AN ABSTRACT OF THE THESIS OF

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Abstract approved: Kim K. Ching

A comparative intraspecific karyotype analysis was performed on European black pine (Pinus nigra Arn.) seed sources (countries) by examining root tip meristemic cells of young seedlings (two weeks old). By a combination of Feulgen and Aceto-carmine staining methods, metaphase chromosomes range (12.93 $\pm$ 1.33) seedlings from two to three seed collections each of French, Austrian, Yugoslavian, Greek and Turkish seed sources were studied. Variables including chromosome number, short arm length (SL), long arm length (LL), total chromosome length (TL), arm ratio (AR), relative chromosome length (RL), centromere index (CI), morphological index (MI), and secondary constriction and satellite occurrences for each cell were measured. The chromosome number was 2n = 24 for all seed sources. However, aneuploidy 2n = 18 was observed in one seedling of each of the Yugoslavian and Greek seed sources. Analyses of variances for the means of chromosomal variables (F-tests were conducted for the variables SL, LL, TL, CI and MI) indicated that there were significant ($P < .05$ or $P < .01$) differences among the seed sources. Chromosomes XI and XII were especially variable among seed sources, with significant ($p < .01$) variation

found for SL, LL, TL, AR, CI and MI. The frequency of secondary constriction occurrences was relatively higher on the long arms of chromosomes and on the longer chromosomes. At low frequency, satellite occurrences were only observed in the Yugoslavian and Greek seed sources, especially on their longer chromosomes. French and Austrian seed sources had only one submetacentric chromosome (chromosome-XII) while the others had two (chromosome-XI and XII). By integrating all the variables measured on all haploid chromosomes, a cluster analysis of the seed collections was performed. Seed sources from Austria and France appear clustered close together, as did Greece and Yugoslavia. The Turkish seed source, however, was the least similar to any of the other sources. Seed collections generally clustered within seed sources.

A Statistical Karyotype Analysis of European Black Pine (*Pinus nigra* Arn.) Seed Sources

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INTRODUCTION

European black pine (Pinus nigra Arn.) is a widespread commercial tree species found in the mountains of southern Europe and Asia Minor, with outliers in many of the Mediterranean islands and in northern Africa (Figure 1) (Vidakovic, 1974). The species shows high variability in morphology, growth form, adaptability, and monoterpene and isozyme composition. However, while a number of sub-specific categories have been described, there is no general consensus on its taxonomy (Rohrig, 1966; Read, 1976; Wilcox and Miller, 1975; Wheeler et al., 1976; Vidakovic, 1974; Arbez et al. 1974; Bonnet-Masimbert and Bikay-Bikay, 1978).

Karyotype studies involving P. nigra have only been at the interspecific level (Saylor, 1964; Pederick, 1970; MacPherson and Filion, 1981) or on an individual tree basis (Borzan and Papes, 1978; Borzan, 1981). Apparently, to date, no studies on P. nigra have been carried out to provide extensive karyotypic information at the intraspecific level. Generally, it appears from the literature that there is a lack of karyotypic information at the intraspecific level in Pinus species (Borzan and Papes, 1978). Traditionally, karyotypic relationships among and within pine species have been studied by comparing idiograms of haploid chromosome sets and by individually analyzing chromosomal variables (in most cases, only arm ratios and relative length of chromosomes) (Saylor, 1961, 1964, 1972; Pederick, 1967, 1970; Borzan and Papes, 1978; Borzan, 1981; MacPherson and Filion,

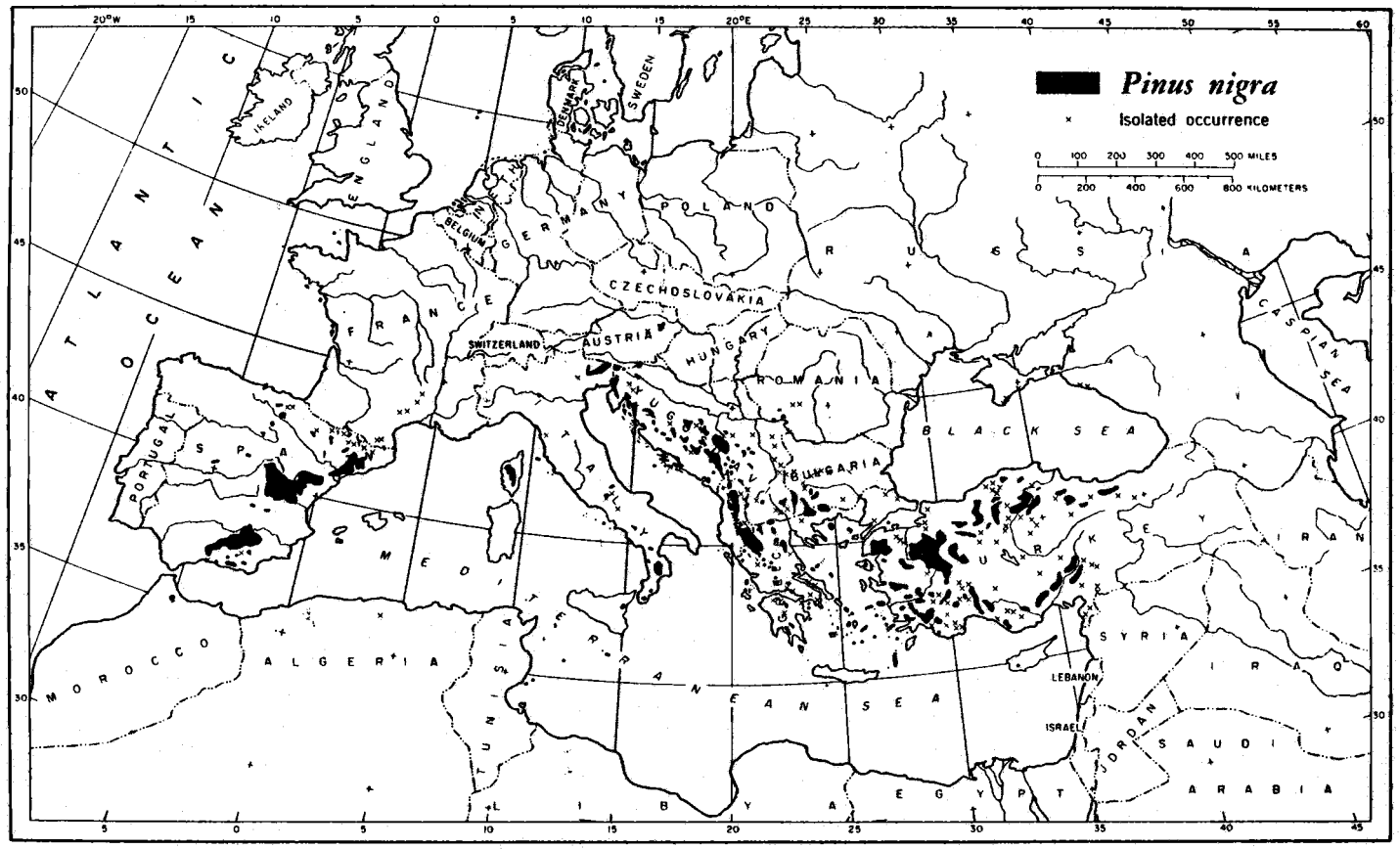

Figure 1. Geographic distribution of European black pine (Critchfield and Little, 1966).

1981). There has been no attempt to interpret statistically karyotypic information involving a number of traits and to explore relationships among populations of species. The objectives of this study were (1) to determine the degree and kinds of intraspecific karyotypic variation in *P. nigra* and, (2) to develop a statistical procedure for use in such karyotype studies.

MATERIALS AND METHODS

In order to meet the objectives of the study, an attempt was made to obtain seeds from the entire range of Pinus nigra Arn. However, seeds could be obtained only from Austria, France, Greece, Yugoslavia, and Turkey (seed sources) because of difficulties in communication (Table 1). The French, Greek, Yugoslavian and Turkish seed sources are each represented by three seed collections, but only two collections could be obtained from Austria. Despite the lack, in most cases, of specific information on location, seed collections within each seed source are known to have come from well separated regions. Two of the seed collections from France (Noceta and Tartagine) came from Corsica, while the St. Guilhem seed collection was from interior France. The two seed collections from Austria came from areas separated by a distance of 40 km. The three seed collections from Greece (Drama-Sparta-Kalambaka), Yugoslavia (Kozina-Visegrad-Zavidovici) and Turkey (Adana-Balikesir-Kütahya) also were well-distributed within the range of P. nigra in these countries (personal communication with seed suppliers) (Table 1). However, other information about the seed collections is incomplete. For example, the seed collections were bulk samples and it is not known how many individual trees were involved per seed collection. Nevertheless, it is likely that about 50 or more parent trees are represented in each seed collection (assuming that the seeds were originally collected for reforestation purposes).

Roughly 200 seeds from each collection were soaked in 1% H_2O_2 for two days in order to speed up and assure more uniform germination.

Table 1. *Pinus nigra* Arn. seed sources and collections studied for the karyotypic analyses.

SEED Sources	Collection	LATITUDE	ELEVATION
AUSTRIA	ALLAND	----	400-900m
	HERNSTEIN	----	500m
TURKEY	ADANA	37°30'N	1250 m
	BALIKESIR	40°20'N	500-1000m
	KUTAHYA	39°21'N	1000-1200m
YUGOSLAVIA	ZAVIDOVICI	44°20'N	600m
	KOZINA	45°31'N	500m
	VISEGRAD	43°50'N	400-650m
FRANCE	NOCETA	----	(COAST)
	TARTAGINE	----	(COAST)
	ST. GUILHEM	----	(INLAND)
GREECE	DRAMA	----	----
	SPARTA	----	----
	KALAMBAKA	----	----

Then the seeds were germinated in the dark in petri dishes in an incubator kept at a constant 25°C. After the radicles of the germinated seeds reached about 5 mm, they were transplanted into pots filled with soil and allowed to grow in a greenhouse for two weeks.

Karyotypes were determined by studying mitotic chromosomes in squash preparations of root tip meristems in two week old seedlings. The root tips were soaked in mono-bromo naphthalene for 24 hours at room temperature, fixed in 3:1 ethyl alcohol-acetic acid for 4-6 hours, hydrolyzed in 1N HCl for 12-15 minutes at 60°C, and stained with a combination of Feulgen and Aceto-carmine (Doerksen and Ching, 1972).

Over 100 slides were made from each seed collection. However, larger numbers of slides were prepared from seed collections which showed the most variation in chromosome morphology. Only one slide per seedling was made in each seed collection. All slides were examined for cells with metaphase chromosomes, but some of the slides had cells in different stages of cell division. One cell with well-spread metaphase chromosomes from each slide was chosen for analysis, and a photograph of the cell (color transparency Kodachrome 200 day light film) was taken through a phase contrast microscope. The following number of cells were photographed (and subsequently analyzed) from each seed collection and seed source:

French seed sources		40
Noceta	10	
Tartagine	10	
St. Guilhem	20	
Austrian seed sources		32
Alland	16	
Hernstein	16	

Yugoslavian seed sources		31
Kozina	10	
Zavidovici	10	
Visegrad	11	
Greek seed sources		47
Drama	15	
Sparta	15	
Kalambaka	17	
Turkish seed sources		51
Balikesir	15	
Kütahya	15	
Adana	21	

Diagrams of the chromosomes in each cell analyzed were drawn from photographs magnified to 3755.7X by using a standard slide projector which was set at a constant distance (250 cm) throughout the course of the experiment. Then, the chromosomes of a given cell were arbitrarily numbered from 1 to 24 and the following data taken from the diagrams for each chromosome: (1) short arm length (SL), (2) long arm length (LL), (3) total chromosome length (TL = SL + LL), (4) presence of a secondary constriction on the short arm (S_{sec}), (5) presence of a secondary constriction on the long arm (L_{sec}), and (6) the presence of a satellite (SAT) (see Figures 2 and 3 for examples of SL, LL, L_{sec} and SAT).

To assign chromosomes to homologous pairs, chromosomes were compared in a pairwise fashion, based on SL, LL, TL, S_{sec}, L_{sec}, SAT, arm ratio (AR = SL/LL, Saylor, 1961) and relative chromosome length (RL = $TL_i/(\Sigma TL_i/n) \times 100$). Once pairwise matching was complete, the mean of SL, LL, and TL and the frequency of S_{sec}, L_{sec} and SAT was computed for each pair. In addition to the above variables, the values of AR, RL, morphological index (MI = SL/LL x TL, Giannelli and Howlett, 1967) and centromere index (CI = SL/TL x 100, Stephenson et al., 1972) were

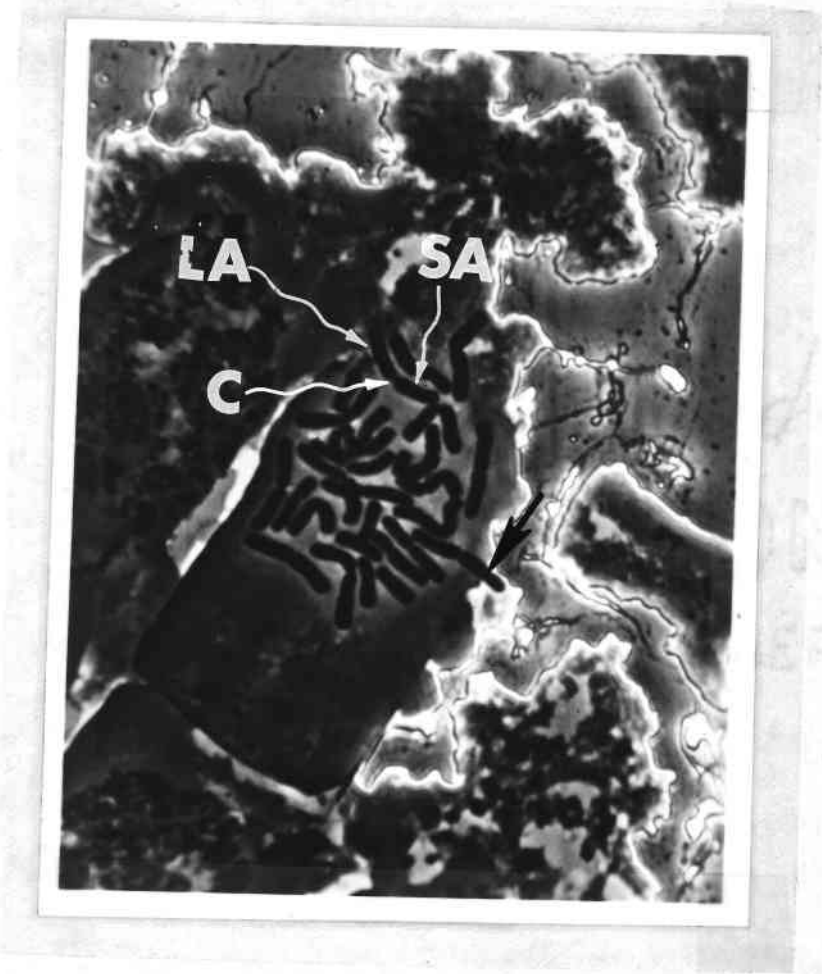

Figure 2. Microphotograph of metaphase chromosomes of P. nigra from root tip meristems, with an example of a centromere (C), short-arm (SA), long arm (LA) and secondary constriction (black arrow) illustrated. Magnification: 2692.4X.

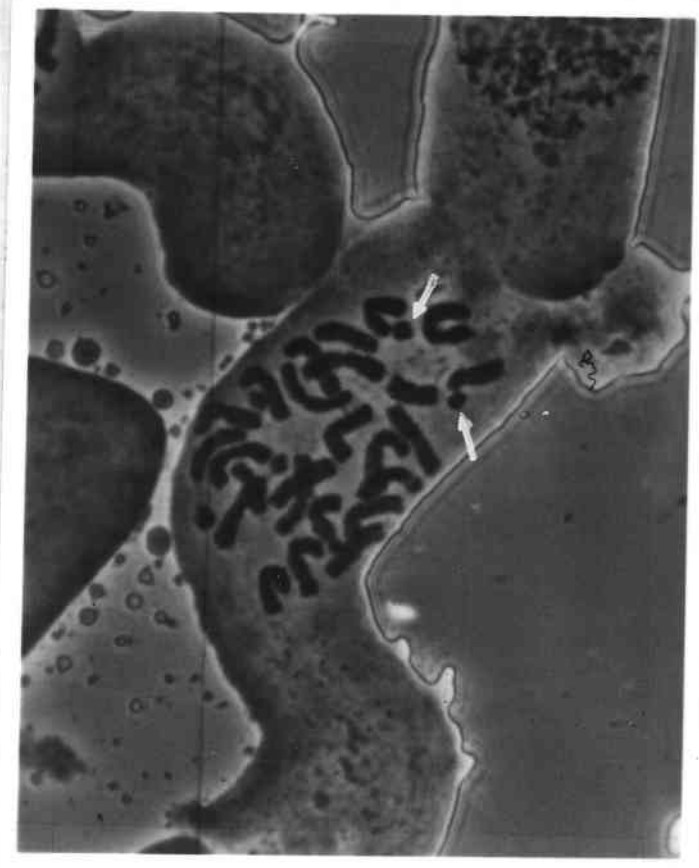

Figure 3. Microphotograph of the chromosomes from the Greek seed source of Pinus nigra showing the occurrence of satellites (arrows). Magnification: 2692.4X.

derived for each pair. The haploid set of chromosomes was numbered in descending order from -I to -XII with chromosome-I being the longest and chromosome-XII the shortest. Therefore, the karyotypic data in each cell was analyzed in the form of 12 haploid chromosomes, each with 10 variables.

STATISTICAL ANALYSIS

Mean values of SL, LL, TL, AR, RL, CI, and MI and frequencies of occurrence S_{sec}, L_{sec} and SAT were calculated for each of the 12 chromosomes in each of the 14 seed collections, i.e., the mean of SL for chromosome-I in the French St. Guilhem seed collection was computed by averaging the SL values of 20 studied cells.

Analyses of variance (ANOVA) of 7 of the 10 seed collection variables were conducted for each of the 12 haploid chromosomes. Only variables, based on seed collection means, S_{sec}, L_{sec} and SAT were not analyzed by ANOVA because the appearance of secondary constrictions was greatly affected by cytological technique such as squashing. On the other hand, SAT frequencies were too small. Moreover, SATs were only observed in 2 of the 5 seed sources. Analyses of variance were carried out using a nested design with seed sources (countries) as treatments (degree of freedom (df) = 4) and seed collections within seed sources as subsamples (df = 9). Both seed sources and seed collections within seed sources were assumed to be random variables in the analyses.

In addition to analyses of variance, a "cluster analysis for cases" was conducted on each of the 12 chromosomes, utilizing seed collection and seed source means of 10 variables (in the case of S_{sec}, L_{sec}, and SAT, the frequency of these was used instead of means). Cluster analysis basically forms clusters of cases based on one of the several possible distance measures (the Euclidean distance, Chi-square statistics, Phi-square, etc.). In this study, two separate cluster

analyses were performed to determine clustering pattern of chromosomes between seed sources as well as seed collections within seed sources. In conducting cluster analyses between seed collections within seed sources, "cases" were chromosomes of seed collections while in cluster analyses between seed sources, "cases" were chromosomes of seed sources (i.e., when a cluster analysis for chromosome-I was performed between 5 seed sources, there were 5 cases and 10 determined variables for each case). All of the 10 variables were utilized in the cluster analyses at one time.

For this study, the Euclidean distance measure (square root of the sum of squares of differences between values of variables for two cases) was chosen as the basis for clustering. Initially, each case was considered to be in a cluster of its own. At each step, the two clusters with the shortest distance between them were combined (amalgamated) and treated as one cluster. This process of combining clusters continued until all the cases were combined into one cluster. This algorithm is called "average distance" or "average linkage." The theoretical basis of cluster analysis is thoroughly discussed in Johnson and Wichern (1982).

By using the P2M:BMDP (cluster analysis for cases: Biomedical computer programs, Engelman, 1979) statistical package, cluster analysis of the 14 seed collections and 5 seed sources of European black pine were performed. Also amalgamation distances (similarity indices) for clusters of each of the 12 chromosomes of the 5 seed sources of this species were calculated.

RESULTS AND DISCUSSION

I. Description of the Average Pinus nigra Arn. Karyotype

The great majority (99%) of the seedlings sampled had the expected diploid chromosome number of 24. However, one seedling in each of the Yugoslavian and Greek seed sources had only 18 chromosomes in all the cells that were observed (Figure 4). So far as I have been able to determine, abnormalities in the chromosome number of Pinus nigra have not been previously reported. While rare, however, irregularities in chromosome numbers have been observed in numerous other pine species. For example, Pederick (1967) reported the occurrence of trisomics (2n = 25) in Pinus radiata D. Donn and Mergen (1958) found tetraploids (2n = 48) in Pinus eliottii Engel. In addition, working with polyembryonic seeds, Ching and Simak (1971) identified the occurrence of mosaic aneuploids ranging in chromosome number from 2n = 12 to 24 in Picea abies (L) Karst. Aneuploids found in the present study were unlikely to have originated from polyembryonic seeds because diploid species could hardly tolerate such chromosome loss. The loss of three pairs of chromosomes in diploid P. nigra would be quite detrimental to individuals (Burnham, 1962). The origin of aneuploids in the present study is possibly unequal translocation (i.e., essential parts of one chromosome may be transferred to another without being detrimental to the individual) or centromeric fusion of chromosomes (Robertsonian fusion) through the natural hybridization at intra- and interspecific levels (Stebbins, 1971; Grant, 1981; Schulz-Schaeffer, 1980; Swanson et al., 1981). Nevertheless, further

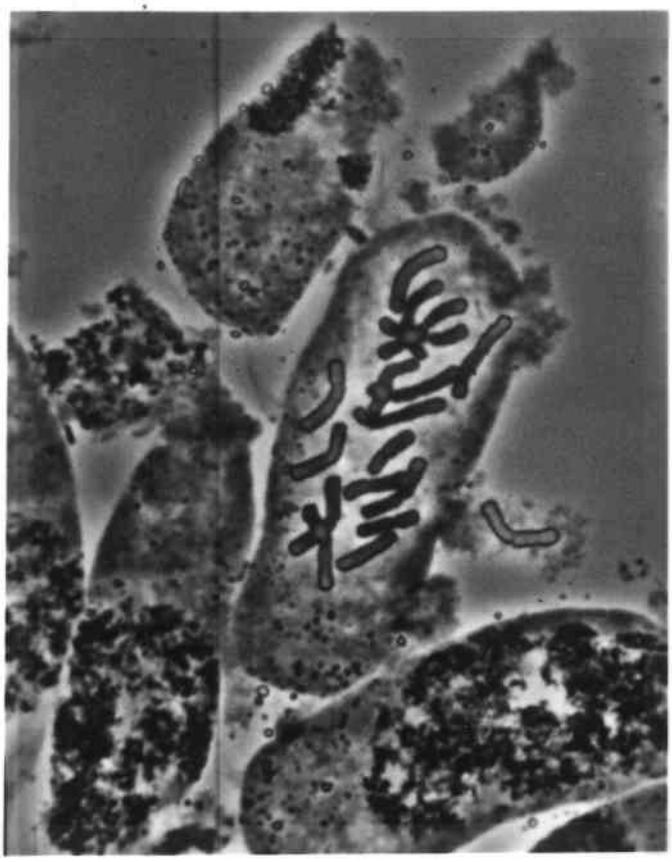

Figure 4. Microphotograph showing aneuploidy (2n = 18 instead of 2n = 24 chromosomes) in one seedling of the Greek seed source of Pinus nigra. Magnification: 2692.4X.

study is needed to be certain how frequently such irregularities in diploid chromosome numbers occur in this species.

Centromeres of the two smallest chromosomes (chromosome-XI and XII) in P. nigra were found to be in the submedian position, while the remaining 10 chromosomes had their centromeres located in the median position (Table 2 and Figure 5). In all pine species studied to date (except group Lariciones), 11 of the 12 chromosomes have consistently been reported to have a median centromere position and the 12th one (the shortest one) a submedian centromere position (Sax and Sax, 1933; Mehra and Khoshoo, 1956; Saylor, 1961, 1964, 1972; Pederick, 1967, 1970; Borzan and Papes, 1978; Borzan, 1981; MacPherson and Filion, 1981). However, previous reports for members of group Lariciones, in which P. nigra is included, have indicated that in the two shortest chromosomes with submedian centromere position (Saylor, 1964). Thus, the results of the present study support earlier findings in P. nigra and closely related species.

The range in mean total chromosome length (TL) was almost two fold, varying from 51.17 mm in chromosome-I to 28.30 mm in chromosome-XII (values obtained from projection drawings by magnification of 3755.7), and relative chromosome length (RL) varied from 123.1 to 68.2. Actually, chromosome length in pairs of subsequently numbered chromosomes often differed by less than 2 mm (Table 2, Figure 5). Separately identifying individual chromosomes in P. nigra, as well as in other pines, is very difficult because of their morphological similarity. Only the longest chromosome (chromosome-I) and the two shortest chromosomes (XI and XII, with submedian centromeres), can be readily identified by visual means in P. nigra, the identification

Table 2. Mean values and standard errors of chromosomal variables of Pinus nigra Arn. over all seed sources.

Variables[1]	Chromosome Number I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
SL	24.51 ± .49	23.19 ± .54	22.44 ± .52	21.91 ± .53	21.12 ± .46	20.67 ± .47	20.06 ± .34	19.36 ± .30	18.48 ± .29	17.34 ± .36	14.48 ± .41	11.44 ± .40
LL	26.66 ± .89	25.23 ± .85	24.42 ± .87	23.52 ± .73	23.18 ± .73	22.56 ± .62	21.92 ± .68	21.22 ± .58	20.43 ± .55	19.46 ± .50	18.40 ± .46	16.86 ± .40
TL	51.17 ±1.36	48.42 ±1.37	46.86 ±1.20	45.43 ±1.18	44.30 ±1.08	43.23 ±1.01	41.98 ± .87	40.58 ± .84	38.91 ± .84	36.80 ± .70	32.88 ± .75	28.30 ± .75
AR	0.92 ± .013	0.92 ± .013	0.92 ± .014	0.93 ± .006	0.91 ± .011	0.91 ± .006	0.92 ± .013	0.91 ± .011	0.90 ± .009	0.89 ± .009	0.78 ± .025	0.68 ± .016
RL	123.1 ± .66	116.4 ± .60	112.7 ± .60	109.2 ± .32	106.5 ± .33	104.0 ± .12	101.0 ± .25	97.6 ± .40	93.7 ± .47	88.5 ± .34	78.2 ± .95	68.2 ± .62
CI	47.9 + .37	47.9 + .33	47.9 + .36	48.2 + .12	47.7 + .28	47.9 + .23	47.8 + .37	47.7 + .32	47.4 + .26	47.1 + .26	44.0 + .79	40.4 + .57
MI	47.1 ± .76	44.6 ± .91	43.0 ± .80	42.4 ± .96	40.5 ± .64	39.6 ± .85	38.4 ± .50	37.0 ± .45	35.2 ± .33	32.8 ± .65	25.9 ± .99	19.2 ± .81
S_{sec}	.264	.228	.190	.224	.104	.140	.160	.124	.100	.070	.060	.005
L_{sec}	.428	.333	.260	.280	.209	.250	.280	.184	.124	.120	.080	.035
SAT	.030	.045	.025	.025	.030	.045	.010	.000	.005	.005	.005	.000

[1] SL = Short arm length (mm) (values obtained from projection drawings by magnification of 3755.7).
LL = Long arm length (mm).
TL = Total length (mm).
AR = Arm ratio (short arm length/long arm length).
RL = Relative length (see text for description).
CI = Centromere index (see text for description).
MI = Morphological index (see text for description).
S_{sec} = Frequency of secondary constrictions on short arm.
L_{sec} = Frequency of secondary constrictions on long arm.
SAT = Frequency of satellites.

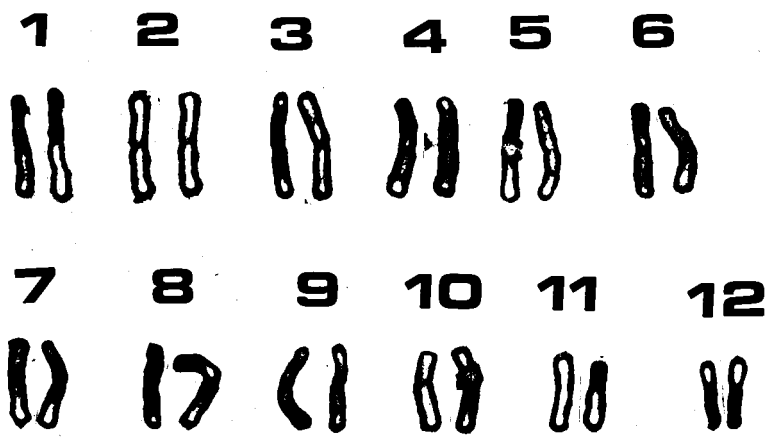

Figure 5. Idiogram of Pinus nigra Arn. Chromosomes were arranged in descending order of length. Magnification: 1325X.

of the remaining 9 chromosomes being possible only by measurements and relative ranking according to total length.

On the average, the frequency of secondary constrictions increased with the overall length of chromosomes, and was greater on long arms than short arms. Secondary constrictions were especially infrequent on the two shortest chromosomes (chromosome-XI and XII) (Table 2). These findings are parallel to the results of earlier studies (Saylor, 1964; Borzan and Papes, 1978; Borzan, 1981; MacPherson and Filion, 1981). Frequently secondary constrictions have been used in pine karyotyping (Saylor, 1961, 1964, 1972; Pederick, 1967, 1970; Borzan and Papes, 1978; Borzan, 1981). However, as seen in this study (Table 2), secondary constrictions have been found to occur only sporadically on pine chromosomes. Perhaps this can be partially attributed to the cytological techniques used in karyotyping. On the other hand, the association between secondary constrictions and the Nucleolus Organizer Region (NOR) on chromosomes has been known for a long time. Recently, MacPherson and Filion (1981) studied thoroughly the association between the distribution of C-heterochromatin and the location of secondary constrictions in some pine species including *P. nigra*. It was apparent from their study that there were more C-bands than the number of maximum nucleoli. They speculated that some of C-banded sites may be inactive NORs. Similarly, Sato *et al.* (1980), in their study of *Allium sativum* L., reported that the morphological appearance of secondary constrictions was not always a perfect indication of the presence of the NORs since the extremely low activity of some NORs fails to produce secondary constrictions. They also pointed out that the size of secondary constrictions reflected the size of the

nucleoli which varied within the same cell as did secondary constrictions.

Saylor (1964) points out that the inconsistency of secondary constrictions from study to study may be the result of chromosomal materials reacting differently to treatments used in cytological study. Therefore, secondary constrictions should not be considered as a major criterion in karyotyping of pines, but can be used as additional chromosome markers. From this study, as well as earlier ones, it is apparent that at least two conventional staining techniques should be used in the same study concerning pine species in order to gain a better understanding of secondary constriction appearances on chromosomes of P. nigra.

Satellites were only rarely observed, but generally were most frequent on longer chromosomes (Table 2). Only a rare occurrence of satellites has been reported in Coniferales. Although the presence of satellites was reported in Abies sachalinensis (Fr. Schm.) Mast (Mergen and Lester, 1961), in Larix decidua Mill (Simak, 1962) and in Sequoiadendron giganteum (Lindle) Decne (Schlarbaum and Tsuchiya, 1975), no study to date has reported satellite occurrences in P. nigra. Stebbins (1971) points out that satellites are heterochromatinized regions on chromosomes and are connected to chromosomes with NORs. While the low frequency of satellites (Table 2) on any one chromosome limits their use for identifying chromosomes, they may be useful for separating P. nigra karyotypes from karyotypes of other closely related pines.

II. Karyotypic Variation Among Seed Sources

Differences in chromosome morphology among seed sources were quantified by the measured chromosomal variables, i.e., short arm length (SL), long arm length (LL), total chromosome length (TL), etc., for each haploid chromosome. Karyotypes of the 14 seed collections of Pinus nigra were determined by averaging karyotypic variables of all cells studied within each seed collection. The karyotype of each of the 5 seed sources was then derived from the karyotypes of seed collections by averaging the values of their chromosomal variables. Idiograms of chromosomes for each chromosome set (chromosome set I to XII) were compared among the seed sources (Appendix 1). Mean values of SL, LL, TL, AR, RL, CI, and MI with standard errors were also determined (Appendix 2, Tables 1-12).

a. Chromosome lengths

Total chromosome length (TL) varied significantly ($P < .05$) among seed sources in all 12 chromosome sets (except for chromosome-VII, VIII, IX) (Table 3). On the average, chromosomes in the French seed source were on the average the shortest while all chromosomes in the Turkish seed source were the longest of any source in the study (Figure 6, Appendix 2, Tables 1-12). Chromosome-III had the greatest range in length among seed sources. For example, TL for chromosome-III ranged from 51.83 ± 2.67 mm in the Turkish source to 44.33 ± 2.47 mm in the French seed source (Appendix 2, Table 3).

As far as intraspecific variation in chromosome length of Pinus species is concerned, only one study has been reported previously

Table 3. F-ratios[1] (mean square for seed sources/mean square for seed collections within sources) for seven variables measured on the 12 haploid chromosomes of Pinus nigra.

	CHROMOSOME SETS											
Variables[2]	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
SL	2.65	3.63	2.72	3.66*	2.59	2.83	1.91	1.32	1.53	2.64	14.37**	8.61**
LL	6.22*	7.24**	10.49**	5.86*	7.29**	5.97*	5.25*	4.54*	5.71*	6.58**	7.27**	9.27**
TL	4.55*	5.53*	5.73*	4.74*	4.62*	4.36*	3.60	2.91	3.50	4.25*	7.06**	9.06**
AR	7.76**	6.42*	6.73**	2.52	12.00**	1.67	10.83**	3.73*	2.87	4.48*	31.09**	9.05**
RL	1.62	4.09*	9.32**	1.91	3.10	0.76	2.69	4.43*	6.02*	1.32	3.49	1.38
CI	7.54*	6.35*	6.54**	2.52	11.85**	1.67	10.84**	3.77*	2.93	4.44*	30.24**	9.19**
MI	1.54	2.43	1.53	2.88	1.48	1.97	0.92	0.56	0.58	1.92	23.87**	8.55**

[1]All F-ratios have (4, 9) degrees of freedom.

[2]Descriptions of variables are the same as in Table 2.

*Significant at $\alpha = .05$ level of probability.

**Significant at $\alpha = .01$ level of probability.

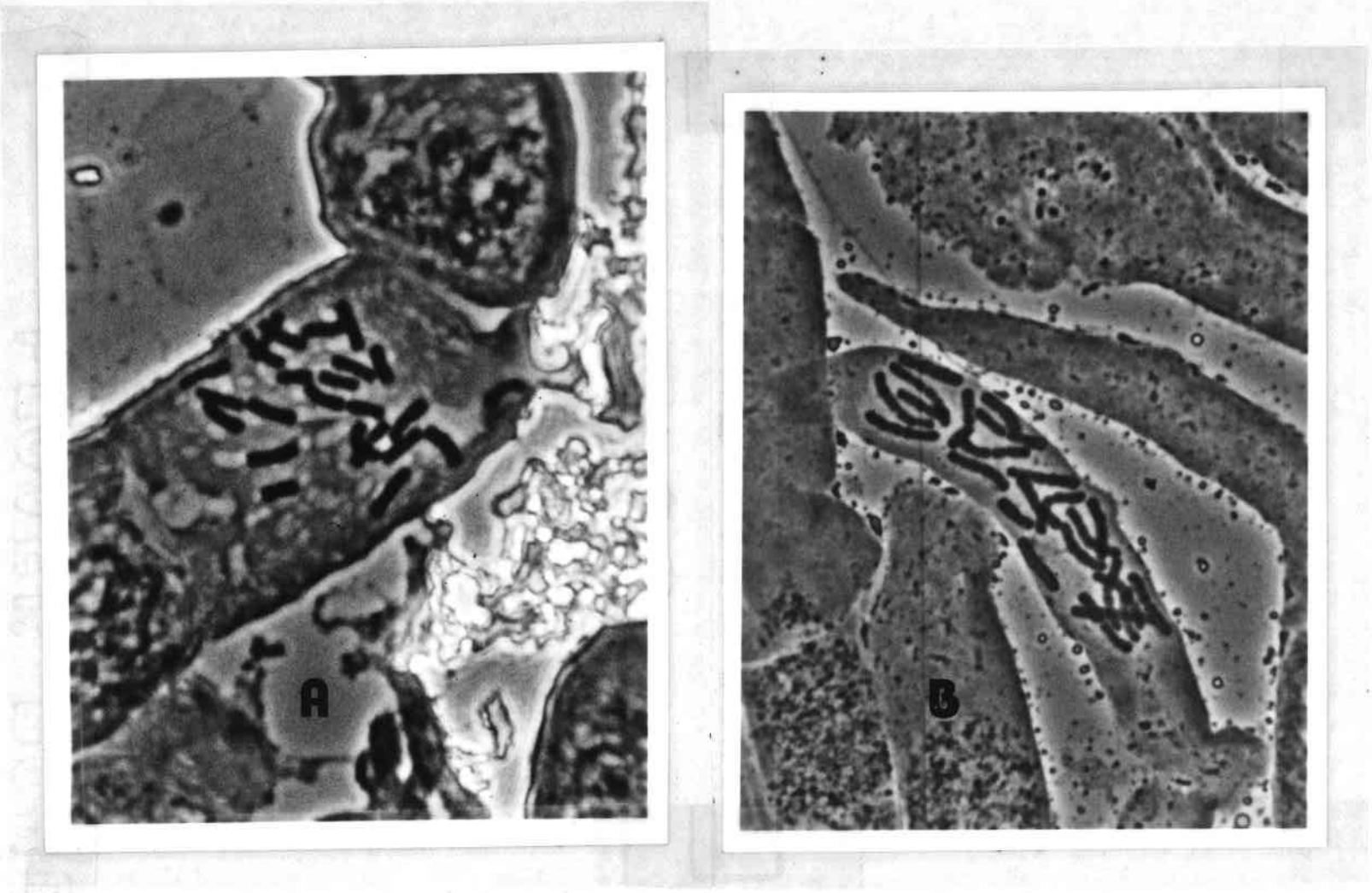

Figure 6. Microphotographs of Pinus nigra chromosomes from France (A) and from Turkey (B). Magnified by 2692.4.

(Borzan and Papes, 1978). Working with two Pinus nigra trees, these authors found that one of the trees (ni 221) had a longer chromosome-XII than the other tree (ni 47), which showed interspecific hybrid incompatability with Scots pine. No compelling explanation for intraspecific variability in chromosome length has been offered to date. However, Miksche (1967, 1968) points out that duplication or deletion processes could cause a lengthening or shortening of chromosomes. Previous studies in plants have shown that the amount of DNA in cells can vary with geographical location. For example, in a number of species, DNA content has been found to increase clinally with latitude (Miksche, 1967, 1968; Sziklai and De-Vesconi, 1978; Stebbins, 1976). Furthermore, Stebbins (1964) reported that species adapted to warm tropical climates are characterized by very small chromosomes while those growing in temperate climates have generally much larger chromosomes. On the other hand, variation among geographic locations in DNA content is not always clinally related to latitude (Dhir and Miksche, 1974; Price et al., 1981a, 1981b). In Microseris species (Price et al., 1981a, 1981b) populations with higher DNA content were restricted to the more mesic sites, generally with well-developed soils, while populations with lower DNA content were restricted to stressful (drier) environments. Price et al. (1981a, 1981b) also pointed out that marginal populations of Microseris species had lower DNA content in their nuclei. Strong association of DNA content within species with environment suggests that chromosome length differences (or differences in DNA content) at the intraspecific level may have adaptive value in the establishment

of species populations (Dhir and Miksche, 1974; Stebbins, 1976; Price et al., 1981a, 1981b).

Information about the precise origins of the seed sources in this study is incomplete (Table 1). However, according to the results of Price et al. (1981a, 1981b), marginal populations may contain less DNA than centrally located populations. The assumption that there is a positive relation between chromosome length and DNA content may help to explain differences observed in chromosome length between the French seed source and Turkish seed source. Thus, the presence of longer chromosomes in the Turkish seed source than in the French seed source may be associated with geographical location of seed sources, since two of the French seed collections came from marginally located populations (from Corsica) while all three Turkish seed collections came from centrally located populations (personal communication with seed suppliers). However, more study is necessary before one can be certain of this.

b. Centromere positions (AR, CI)

Mean values of AR and CI were significantly different ($P < .05$ or $P < .01$) in chromosome sets I, II, III, V, VII, VIII, X, XI and XII among the seed sources (Table 3). Moreover, AR values in chromosome sets I to X were smaller in the Turkish seed source than in the other sources (Appendix 2, Tables 1-12). Median and submedian centromere positions were defined with AR values of .80-1.00 and .50-.80, respectively.

Previous studies on Pinus nigra karyotypes report that the two smallest chromosomes (chromosome-XI and XII) have submedian

centromeres while the 10 remaining chromosomes have median centromeres (Saylor, 1964; Pederick, 1970; Borzan and Papes, 1978; Borzan, 1981; MacPherson and Filion, 1981). However, while in this study, all sources had submedian centromere positions in chromosome-XII, in two of the five sources (France and Austria) the centromere of chromosome-XI was found to be in the median position. For example, AR values of chromosome-XI were (.86 $\pm$ .01) in the French seed source, (.81 $\pm$ .007) in the Austrian, (.71 $\pm$ .01) in the Yugoslavian, (.77 $\pm$ .02) in the Greek and (.78 $\pm$ .01) in the Turkish (Appendix 2, Table 11).

Variation among seed sources in the position of the centromere of a particular chromosome could originate from pericentric inversions or unequal translocations (Stebbins, 1971; Saylor, 1969; Pederick, 1969). Either one of the above causes would lead to irregularities in chromosome pairing during meiotic division and decrease viability in gametes of F_1 hybrids between seed sources with different centromere positions (Schulz-Schaeffer, 1980; Lewis, 1962). It would be interesting to study the behavior of chromosome-XI at meiosis of F_1 hybrids between the French or Austrian seed sources and the remaining sources. Moreover, it would be useful information for future tree improvement work in P. nigra to determine if seed production is reduced in F_1 hybrids between these sources.

c. Occurrence of secondary constrictions (S_{sec}, L_{sec}) and satellites (SAT)

Frequency of secondary constrictions on short and long arms of the 12 chromosomes for the different seed sources is given in Table 4. Secondary constrictions on chromosome-XII were not observed in the

Table 4. Frequencies of secondary constrictions and satellites (SAT) on Pinus nigra chromosomes from five seed sources (on short arm, S_{sec}; on long arm, L_{sec}).

Seed Source	Variables	CHROMOSOME I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
France	S_{sec}	.20	.27	.17	.30	.15	.15	.12	.10	.10	.07	.05	.02
	L_{sec}	.40	.27	.22	.12	.35	.17	.10	.18	.12	.15	.05	0
	SAT	0	0	0	0	0	0	0	0	0	0	0	0
Austria	S_{sec}	.31	.19	.03	.25	0	.12	.12	.12	.09	.09	.06	.06
	L_{sec}	.44	.34	.12	.41	0	.25	.28	.22	.09	.09	.03	.19
	SAT	0	0	0	0	0	0	0	0	0	0	0	0
Yugoslavia	S_{sec}	.22	.13	.32	.16	.22	.16	.13	.09	.13	.06	.09	0
	L_{sec}	.48	.23	.29	.23	.29	.26	.42	.13	.09	.19	.19	0
	SAT	.16	.06	.09	.09	.06	.13	.09	.06	0	.03	0	0
Greece	S_{sec}	.32	.32	.45	.23	.17	.15	.13	.19	.06	.02	.08	0
	L_{sec}	.53	.40	.38	.28	.38	.30	.28	.13	.15	.06	.11	0
	SAT	.15	.08	.13	.04	.06	.04	.13	0	0	0	.02	0
Turkey	S_{sec}	.22	.18	.18	.10	0	.08	.23	.08	.08	.12	0	0
	L_{sec}	.29	.37	.25	.25	0	.33	.27	.20	.19	.08	0	0
	SAT	0	0	0	0	0	0	0	0	0	0	0	0

Greek, Yugoslavian and Turkish seed sources. Morever, no secondary constrictions were found on chromosome-V in the Austrian and on chromosome-V and XI in the Turkish seed sources (Table 4). The lack of secondary constrictions on chromosome-V may be diagnostic of Austrian and Turkish seed sources of Pinus nigra.

Interestingly, satellites were only observed in the Yugoslavian and Greek seed sources. Long chromosomes of these seed sources (i.e., chromosomes-I to VIII) had relatively higher frequencies of satellites than the shorter ones (i.e., chromosome-XI to XII). In fact, chromosomes-IX to XII had either no satellites or only a rare (< 5%) occurrence of them in these two sources (Table 4). The occurrence of satellites only in the Yugoslavian and Greek seed sources might be useful in distinguishing them from other seed sources of P. nigra.

III. Karyotypic Relationships Among Seed Sources

Cluster analyses were performed for each of 12 chromosomes among seed collections by utilizing all variables (SL, LL, TL, AR, RL, MI, CI, S_{sec}, L_{sec}, SAT) at one time. A general clustering pattern among seed collections was developed by taking into consideration all the cluster analyses together. The similarity indices in Figure 7 were computed by averaging the values for amalgamated distances of the 12 cluster analyses conducted between seed collections. Cluster analyses indicated that the seed collections within the seed sources were uniform in terms of their karyotypes since the seed collections within seed sources tended to cluster together. It is evident from Table 3 that the variables (SL, LL, TL, AR, RL, CI and MI) on some chromosomes among the seed sources showed significant variation. In order to

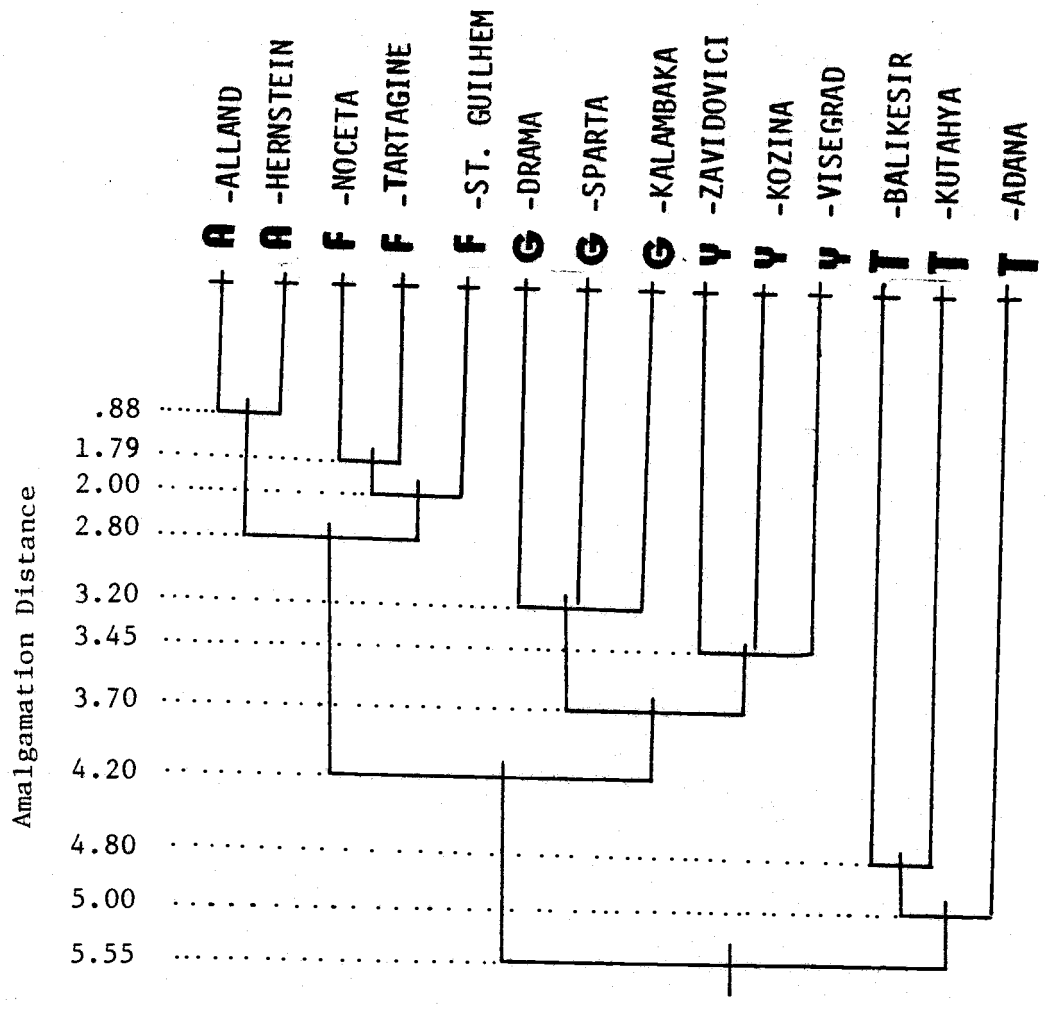

Figure 7. Dendrogram showing the general clustering pattern among the seed collections of five seed sources of European black pine (A = Austria, F = France, G = Greece, Y = Yugoslavia, and T = Turkey) (note: dendrogram not to scale).

determine karyotypic variation pattern among seed sources, cluster anlayses for each chromosome set between seed sources were conducted. In more detail, the results of separate cluster analysis for each chromosome between seed sources are presented in Appendix 1, Figure 1. As far as karyotypes of seed sources were concerned, the French and Austrian seed sources appeared to have the most similar chromosomes, with the Greek and Yugoslavian seed sources being quite similar to those two sources. The Turkish source, however, almost always clustered outside the other four sources and, thus, displayed the least chromosome similarity with the others (Figure 7 and Appendix 1, Figure 1).

On the other hand, one should avoid misinterpreting karyotypic similarities or variations based on chromosome morphology since chromosomes with similar morphology may have totally different gene content. However, karyotypic similarities between seed sources do provide us with some indication of the possible behavior of chromosomes in meiosis.

It would be possible to obtain maximum gene recombination from hybrids between karyotypically similar seed sources. On the other hand, hybrids between the French and Turkish or Greek and Turkish seed sources may not yield the maximum recombinations of genes because of differences in arm ratio and in length of chromosomes, presence or absence of satellites, secondary constrictions, etc., which may indirectly reduce the chiasma frequency in meiosis. From the literature, it appears that there is a great potential for tree improvement in Pinus nigra since variation in growth performance and resistance to cold and disease between seed sources have been reported (Wilcox

and Miller, 1975; Wheeler et al., 1976; Read, 1976; Vidakovic, 1974). Therefore, to maximize the genetic recombination in hybrids between seed sources, it is important for tree breeders to recognize the occurrence of karyotypic variation at the intraspecific level in P. nigra.

Traditionally, karyotypic information has been presented by just giving idiograms of chromosomes, along with AR and RL values. This kind of analysis of karyotypic data lacks a quantitative measure of karyotypic information. Especially, when large numbers of populations (species) and variables are involved in a karyotype study, the interpretation of karyotypic data will be more difficult. Unlike an idiogrammatic presentation of karyotypes, cluster analysis used here seems to be a powerful tool in determining karyotypic similarities and differences between populations within species by providing a numerical analysis. Therefore, using cluster analysis, along with conventional methods, is recommended in future karyotype studies when there are large numbers of variables and populations to be analyzed.

IV. Summary and Conclusions

Previous karyotypic studies in Pinus nigra, as well as in other pine species, have not reported any karyotypic variation at the intraspecific level for chromosome traits such as chromosome number, chromosome length, centromere position, etc. (Mehra and Khoshoo, 1956; Saylor, 1961, 1964, 1972; Pederick, 1967, 1970; MacPherson and Filion, 1981). However, in contrast to findings of many of these earlier studies in pine species, considerable intraspecific variation in chromosome morphology was found in this study. This was probably because a

more intense sampling was practiced at the intraspecific level in this investigation. The most significant findings of this study were:

1. One seedling in each of the Yugoslavian and Greek seed sources had an aneuploid chromosome number of 2n = 18. However, further study is needed to be certain of how frequently such irregularities in diploid chromosome number occur in this species.
2. Chromosome lengths among the seed sources showed significant variation. Generally, the French seed source had the shortest, while the Turkish had the longest chromosomes among the seed sources. To see if there is a positive correlation between the amount of DNA and chromosome length, a DNA study which included different seed sources of P. nigra in the sampling would be interesting.
3. In the Greek, Yugoslavian, and Turkish seed sources, both chromosome-XI and XII had a submedian centromere position, whereas in the French and Austrian seed sources only chromosome-XII had such a centromere position. It would be interesting to study meiotic behavior of chromosome-XI in hybrids of the French (or Austrian) and Greek (or Turkish or Yugoslavian) seed sources to determine if differences among these sources is great enough to result in severe pairing abnormalities.
4. Secondary constrictions were observed in greater frequency in longer chromosomes of all seed sources. However, chromosome-V from the Turkish and Austrian seed sources did not show any secondary constriction occurrences. This can be

used as a diagnosis of the Turkish and Austrian seed sources of P. nigra.

5. Satellites, at very low frequency, were observed in the Greek and Yugoslavian seed sources. This could be a useful marker in the diagnosis of these seed sources in P. nigra.

On the basis of cluster analyses, the Austrian and French seed sources appeared to have the most similar chromosomes, with the Greek and Yugoslavian seed sources being the closest to them. However, the Turkish seed source displayed the least similarity with the other seed sources. This karyotypic information can be used in future hybridization studies between P. nigra seed sources.

In the light of the results of this study, the following conclusions can be made on the cytological techniques and statistical procedures used in karyotyping of Pinus:

1. Since an inconsistent appearance of secondary constrictions on chromosomes was observed in this study, using two conventional stainings (i.e., C-banding with Giemsa staining) in the karyotyping of this species as well as other pine species may yield more consistent data on secondary constrictions.
2. Cluster analysis used here seems to be a very powerful tool in determining karyotypic similarities and differences at the intraspecific level. Hence, cluster analysis, along with traditional methods, is recommended in future karyotype studies when there are large numbers of variables and populations to be analyzed.

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APPENDIX 1

The results of cluster analyses and idiogramatic comparisons for each of 12 chromosomes among seed sources.

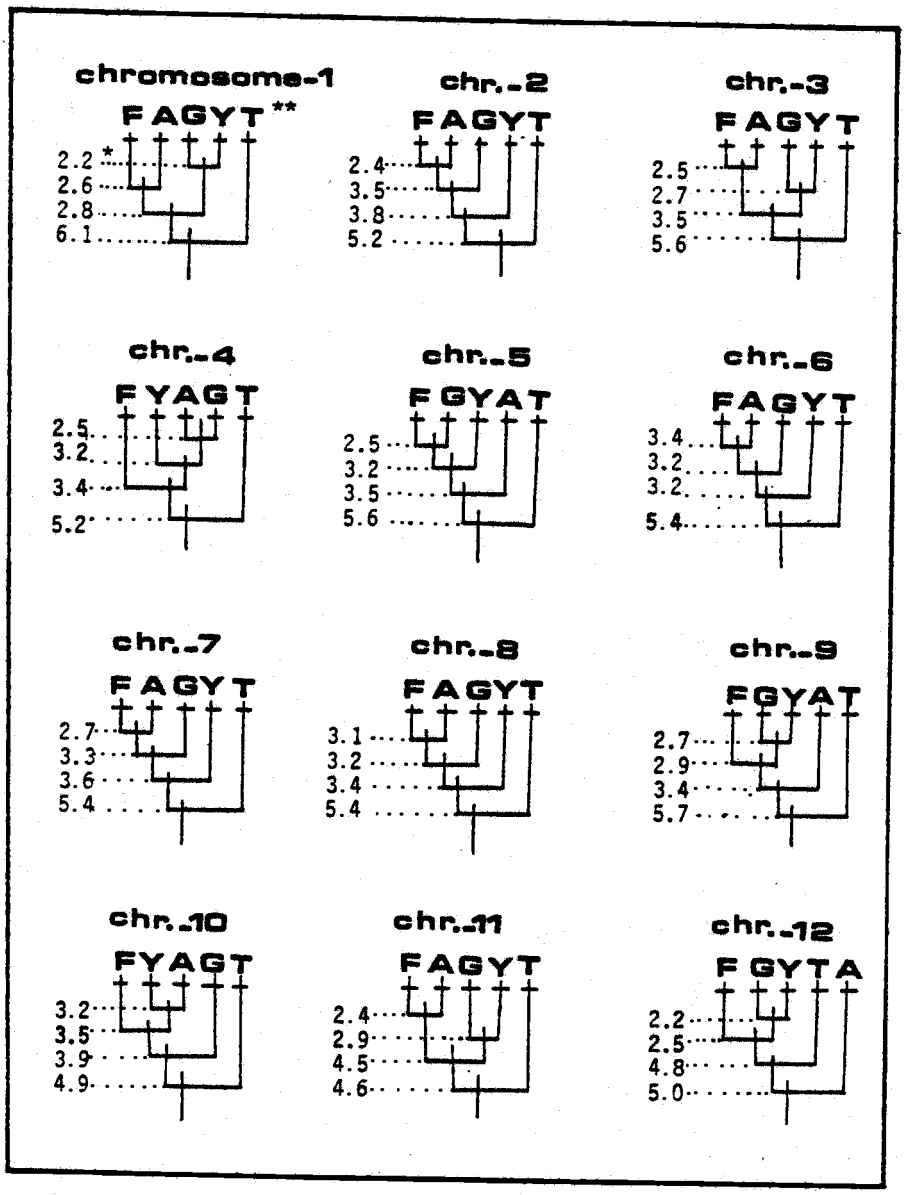

Figure 1. Cluster analysis of haploid chromosomes (n = 12) from five seed sources of European black pine (Pinus nigra Arn.) (note: dendrograms not in scale).

*Amalgamation distances for clusters.

**Seed sources: F = France, A = Austria, Y = Yugoslavia, and T = Turkey, G = Greece

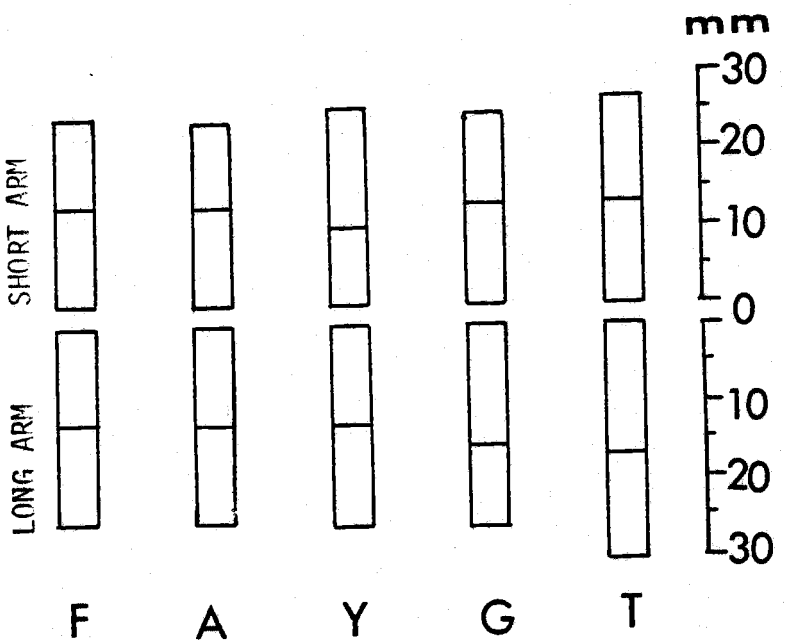

Figure 2. The idiograms of chromosome-I in five seed sources of European black pine (Pinus nigra Arn.). Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, and T = Turkey). Magnification: 3755.7X.

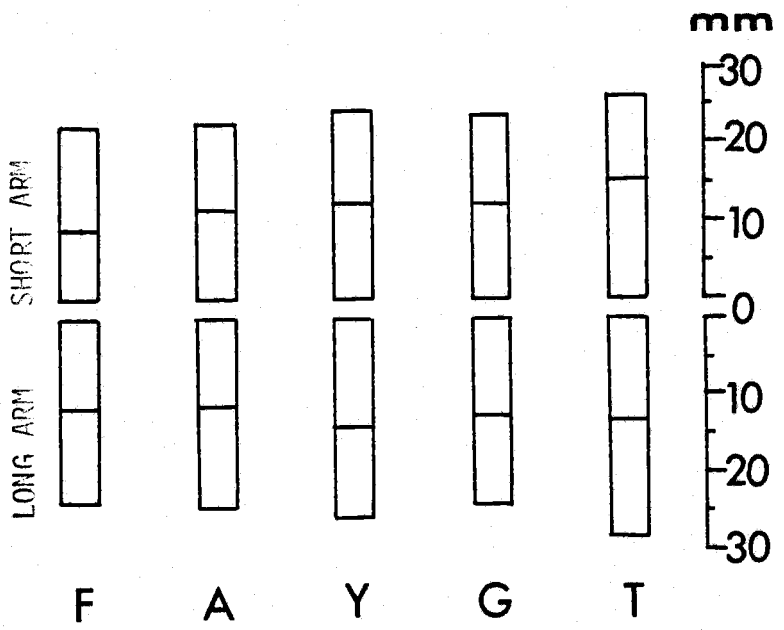

Figure 3. The idiograms of chromosome-II in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

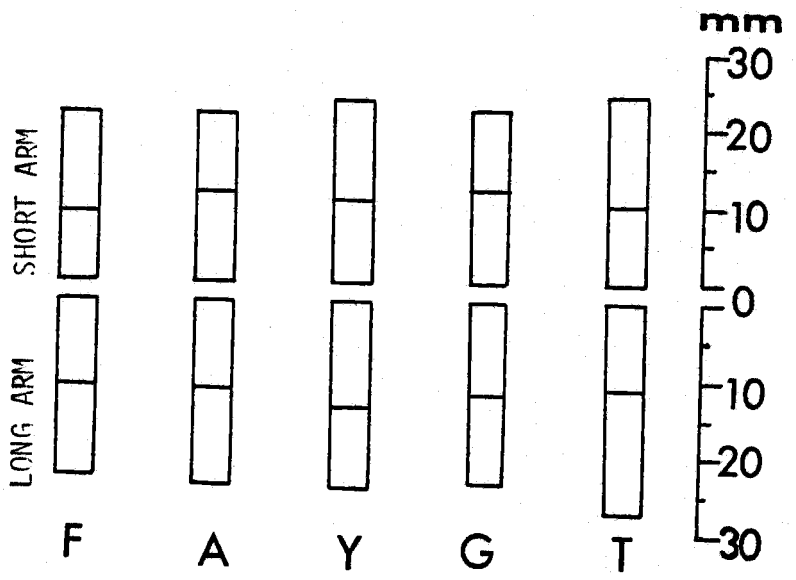

Figure 4. The idiograms of chromosome-III in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

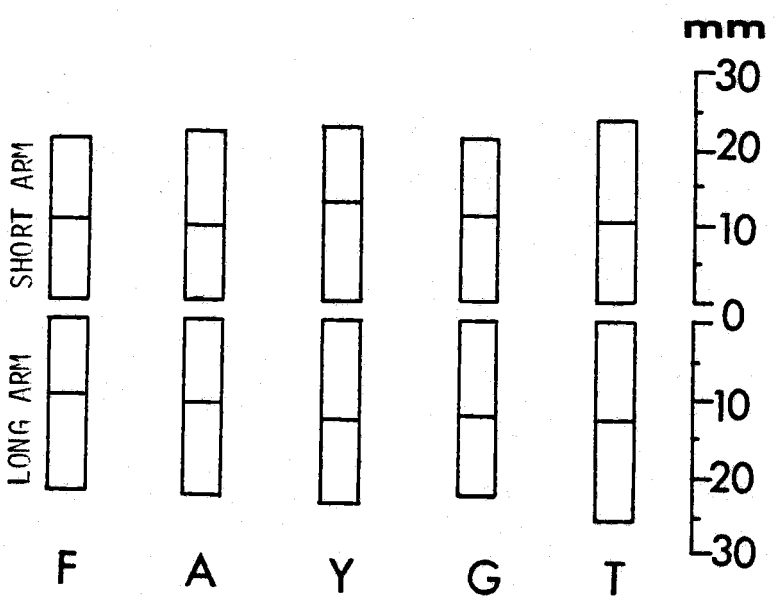

Figure 5. The idiograms of chromosome-IV in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

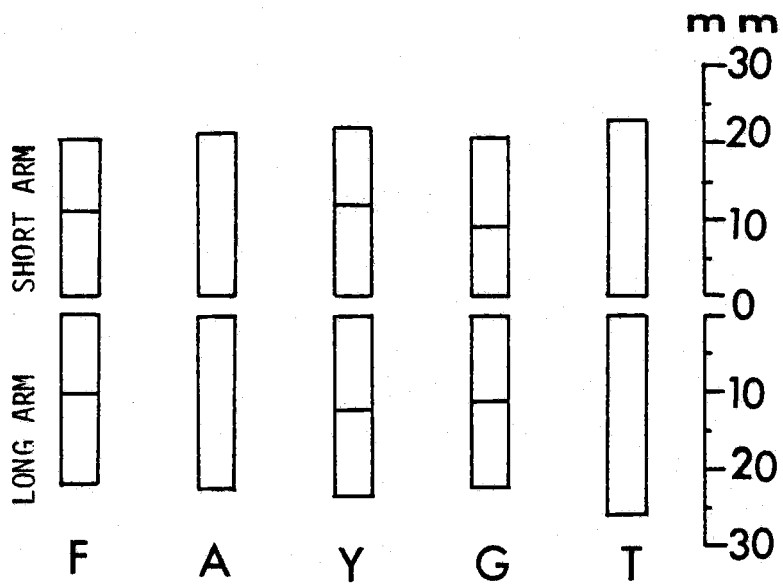

Figure 6. The idiograms of chromosome-V in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

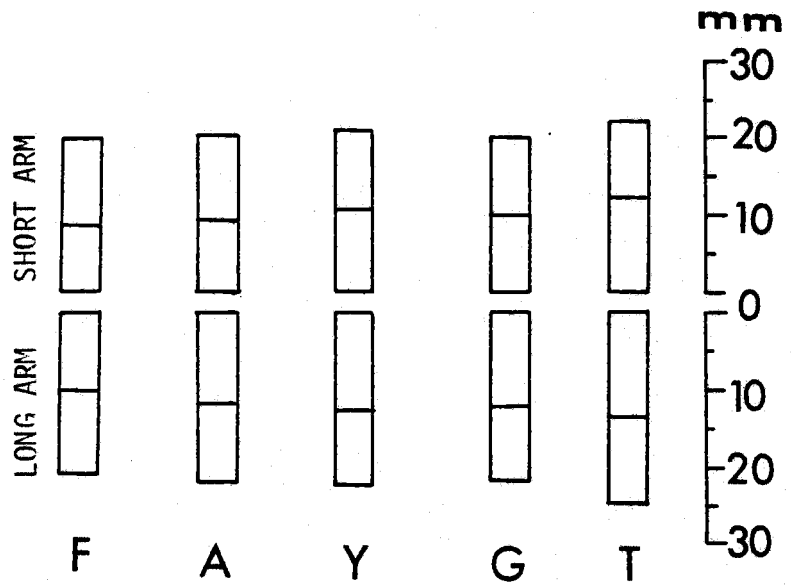

Figure 7. The idiograms of chromosome-VI in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

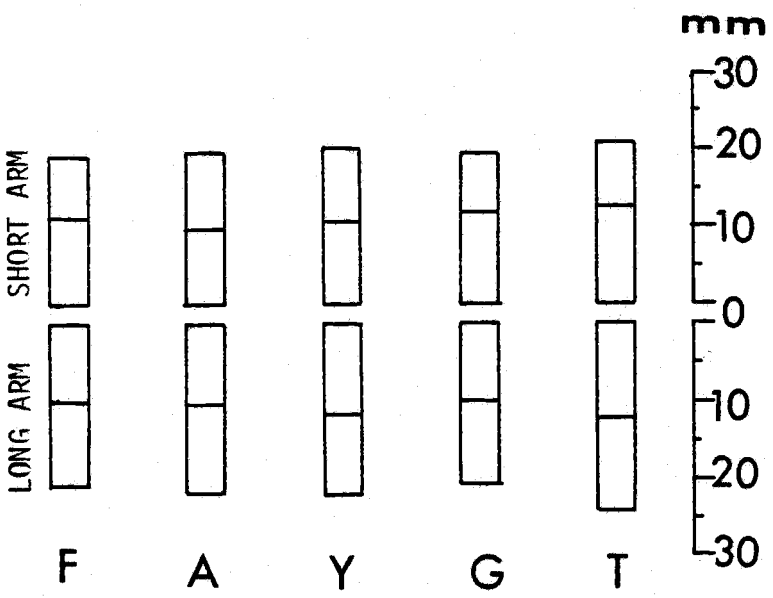

Figure 8. The idiograms of chromosome-VII in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

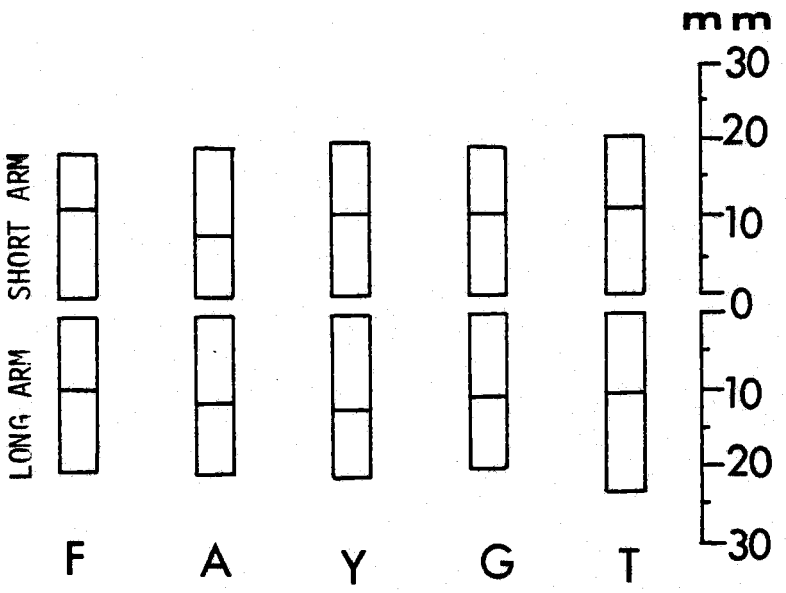

Figure 9. The idiograms of chromosome-VIII in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

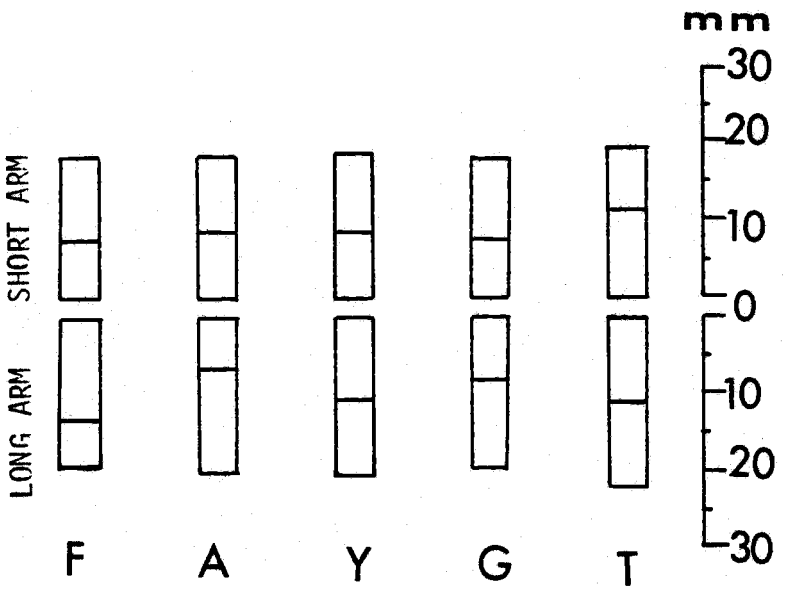

Figure 10. The idiograms of chromosome-IX in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

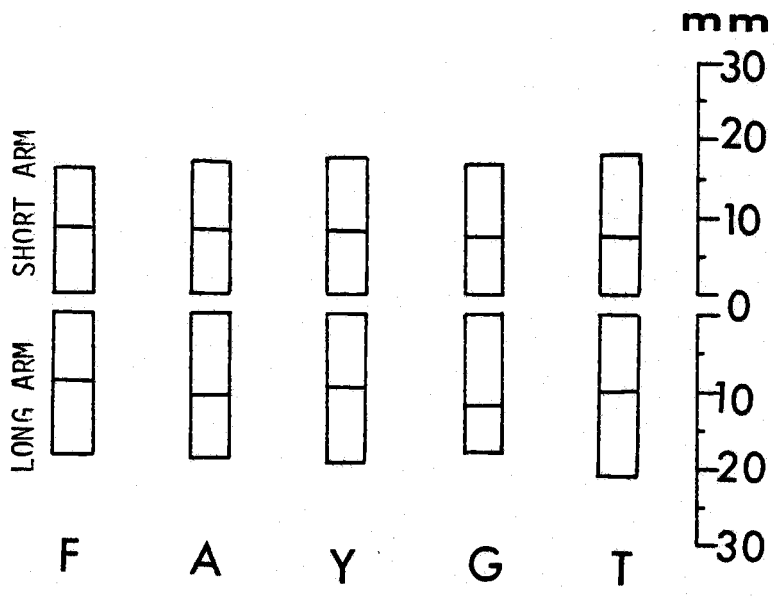

Figure 11. The idiograms of chromosome-X in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

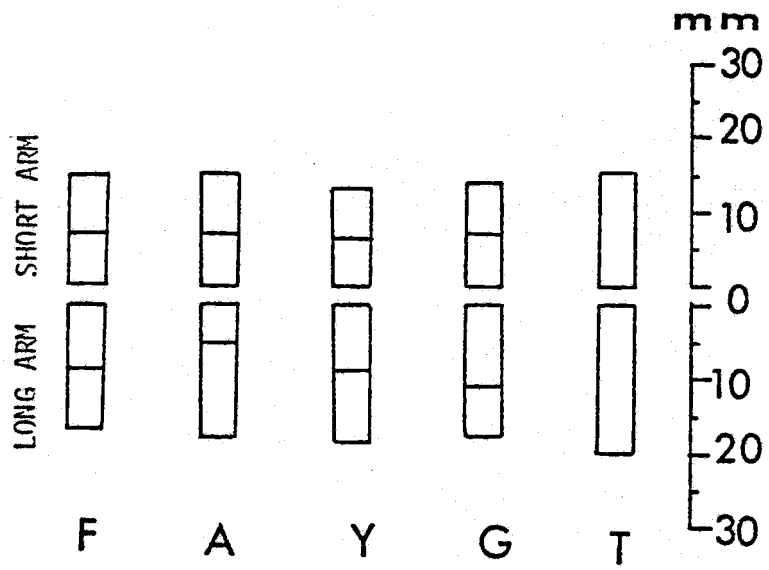

Figure 12. The idiograms of chromosome-XI in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

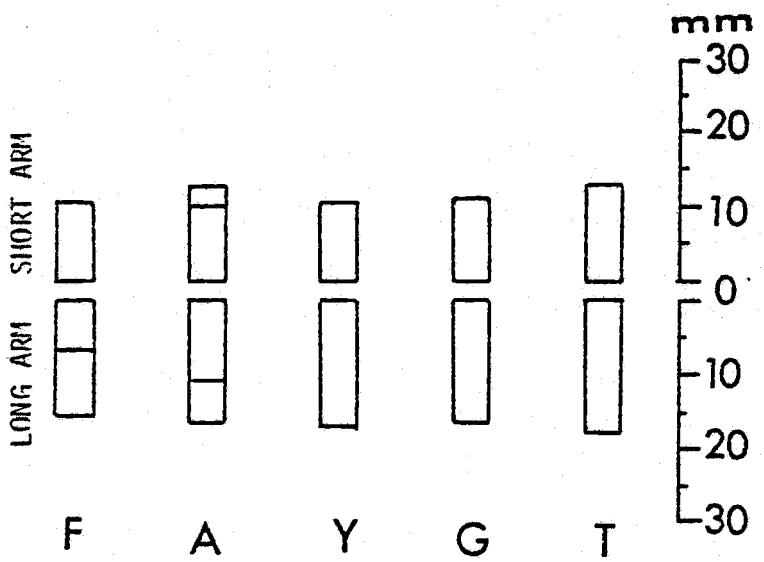

Figure 13. The idiograms of chromosome-XII in five seed sources of European black pine. Bands on chromosome arms indicate the average location of secondary constrictions (F = France, A = Austria, Y = Yugoslavia, G = Greece, and T = Turkey). Magnification: 3755.7X.

APPENDIX 2

The results of analysis of variances for means of chromosomal variables on each of 12 chromosomes among seed sources.

Table 1. Comparative karyotypic information for haploid chromosome-I from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources: France	Austria	Yugoslavia	Greece	Turkey	F-Statistics values[2] (df=4, 9)
SL (mm)[3]	23.79±1.74	23.63±.26	25.00±.50	23.91±.71	26.24±.78	2.65
LL (mm)	25.49±1.73	25.65±.26	26.28±.43	25.68±.62	30.19±1.66	6.22*
TL (mm)	49.28±3.46	49.28±.52	51.28±.93	49.59±1.33	56.43±2.44	4.55*
AR	0.93±0.006	0.92±0.00	0.95±0.006	0.93±.01	0.87±.03	7.74**
RL	123.8±2.11	120.8±.15	122.4±1.52	123.8±1.01	124.5±1.47	1.62
CI	48.2±0.14	48.0±0.00	48.7±.10	48.2±.20	46.5±.96	7.54**
MI	46.4±3.24	45.3±.50	48.8±1.03	46.1±1.53	49.7±1.51	1.54
S_{sec}	0.2	0.31	.22	.32	0.22	Not subjected to F-test
L_{sec}	0.4	0.44	.48	.53	.29	" " " "
SAT	--	--	.16	.15	--	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 2. Comparative karyotypic information for haploid chromosome-II from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources: France	Austria	Yugoslavia	Greece	Turkey	F-Statistics values[2] (df=4, 9)
SL (mm)[3]	22.17±1.24	22.28±.34	23.88±.42	22.64±.63	24.97±1.30	3.63
LL (mm)	23.92±1.44	24.34±.14	25.38±.65	24.03±.59	28.46±1.45	7.24**
TL (mm)	46.09±2.67	46.62±.48	49.26±1.05	46.67±1.22	53.43±2.61	5.53*
AR	0.93±.006	0.91±.007	0.94±.01	0.94±.004	0.88±.03	6.42*
RL	116.1±1.29	114.4±.15	117.6±.87	116.5±.40	117.6±.98	4.09*
CI	48.1±.15	47.8±.25	48.5±.30	48.5±.09	46.7±.84	6.35*
MI	42.7±2.30	42.6±.65	46.4±.72	44.1±1.27	46.97±2.78	2.43
SCS	.27	.19	.13	.32	.18	Not Subjected to F-test
SCL	.27	.34	.23	.40	.36	" " " "
SAT	0	0	.06	.08	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

*Significant at $\alpha = .05$ level of probability.
**Significant at $\alpha = .01$ level of probability.

Table 3. Comparative karyotypic information for haploid chromosome-III from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources: France	Austria	Yugoslavia	Greece	Turkey	F-Statistics values[2] (df=4, 9)
SL (mm)[3]	21.49±1.32	21.56±.25	23.16±.58	21.86±.59	24.11±1.48	2.72 NS at .05
LL (mm)	22.84±1.15	23.69±.43	24.48±.56	23.35±.43	27.72±1.31	10.49**
TL (mm)	44.33±2.47	45.25±.68	47.66±1.14	45.21±1.02	51.83±2.67	5.73*
AR	0.94±.01	0.91±.007	0.95±.006	0.93±.006	0.87±.04	6.73**
RL	111.6±.69	111.0±.40	113.7±.55	112.9±.04	114.1±.89	9.32**
CI	48.4±.29	47.8±.25	48.6±.11	48.3±.26	46.6±.87	6.54**
MI	41.7±2.81	41.2±.15	44.7±.94	42.2±1.23	45.1±3.27	1.53 NS at .05
S_{sec}	.17	.03	.32	.45	.18	Not Subjected to F-test
L_{sec}	.22	.12	.29	.38	.25	" " " "
SAT	0	0	.09	.13	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

NS = not significant.
*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 4. Comparative karyotypic information for haploid chromosome-IV from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources					F-Statistics values[2] (df=4, 9)
	France	Austria	Yugoslavia	Greece	Turkey	
SL (mm)[3]	20.83±1.32	21.44±.16	22.33±.52	21.14±.48	23.79±1.27	3.66*
LL (mm)	22.37±1.31	22.75±.28	23.85±.60	22.61±.56	26.00±1.17	5.86*
TL (mm)	43.20±2.63	44.19±.44	46.18±1.12	43.75±1.03	49.79±2.41	4.74*
AR	0.93±.006	0.95±.007	0.94±.006	0.93±.006	0.91±.02	2.52 NS at .05
RL	108.7±1.04	108.4±.20	110.2±1.04	109.2±.15	109.6±.43	1.91 " " "
CI	48.2±.17	48.5±.70	48.3±.09	48.4±.18	47.8±.44	2.52 " " "
MI	40.3±2.66	41.8±.18	43.3±1.02	40.8±.96	45.6±2.67	2.88 " " "
S_{sec}	.30	.25	.16	.23	.10	Not Subjected to F-test
L_{sec}	.12	.41	.23	.28	.25	" " " "
SAT	0	0	.09	.04	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7

NS = not significant.
*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 5. Comparative karyotypic information for haploid chromosome-V from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources: France	Austria	Yugoslavia	Greece	Turkey	F-Statistics values[2] (df=4, 9)
SL (mm)[3]	20.21±1.28	20.58±.36	21.64±.53	20.51±.50	22.67±1.31	2.59 NS at .05
LL (mm)	21.85±1.22	22.54±.47	23.45±.70	22.14±.48	25.91±1.18	7.29**
TL (mm)	42.06±2.50	43.12±.83	45.09±1.23	42.65±.98	48.58±2.47	4.62*
AR	0.93±.006	0.92±.007	0.92±.006	0.93±.004	0.87±.02	12.00**
RL	105.8±.71	105.8±.80	107.5±.76	106.5±.06	106.9±.55	3.10 NS at .05
CI	48.1±.18	47.8±.20	48.0±.73	48.03±.03	46.6±.52	11.85**
MI	39.0±2.56	40.0±.50	41.6±.93	39.5±1.05	42.4±2.67	1.48
S_{sec}	.15	0	.22	.17	0	Not Subjected to F-test
L_{sec}	.35	0	.29	.38	0	" " " "
SAT	0	0	.06	.06	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

NS = not significant.
*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 6. Comparative karyotypic information for haploid chromosome-VI from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources					F-Statistics values[2] (df=4, 9)
	France	Austria	Yugoslavia	Greece	Turkey	
SL (mm)[3]	19.82±1.24	20.24±.26	21.16±.56	19.85±.52	22.28±1.33	2.83 NS at .05
LL (mm)	21.42±1.15	22.16±.30	22.60±.72	21.69±.65	24.91±.97	5.97*
TL (mm)	41.24±2.4	42.40±.56	43.76±1.28	41.54±1.17	47.19±2.17	4.36*
AR	0.92±.01	0.91± 0	0.93±.01	0.913±.003	0.89±.03	1.67 NS at .05
RL	103.8±.61	104.0±.10	104.4±.23	103.7±.58	104.1±.44	0.76 " " "
CI	48.1±.28	47.7± 0	48.6±.20	47.8±.11	47.2±1.02	1.67 " " "
MI	38.2±2.54	38.6±.50	41.0±1.05	37.9±.93	42.2±3.08	1.97 " " "
S_{sec}	.15	.12	.16	.15	.08	Not Subjected to F-test
L_{sec}	.17	.25	.26	.30	.33	" " " "
SAT	0	0	.13	.04	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7

NS = not significant.
*Significant at α = .05 level of probability.

Table 7. Comparative karyotypic information for haploid chromosome-VII from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources					F-Statistics values[2] (df=4, 9)
	France	Austria	Yugoslavia	Greece	Turkey	
SL (mm)[3]	19.47±1.08	19.66±.21	20.47±.70	19.49±.49	21.22±1.05	1.91 NS at .05
LL (mm)	20.73±1.25	21.70±.26	22.05±.63	20.70±.60	24.42±1.45	5.25*
TL (mm)	40.2±2.33	41.36±.47	42.52±1.33	40.19±1.08	45.64±2.41	3.60 NS at .05
AR	0.94±.006	0.91±.007	0.93±.006	0.94±.006	0.87±.02	10.83**
RL	101.2±.53	101.5±.05	101.4±.26	100.3±.41	100.4±.71	2.69 NS at .05
CI	48.4±.18	47.6±.05	48.2±.14	48.5±.15	46.5±.74	10.84**
MI	37.7±2.04	37.4±.20	39.5±1.50	37.8±.98	39.8±2.04	0.92 NS at .05
S_{sec}	.12	.12	.13	.13	.23	Not Subjected to F-test
L_{sec}	.10	.28	.42	.28	.27	" " " "
SAT	0	0	.09	.13	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7

NS = not significant.
*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 8. Comparative karyotypic information for haploid chromosome-VIII from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources					F-Statistics values[2] (df=4, 9)
	France	Austria	Yugoslavia	Greece	Turkey	
SL (mm)[3]	18.65±.87	19.17±.40	19.78±.68	18.89±.35	20.32±1.35	1.32 NS at .05
LL (mm)	20.16±1.23	20.91±.01	21.35±.80	20.31±.53	23.38±1.13	4.54*
TL (mm)	38.81±2.10	40.08±.41	41.13±1.48	39.20±.88	43.69±2.33	2.91 NS at .05
AR	0.93±.01	0.92±.01	0.92±.006	0.93±.006	0.87±.04	3.73*
RL	97.7±.46	98.4±.15	98.1±.74	97.9±.23	96.1±.87	4.43*
CI	48.1±.41	47.8±.50	48.1±.09	48.2±.20	46.5±1.12	3.77*
MI	35.9±1.43	36.7±.95	38.1±1.24	36.4±.61	38.1±3.10	0.56 NS at .05
S_{sec}	.10	.12	.09	.19	.08	Not Subjected to F-test
L_{sec}	.18	.22	.13	.13	.20	" " " "
SAT	0	0	.06	0	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

NS = not significant.
*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 9. Comparative karyotypic information for haploid chromosome-IX from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources: France	Austria	Yugoslavia	Greece	Turkey	F-Statistics values[2] (df=4, 9)
SL (mm)[3]	17.79±.93	18.42±0.0	18.56±.64	18.10±.36	19.51±1.18	1.53 NS at .05
LL (mm)	19.28±.87	20.21±.52	20.66±.71	19.60±.33	22.41±1.10	5.71*
TL (mm)	37.07±1.80	38.63±.52	39.22±1.33	37.70±.68	41.92±2.15	3.50 NS at .05
AR	0.92±.02	0.91±.02	0.90±0.01	0.92±.006	0.87±.03	2.87 " " "
RL	93.4±.69	95.2±.55	93.5±.56	94.1±.43	92.3±.65	6.02*
CI	48.0±.41	47.7±.63	47.3±.30	47.7±.38	46.5±.93	2.93 NS at .05
MI	34.7±2.00	35.1±.30	35.2±1.28	34.7±.80	36.5±2.57	0.58 " " "
S_{sec}	.10	.09	.13	.06	.08	Not Subjected to F-test
L_{sec}	.12	.09	.09	.15	.19	" " " "
SAT	0	0	0	0	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

NS = not significant.
*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 10. Comparative karyotypic information for haploid chromosome-X from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources: France	Austria	Yugoslavia	Greece	Turkey	F-Statistics values[2] (df=4, 9)
SL (mm)[3]	16.53±.91	17.22±.37	17.82±.66	16.68±.25	18.44±1.04	2.64 NS at .05
LL (mm)	18.75±.70	19.23±.27	19.49±.74	18.51±.29	21.34±.87	6.58**
TL (mm)	35.28±1.61	36.45±.65	37.31±1.40	35.19±.53	39.78±1.85	4.25*
AR	0.88±.02	0.90±.01	0.91±.006	0.90±.006	0.86±.03	4.48*
RL	88.9±.61	89.4±.50	88.9±1.16	87.9±.76	87.6±1.19	1.32 NS at .05
CI	46.8±.43	47.3±.15	47.8±.03	47.4±.18	46.3±.72	4.44*
MI	31.1±1.96	32.6±.77	34.1±1.23	31.7±.55	34.4±2.23	1.92 NS at .05
S_{sec}	.07	.09	.06	.02	.12	Not subjected to F-test
L_{sec}	.15	.09	.19	.06	.08	" " " "
SAT	0	0	.03	0	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

NS = not significant.
*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 11. Comparative karyotypic information for haploid chromosome-XI from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources					F-Statistics values[2] (df=4, 9)
	France	Austria	Yugoslavia	Greece	Turkey	
SL (mm)[3]	14.79±.29	14.98±.11	13.30±.45	13.79±.22	15.52±.47	14.37**
LL (mm)	17.14±.44	18.38±.06	18.76±.71	17.84±.55	19.88±.69	7.27**
TL (mm)	31.93±.73	33.36±.17	32.06±1.14	31.63±.61	35.44±1.10	7.06**
AR	0.86±.01	0.81±.007	0.71±.01	0.77±.02	0.78±.01	31.09**
RL	80.7±2.55	81.8±.55	76.4±1.02	79.0±.29	78.1±1.89	3.49 NS at .05
CI	46.3±.26	44.9±.11	41.5±.38	43.6±.87	43.9±.35	30.24**
MI	27.6±.48	27.2±.30	22.73±.74	24.4±.56	27.6±.84	23.87**
S_{sec}	.05	.06	0.9	.08	0	Not subjected to F-test
L_{sec}	.05	.03	.19	.11	0	" " " "
SAT	0	0	0	.02	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

*Significant at α = .05 level of probability.
**Significant at α = .01 level of probability.

Table 12. Comparative karyotypic information for haploid chromosomes-XII from five Pinus nigra Arn. seed sources.

Variables[1]	Seed Sources					F-Statistics values[2] (df=4, 9)
	France	Austria	Yugoslavia	Greece	Turkey	
SL (mm)[3]	11.03±.33	11.90	10.69±.59	10.79±.37	12.79±.54	8.61**
LL (mm)	15.97±.48	16.53±.07	16.99±.68	16.46±.34	18.34±.25	9.27**
TL (mm)	27.00±.82	28.43±.07	27.68±1.27	27.25±.60	31.13±.69	9.06**
AR	0.69	0.72	0.63±.01	0.66±.02	0.696±.03	9.05**
RL	68.2±2.73	69.8±.65	66.0±1.16	68.1±.89	68.7±1.59	1.38 NS at .05
CI	40.9±.06	41.8±.15	38.6±.40	39.6±.79	41.1±.89	9.19**
MI	18.6±.57	20.5±.06	17.43±1.07	17.9±.84	21.7±1.28	8.55**
S_{sec}	.02	.06	0	0	0	Not subjected to F-test
L_{sec}	0	.19	0	0	0	" " " "
SAT	0	0	0	0	0	" " " "

[1]Descriptions of variables are the same as in Table 2.

[2]Analysis of variance was carried out with a nested design with seed sources (countries) as treatments and seed collections as a subsample.

[3]From projection drawings magnified by 3755.7.

NS = not significant.
**Significant at α=.01 level of probability.