromosomes with secondary constrictions vary accordingly: the chromosome pair with frequency occurrence 10.8% occupy the fourth place; with 40.9% — the fifth place; with 27.9% — the sixth place; with 16.1% — the seventh place; with 4.3% the eighth place by data Gabrilavichus (1973). Eight chromosome pairs are metacentric and four chromosome pairs (IX—XII) are sub-metacentric. The values of chromosomes pairs nine and ten do not differ very much from each other though the ninth pair is a little longer than the tenth pair and is more closer by arm ratio value to the previous group. The tenth chromosome pair has the secondary constriction with the frequency of — 64.4%.

These chromosomes are difficult to distinguish on a polykaryogram, but taking the additional properties into consideration they could be plotted separately (*Fig. 4*). The eleventh chromosome pair is picked out as an individual homologous pair. Its mean arm ratio is equal to 43.1% in some cases it looks nearly to be metacentric. The twelveth chromosome pair is always easy to identify microscopically or with photomicrographs. This chromosome pair displays a constant arm ratio and absolute and relative length. The respective coefficient of variation is small. Two taxons of European spruce with its form accuminata are growing in common in the Lithuanian spruce populations. The karyological difference between these two taxons was not found in comparison analysis and therefore the *fig. 4a* gives us one general polykaryogram.

Four chromosome groups are picked out in the Karelian populations: I, VIII, IX, XII are identified separately; X and XI (*Fig. 4b*) are combined in one group.

Chromosome pairs of the karyotype population of Perm region (*Fig. 4c*) are combined into two groups: I—VIII and IX—X pairs except the chromosome pairs XI and XII. The karyotype of Altai population of Siberian spruce is characterized by the individual chromosomes XI and XII; I—VIII and IX—X pairs are combined in groups (*Fig. 4d*).

In the allopatric populations of Siberian spruce (*Fig. 4d*, Altai) all the metacentric chromosome groups I—VIII and one sub-metacentric chromosome group have the secondary constrictions. The hybrid populations (the Karelian ASSR and Perm region) have the secondary constrictions in all the metacentric chromosomes (the first eight pairs), but the frequency occurrence of the secondary constrictions in each pair is extremely variable (see *Table 2*), demonstrating heterogeneity and their hybrid nature.

Table 2. — The secondary constrictions frequency

Populations	Chromosome groups / The secondary constrictions frequency, %						
Lithuania	I	II—III	IV—VIII	IX	X	XI	XII
	10	82	24	23	63	2	0
Karelia		I—VIII		IX	X—XI		XII
		43		15	13		0
Perm		I—VIII		IX—X	XI		XII
		43		38	0		0
Altai		I—VIII		IX—X	XI		XII
		35		30	0		0

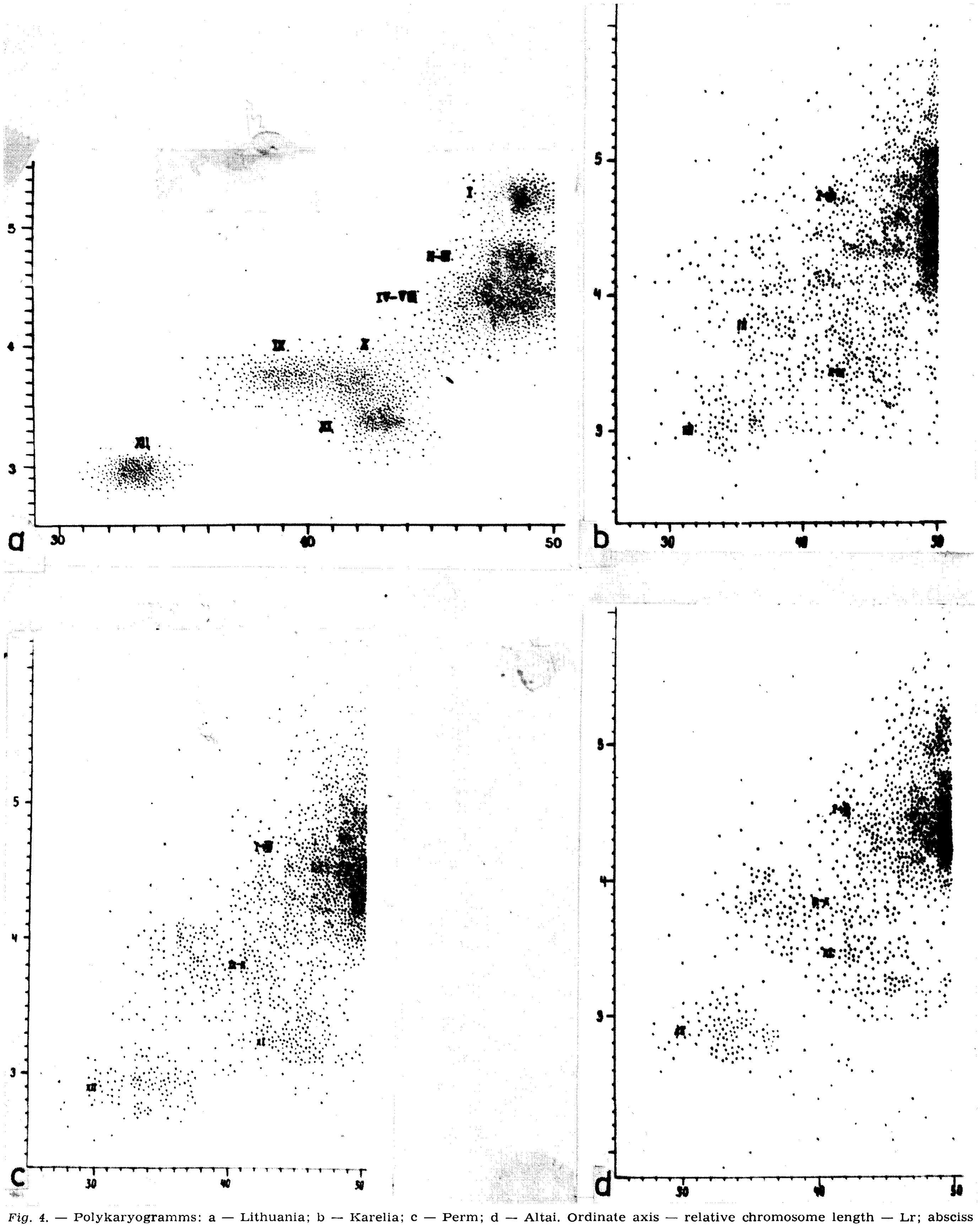

Fig. 4. — Polykaryogramms: a — Lithuania; b — Karelia; c — Perm; d — Altai. Ordinate axis — relative chromosome length — Lr; absciss axis — centromere index — I^c.

The variation of chromosome spiral rate in the studied karyotypes is presented graphically as frequency distribution *(Fig. 5)*.

The comparison of the frequency distribution graphs *(Fig. 5 a, b, c, d)* of allopatric and sympatric populations facilitates the use of this technique for comparative ana-

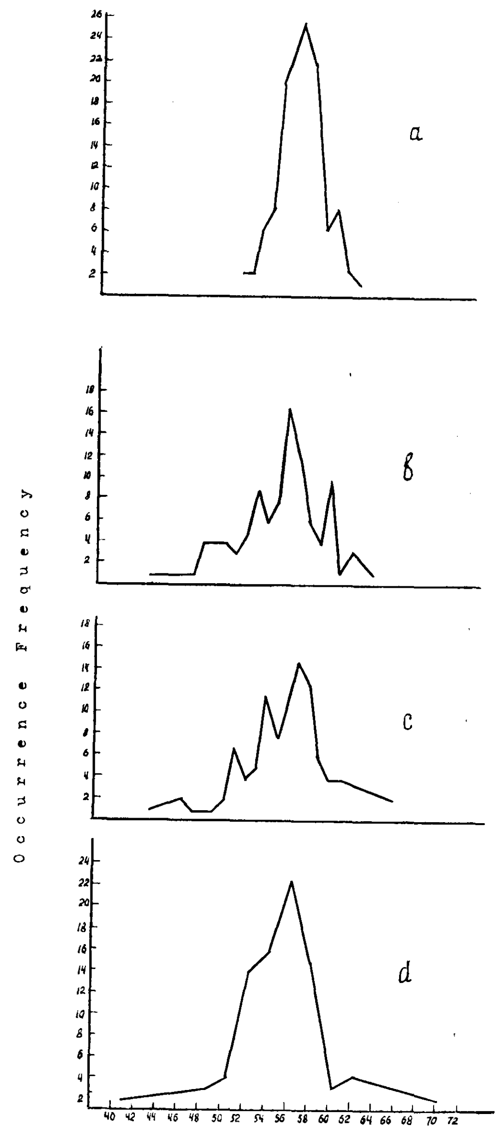

Fig. 5. — Distribution of chromosome complements by spiral index in allopatric populations — a, d, and in sympatric populations — b, c: a — Lithuania; b — Karelia; c — Perm; d — Altai. Ordinate axis — occurrence frequency, absciss axis — spiral index, %.

lysis of populations. The allopatric populations are presented as curves with one peak while the sympatric populations are presented as curves with many peaks.

This point of view does not contradict BURLEY's (1965) statement who supposed the possibility of inadequate chromosome reaction to colchicine.

Summary

1. The karyotype studies of *Picea abies* (L.) KARST. *P. obovata* LEDEB. and *P. fennica* (BOBROV) were carried out by the same method used by different scientists.

2. The populations of *Picea abies* (L.) KARST., and *P. obovata* LEDEB. are allopatric, the panmixia among them is absolutely excluded. The populations of *P.* × *fennica* are sympatric and panmixia among them took place continually since the post-glacial for 4—5 thousand years and strong heterogeneity is evident.

3. The karyotype difference of allopatric populations appears not only in the chromosome morphology, but in chromosome number as well; additional chromosomes 1B, 2B, 3B were found in the karyotypes of Siberian spruce. The additional chromosomes were not found in the karyotypes of *Picea* × *fennica*. The karyotypes of sympatric populations are characterized by high heterogeneity that is clearly seen in double-and-more peaked curves of spiral index and at the same time it shows the hybrid nature of the transitional populations as a result of introgression.

Key words: Picea abies (L.) KARST., *Picea obovata* LEDEB., *Picea* × *fennica* (REGEL) Komarov, Chromosome.

Zusammenfassung

Zytologische Untersuchungen (Karyotyp) bei Herkünften von *Picea abies* (L.) KARST., *Picea obovata* LEDEB. und *Picea fennica* (BOBROV) (*P. obovata fennica* HENRY) in der UdSSR ergaben, daß *Picea abies* und *Picea obovata* als allopatrische Populationen anzusehen sind, wobei Panmixie auszuschließen ist. Bei *Picea obovata fennica* war Panmixie festzustellen. Bei der sibirischen Fichte konnten z. T. zusätzliche Chromsomen festgestellt werden, was bei *P. obovata fennica* nicht der Fall war.

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Controlled Pollinations among Pine species in Greece

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Introduction

The degree of relationship among pine species has been studied by several investigators and different classification systems were proposed according to the criteria used each time (SHAW 1914, PILGER 1926, DUFFIELD 1952, MIROV 1953, GAUSSEN 1955, 1960, LITTLE and CRITCHFIELD 1969). DUFFIELD (1952) was the first to use the criterion of crossability. He also proposed certain modifications to the Shaw system which is based on morphological characters. According to SHAW (1914) the Lariciones group includes among others the species *P. nigra*, *P. sylvestris* and *P. heldreichii*. These species occur naturally in Greece. The Insignes group includes among other the species *P. halepensis*, *P. brutia* and *P. pinaster*. The first two species occur naturally in Greece while the third does not. In DUFFIELD's modified system, *P. halepensis* (which grows naturally together with *P. brutia*, *P. pinaster* and *P. nigra)* is transferred from the Insignes group of the subgenus Diploxylon to the Lariciones group of the same subgenus.

This paper reports the results of the second phase (1967—1972) of a study which was conducted in order to obtain information on the crossability among a number of pine species growing in Greece with the ultimate purpose of exploring the possibility of transfering characters from one species to the other (because of high genetic variability in the genus *Pinus)* in order to produce superior genotypes having desirable characters and especially a high adaptability to the adverse environmental conditions of this country.

The results of the first phase (1962—1966) of this study were reported by BASSIOTIS (1972). During that phase artificial pollinations were made among pine species of the same group as well as among species belonging to the Insignes and Lariciones groups. BASSIOTIS (1972) found that most cross-pollinations between groups failed to produce filled seeds but some did produce a few. A small number of seedlings were grown from these seeds and were described by the previous author as putative between-groups hybrids. However, the overall failure of the between groups crossing program led us to reject the Duffield modifications of the Shaw system with regard to the position of *P. halepensis* and accept the Shaw system intact, at least for the pine species growing in Greece (BASSIOTIS 1972).

Materials and Methods

The failure to obtain filled seed from the between-groups crosses of the first phase (1962—1966) of the investigation