Karyotype Analysis of Pinus-Group Lariciones[1])

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(Received for publication June 27, 1964)

Introduction

Early karyological studies of the genus Pinus describe pine chromosomes as being similar not only within karyotypes but also among the different species. Recent studies have shown, however, that this regularity is not so general as once thought. An example of karyotypic variability is presented in this paper. Karyotypes of 19 species of pine of the Group Lariciones are described. The pines in this Group are found primarily in the Old World where they range from Western Europe to the Far East. Two species are found in the New World. Pinus resinosa is located in northeastern United States and Canada; Pinus tropicalis is restricted to Cuba and the Isle of Pines.

Review of Literature[3])

The characteristic haploid karyotype of the genus Pinus was first described clearly by SAX and SAX (1933) as consisting of 12 similar chromosomes. The smallest chromosome is somewhat heterobrachial, and the remaining eleven have approximately median centromeres. Later investigators have wbtained the same general karyotypic pattern in various species. In addition some have also found evidence of interspecific differences in chromosome morphology. Although the magnitude of the evidence in the individual studies generally is not great, an analysis of the collective data discloses certain distinctive features. For example, AASS (1957) examined the karyotypes of 24 trees of Pinus sylvestris and found secondary constrictions in the largest pair of chromosomes. From the photographs and drawings presented in the article, it also appears that the second smallest as well as the smallest chromosome has a submedian centromere. A detailed study of the P. sylvestris karyotype was also made by NATARAJAN et at. (1961). Their idiogram shows two pairs of heterabrachial chromosomes and secondary constrictions in the largest chromosome pair. In addition, their idiogram shows secondary constrictions in four other pairs of chromosomes.

In a detailed study based on relative arm lengths, SAYLOR (1961) presented evidence of karyotypic variation. The centromeres of the two smallest chromosomes of Pinus resinosa, a member of the Group Lariciones to which P. sylvestris also belongs, were described as being located in a submedian position. Three other species of Lariciones, P. *nigra*, P. thunbergii, and P. densiflora, also were examined briefly. They possessed the second submedian chromosome characteristic of the karyotype of P. resinosa. These data, combined with the findings of AASS (1957) and NATARAJAN et al. (1961), suggested the submedian location of the centromere of the second smallest chromosome as a diagnostic feature of the karyotypes of species of *Lariciones*. The present study was designed to investigate this possibility.

Material and Methods

The number of species included in the Group *Lariciones* varies frwm 12 to 21 depending on the classification system used. The material available for this study included all but two of the 21 species that might be classified as members of this Group. All attempts to obtain material of Pinus hwangshanensis HSIA (found only in China) have failed thus far. Three seed lots of P. merkusii DEVRIES were acquired, but none of the seeds were viable. Species identification of the material studied was primarily according to information provided by the seed donors. All seedlings used are being grown for positive identification when sufficiently mature.

The karyotypes were determined by studying mitotic chromosomes from squash preparations of root-tip meristems of young seedlings. The root-tips were pretreated in oxyquinoline (0.3 g/l.) for 24—36 hours at 12° C, fixed in 3 : 1 ethyl alcohol-acetic acid for 1—4 hours, hydrolyzed in 1 N HCl for 10—15 minutes at 60° C, and stained in acetocarmine (SAYLOR, 1961).

The chromosomes were drawn at 2000× magnification using a projection apparatus. Measurements were made on the drawings. To facilitate studying and comparing the individual karyotypes, the chromosomes were numbered and arranged from 1—12 by descending order of lengths of the a (short) arm. Divergence of the lengths of the b (long) arms from a normal descending sequence and the locations of the centromeres and secondary constrictions were used as diagnostic features of the karyotypes. It was not possible to identify positively each of the chromosomes because the lengths of the a arms of adjacent chromosomes were often very similar. The lengths of the a arms for two and three chromosomes sometimes differed by only 0.5 mm, and the error term for the microtechnical and drawing procedures was of this magnitude. The patterns found in more than 50 per cent of the plates examined for a given species determined the final arrangement of chromosomes. No evidence of intraspecific ~karyotypicvariation has been detected, so a minimum of six drawings from at least four seedlings was considered adequate for defining the karyotypes of the individual species.

Problems encountered in studying secondary constrictions in pine chromosomes have been discussed previously (SAYLOR, 1961). The achromatic regions are often difficult to detect when chromosomes are contracted to the degree that is necessary to study their general morphology. To help overcome this problem, a greater effort was made in this study to select only those plates in which the achromatic regions were evident and the chromosomes were not overly contracted. This proved to be relatively helpful. Secondary constrictions were observed with sufficient repeatability (i.e., in more than 50 per cent of the plates) in the chromosomes of most species to include them as part of the karyotype.

Results and Discussion

The karyotypes of all species examined are basically similar to the general karyotypic pattern presented for the pines by SAX and SAX (1933). The chromosomes within any

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[1]) Contribution from the Department of Genetics and the Department of Forest Management, North Carolina Agricultural Experiment Station as Journal Paper No. 1766. Supported in part by the National Science Foundation Program in Systematic Biology: Grant G 18715.

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[3]) A more detailed review of the literature was presented in an earlier paper (SAYLOR, 1961).

Table 1. — Mean arm length in millimeters of chromosomes magnified 2000×. See note.

Chromosome	Arms a	Arms b	a/b	Arms a	Arms b	a/b	Arms a	Arms b	a/b	Arms a	Arms b	a/b
	P. densiflora			*P. heldreichii*			*P. insularis*			*P. khasya*		
1	12.7	13.6*	.934	14.5	16.4	.884	14.5	16.4	.884	14.9	16.6	.898
2	12.0	12.8*	.938	14.1	15.1	.934	14.1*	15.1	.934	14.4	15.2	.947
3	11.7	11.8	.992	13.7*	14.1	.972	13.8	14.2	.972	14.0	14.5	.965
4	11.5	12.5	.920	13.5	15.1	.894	13.4	14.7	.912	13.7	15.4	.890
5	11.2	11.6	.966	13.1	13.6	.963	13.1	14.0	.936	13.4	14.0	.957
6	11.1	12.3	.902	12.8	14.9	.859	12.8	14.7	.871	13.0	14.8	.878
7	10.9	11.4	.956	12 6	13.0	.969	12 6	13.5	.933	12.8	13.4	.955
8	10.6	12.1	.876	12.1	13.9	.870	12.2	14.7	.830	12.4	14.6	.849
9	10.4	11.4	.912	11.9	12.8	.930	11.8	13.2	.894	11.9	12.9	.922
10	9.6	10.3	.932	10.9	12.6	.865	10.9	11.8	.924	10.9	11.9	.916
11	7.7	10.6	.726	9.4	13.1	.717	8.4	13.6	.618	8.8	13.5	.652
12	6.3	8.8	.716	6.6	11.8	.559	7.2	11.0	.654	7.7	11.9	.647
	P. luchuensis			*P. massoniana*			*P. montana*			*P. nigra*		
1	13.3	14.4*	.924	13.6*	14.3	.951	12.9	13.4	.963	14.4*	15.4	.935
2	13.0	13.7	.949	13.0	13.4	.970	12.4	13.0	.954	14.0	14.6*	.959
3	12.6	12.9	.977	12.5	12.9	.969	12.2	12.5	.976	13.6	14.2	.958
4	12.5 x	13.8	.906	12 3	13.3	.925	11.9	13.0	.915	13.3	13.6*	.978
5	12.3	12 7	.939	12.1	12.5	.968	11.7	12.1	.967	13.0	13.5*	.963
6	12.1	14.2	.852	11.9	12.9	.922	11.4*	12.6	.905	12.9	14.3z	.902
7	11.8	12.7	.929	11.6	12.2	.951	11.0	11.9	.924	12.6	13.4	.940
8	11.4	12.3	.927	11.3	12.7	.890	10.8	11.2	.964	12.1	12.7	.953
9	11.2	13.3	.842	11.0	12.1	.909	10.3	12.4	.831	11.8	13.0	.908
10	10.4*	11.4	.912	10.1	11.4	.886	9.5	10.5	.905	10.6	12.1	.876
11	8.4	11.8	.712	7.9	12.0	.658	7.6	10.9	.697	8.6	11.9	.723
12	7.1	9.6	.740	6.7	10.3	.650	6.7	9.7	.691	7.1	10.8	.657
	P. resinosa			*P. sylvestris*			*P. tabulaeformis*			*P. taiwanensis*		
1	15.1	15.8*	.956	13.7*	14.7	.931	14.5	15.8*	.918	15.1	15.7	.962
2	14.2	14.9	.953	13.0	14.2*	.915	14.0*	15.1	.927	14.5	15.1	.960
3	13.7	14.2	.965	12.7	13.1	.969	13.9	14.1	.986	14.2	15.0*	.947
4	13.4	14.9z	.899	12.4	12.8	.969	13.6	14.8	.919	13.9	16.0*	.869
5	13.1	13.8	.949	12.1	13.8	.877	13.3	14.1	.943	13.8	14.4	.958
6	12.9	14.8z	.872	11.8	12.3	.959	13.1	13.5	.970	13.5	15.0	.900
7	12.7	13 1	.969	11.5	13.0	.885	12.8	14.6	.877	13.3	13.9	.957
8	12.4	14.2	.873	11.2	11.8	.949	12.5	13.2	.947	12.8	14.9	.859
9	12.1	13.2	.917	10.8	12.4	.871	12.0	13.7	.876	12.2	13.7	.890
10	11.4*	12 6	.905	9.9*	11.2	.884	11.1	12.1*	.917	11.3	13.1	.862
11	8.7	12.9	.674	7.9	11.6	.681	8.9	12.7	.701	9.0	13.4	.672
12	6.8	10.9	.624	6.8	10.2	.667	7.4	10.7	.691	7.6	11.0	.691
	P. thunbergii			*P. tropicalis*			*P. yunnanensis*			*P. halepensis*		
1	12.6	13.8	.913	15.5*	16.6*	.934	14.8*	15.9	.931	16.5	17.3	.954
2	12.4	13.1	.947	15.2	15.8	.962	14.1	15.1	.934	15.5	17.1	.906
3	12.2	12.4	.984	14.8	15 3	.967	13.9	14.4	.965	15.1	16.5	.915
4	12.0	13 3	.902	14.4	16 0	.900	13.5	14.1	.957	14.8	15.7	.943
5	11.9	12.4z	.960	14.2	14.7	.966	13.3	14 4	.924	14.5	15.2	.954
6	11.6	13.3	.872	14.0	15.4	.909	13.1	14.0	.936	14.0	16.1	.870
7	11.4	12.4	.919	13.7	14.5	.945	12.9	13.8	.935	13.8	15.5	.890
8	10.9	11.4	.956	13.5	16.0z	.844	12.4	14.5	.855	13.6*	14.4	.944
9	10.7	12.0	.892	13.0	14.4	.903	12.1	12.9	.938	13.2	16.2	.815
10	10.1*	11.3	.894	12.0	13.1	.916	10.7	12.6	.849	12.4	14.5	.855
11	7.6	11.4	.667	9.4	14.3	.657	9.1	12.8	.711	9.7	15.2	.638
12	6.3	9.1	.692	7.1	11.5	.617	7.7	11.3	.681	7.4	13.7	.540
	P. brutia			*P. pityusa*			*P. pinaster*					
1	16.7	17.7	.944	17.5	18.8*	.931	12.9*	13.9	.928			
2	15.8*	16.9	.935	16.9	17.8	.949	12.4	13.2*	.939			
3	15.4	16.0	.962	16.3	17.7	.921	12.1	12.5	.968			
4	15.1	17.3*	.873	16 1	16 6	.970	11.7	12.2	.959			
5	14.9	15.6	.955	15.7	17.2	.913	11.6*	12.9	.899			
6	14.7	17.0	.865	15.5	16.5	.939	11.4	12.0	.950			
7	14.4	15.8	.911	15.3	16.9	.905	11.2	11.8	.949			
8	14.2	14.9	.933	14.7	15.6	.942	11.0	12.5	.880			
9	13.8	15.8	.873	14.1	16 5	.854	10.6	11.1	.955			
10	12.9	14.7	.878	13.5	15 6	.865	9.6	10.9	.881			
11	9.9	15.3	.647	10 5	16.3	.644	8.4	11.1	.754			
12	8.3	14.3	.580	8.5	15.0	.567	6.6	10.2	.647			

Note: — x, *, and z denote secondary constrictions in the distal, medial, and proximal portion of the arms, respectively.

given complement are similar in size and general morphology. They gradually decrease in length, and they possess either median or submedian centromeres. (Chromosomes with median and submedian centromeres are defined by short-arm : long-arm ratios of 0.75—1.00 and 0.50—0.75, respectively.) The smallest chromosome is always heterobrachial.

The mean lengths of the chromosome arms *(Table 1)* do not provide critical information about differences in chromosome lengths among the various species. The apparent specific differences most likely resulted from unequal pretreatment periods, or from differential reaction of the chromosomes to the pretreatment, or both.

The two smallest chromosomes in the haploid complement of every species of *Lariciones* examined possess submedian centromeres *(Tables 1 and 2; Figures 1 and 2)*. The centromeres of the ten remaining chromosomes are all median. In my overall cytogenetic study of this genus, karyotypes have been determined for 39 species from eight of the twelve Groups listed by SHAW (1914 and 1924). Thus far, only the species of *Lariciones* have the centromeres of both of the smallest chromosomes located in a submedian position *(Tables 2 and 4)*. This evidence confirmes the earlier findings of AASS (1957), NATARAJAN *et al.* (1961), and SAYLOR (1961) and suggests the existence of inter-Group differences in chromosome morphology.

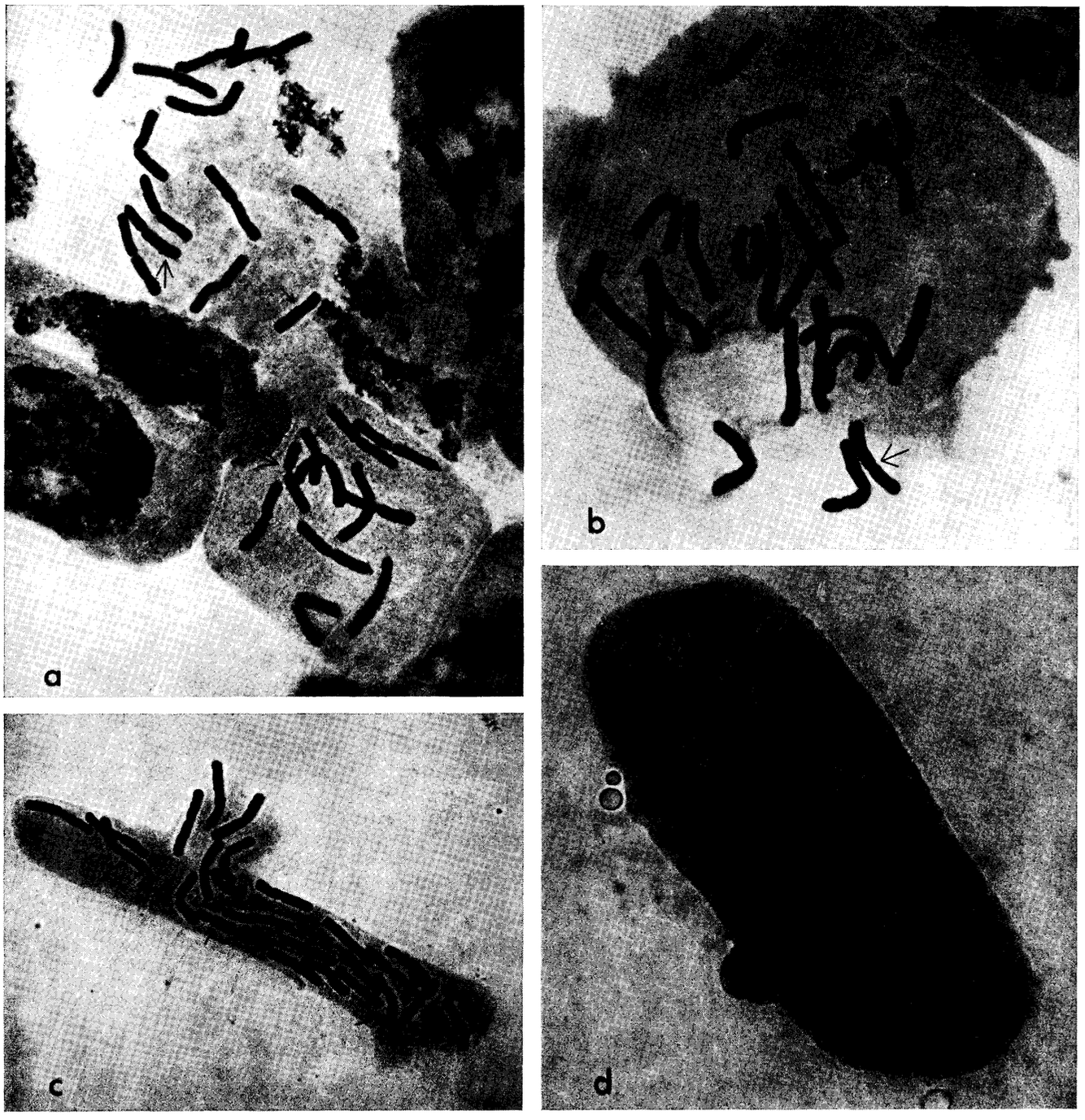

Figure 1. — Chromosomes from aceto-carmine preparations of root-tip meristems. The short and long arrows denote respectively the smallest and second smallest chromosomes, both of which are heterobrachial. (a) *P. tropicalis* (1025×); (b) *P. resinosa* (1225×); (c) *P. heldreichii* (780×); (d) *P. sylvestris* (1125×).

Table 2. — Diagnostic features of karyotypes of species of *Lariciones* obtained by arranging the chromosomes according to descending order of the *a* (shorter) arm lengths as in *Table 1*.

Species	Plates drawn	Plants	Chromosomes showing exceptions to the descending order in *b* (longer) arm lengths	Chromosomes with most median centromeres	Chromosome eleven *a*/*b*	Chromosome twelve *a*/*b*
P. densiflora SIEB. & ZUCC.	10	6	4, 6, 8, 11	3, 5	.726	.716
P. heldreichii CHRIST.	6	4	4, 6, 8, 11	3, 7	.717	.559
P. insularis ENDLICH	6	4	4, 6, 8, 11	3, 5	.618	.654
P. khasya ROYLE	6	4	4, 6, 8, 11	3, 5	.652	.647
P. luchuensis MAYR	8	4	4, 6, 9, 11	3, 5	.712	.740
P. massoniana LAMB.	6	5	4, 6, 8, 11	2, 3	.658	.650
P. montana MILLER	7	5	4, 6, 9, 11	3, 5	.697	.691
P. nigra ARNOLD	6	6	6, 9, 11	4, 5	.723	.657
P. resinosa AIT.	13	8	4, 6, 8, 11	3, 7	.674	.624
P. sylvestris L.	13	8	5, 7, 9, 11	3, 4	.681	.667
P. tabulaeformis CARRIERE	6	4	4, 7, 9, 11	3, 6	.701	.691
P. taiwanensis HAYATA	7	4	4, 6, 8, 11	1, 2	.672	.691
P. thunbergii PARL.	6	6	4, 6, 9, 11	3, 5	.667	.692
P. tropicalis MORELET	8	5	4, 6, 8, 11	3, 5	.657	.617
P. yunnanensis FRANCHET	7	4	5, 8, 11	3, 4	.711	.681
P. halepensis MILLER	6	6	6, 9, 11	1, 5	.638	.540
P. brutia TEN.	6	6	4, 6, 9, 11	3, 8	.647	.580
P. pityusa STEVEN	10	7	5, 7, 9, 11	2, 4	.644	.567
P. pinaster AIT.	10	6	5, 8, 11	3, 4	.754	.647

Table 3. — Number of species in *Table 2* having common patterns of *b* arm lengths and of location of metacentric chromosomes.

b arm patterns		Chromosomes with most median centromere	
Sequence	Species	Positions	Species
4, 6, 8, 11	8	1, 2	1
4, 6, 9, 11	4	1, 5	1
Either of above with one change	3	2, 3	1
Other	4	2, 4	1
		3, 4	3
		3, 5	7
		3, 6	1
		3, 7	2
		3, 8	1
		4, 5	1

Table 4. — Short-arm : long-arm ratio of the second smallest chromosome (number eleven) for species of *Pinus* as arranged by SHAW (1924).

Species	Ratio
Group *Strobi*	
P. flexilis JAMES	.839
P. strobus L.	.875
P. lambertiana DOUGL.	.832
P. flexilis JAMES	.839
Group *Balfourianae*	
P. aristata ENGELM.	.830
Group *Longifoliae*	
P. canariensis SMITH	.769
Group *Pineae*	
P. pinea L.	.860
Group *Australes*	
P. palustris MILL.	.944
P. taeda L.	.920
P. caribaea MORELET	.816
P. elliottii ENGELM.	.929
P. ponderosa LAWS.	.807
P. washoensis MASON and STOCKWELL	.824
P. jeffreyi GREV. and BALF.	.775
P. glabra WALT.	.864
P. engelmanii CARR.	.843
P. cooperi BLANCO	.812
Group *Insignes*	
P. virginiana MILL.	.878
P. clausa (CHAPM.) VASEY	.915
Group *Macrocarpae*	
P. coulteri D. DON	.803
P. torreyana PARRY	.872

The karyotypes of the species within the Group *Lariciones* are not readily distinguishable from each other, although subtle interspecific differences possibly exist. Patterns of length of the *b* arms were determined after arranging the chromosomes of a given karyotype in descending order according to the lengths of the *a* arms *(Table 2)*. The locations of the two chromosomes with the most median centromeres also were compared. According to these features, different karyotypes exist within this Group, although many of the karyotypes are similar. For example, several species appear to have a common karyotype *(Tables 2 and 3; Figure 3)* in which the chromosomes in positions 4, 6, 8, and 11 commonly interrupt the descending order of the *b* arm lengths and in which the most median centromeres occur in positions 3 and 5. Considering the process of speciation these results are not unexpected, and this may be the basic pattern from which the other species in the Group have evolved. If this postulation is true, common patterns may also exist within each of the other Groups.

The location of secondary constrictions is another feature that may be used in determining differences among karyotypes. In 18 of the 19 species examined, secondary constrictions were observed with sufficient repeatability to be included as part of the karyotypes *(Table 1)*. The number of chromosomes in a karyotype having secondary constrictions ranged from one to five. The most consistent results were observed for *P. massoniana;* secondary constrictions were found in the same region of chromosome number 1 in every plate drawn of this species. A similar situation was found for chromosome number 1 in eight out of ten plates of *P. pinaster.*

The location of the achromatic regions was sufficiently variable to suggest that this evidence is not absolutely conclusive. The results are considered accurate enough for indicating where secondary constrictions are most probably located. The value of secondary constrictions as a diagnostic feature of pine karyotypes remains questionable, because the numbers and positions of these regions reported by different investigators frequently vary for any one species. For example, AASS (1957) observed an achromatic region in the longer arm of the largest chromosome of the *P. sylvestris* complement. However, NATARAJAN *et al.*

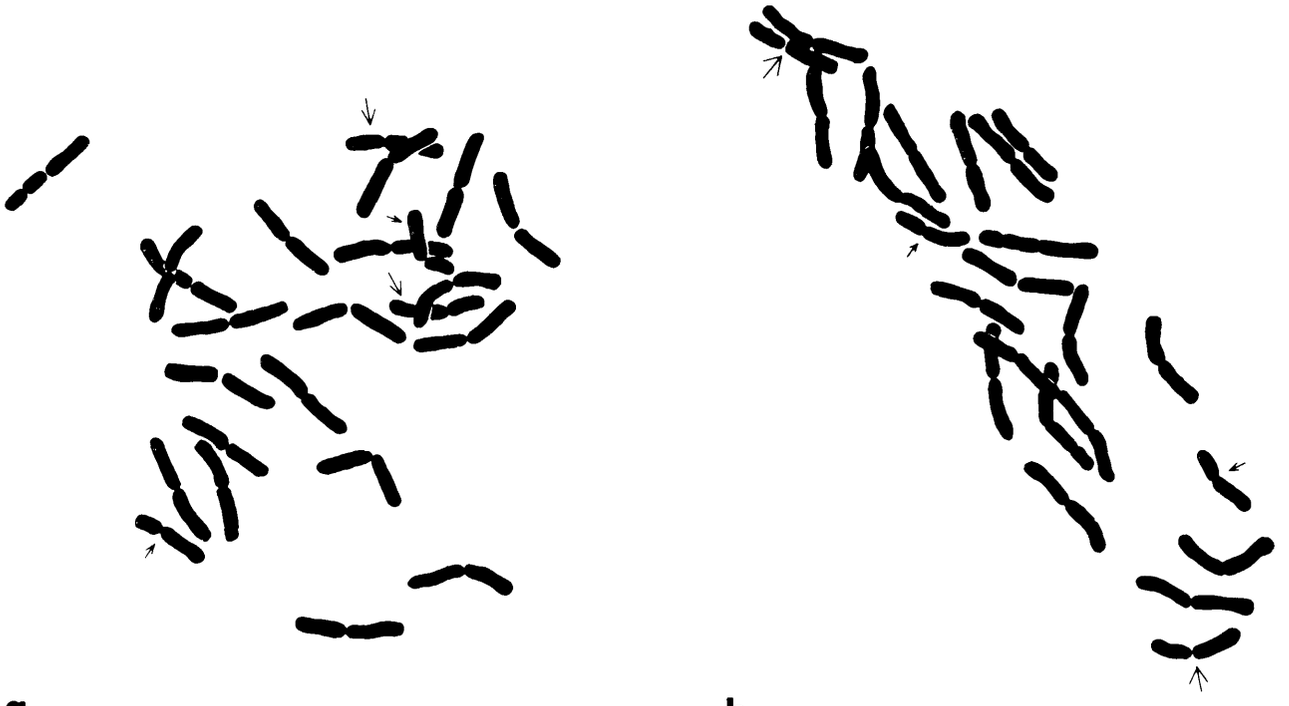

Figure 2. — Projection drawings of chromosomes obtained from aceto-carmine squash preparations of root-tip meristems. The short and long arrows denote respectively the smallest and second smallest chromosomes, both of which are heterobrachial. (a) *P. halepensis* (Reduced to 1200×); (b) *P. pinaster* (Reduced to 1200×).

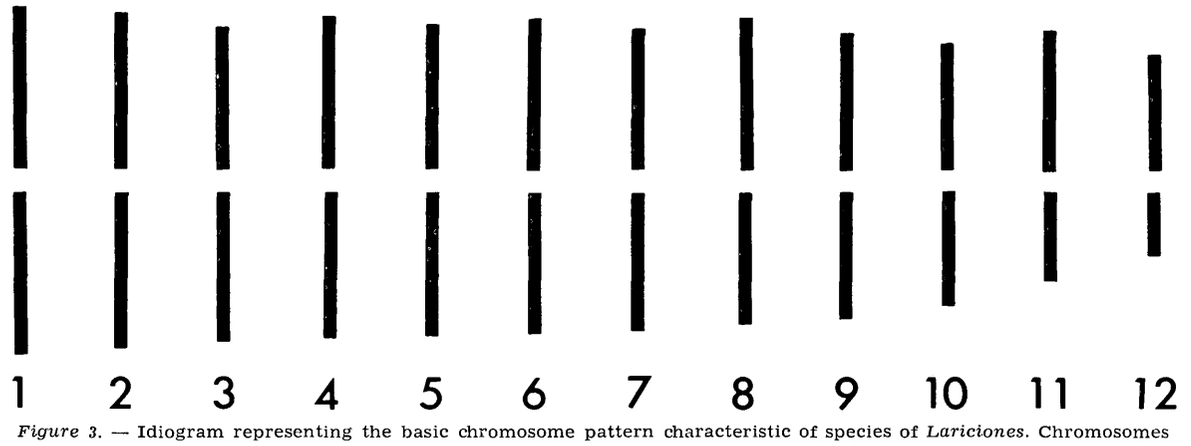

Figure 3. — Idiogram representing the basic chromosome pattern characteristic of species of *Lariciones*. Chromosomes arranged in descending order of length of the shorter arm. (See text for further explanation.)

(1961) placed it in the short arm of the same chromosome, and they described four other secondary constrictions that Aass did not report. The results of this study support the findings of Natarajan *et al.* for chromosomes 1 and 10, but differ for chromosomes 2, 6 and 7. An achromatic region was found in the longer arm rather than the shorter arm of chromosome 2. No secondary constrictions were observed in chromosomes 6 and 7.

Species of pine have been classified according to several different criteria such as seed and cone characteristics (Shaw, 1914), needle numbers (Pilger, 1926), and cross-ability patterns (Duffield, 1952). In general, there is little disagreement among the different classifications with respect to the *Lariciones*. The present study provides karyological data substantiating the interrelationship of the species in this Group. In addition it provides information that should help clarify the taxonomic position of four controversial species.

Pinus halepensis has been placed in various Groups, depending on the characteristics used in the classification. Shaw placed this species in the Group *Insignes*, while Pilger assigned it to the Section *Banksia*.[4]) Duffield concluded, however, from morphological, anatomical, and biochemical data that *P. halepensis* is more closely related to species of *Lariciones*. The presence of the heterobrachial eleventh chromosome, characteristic of the *Lariciones*, in every examined plate of *P. halepensis* supports Duffield's conclusion. Similar evidence was found for *P. brutia* and *P. pityusa*, two taxa often considered varieties of *P. halepensis*, which further corroborates the relationship of *P. halepensis* to the other *Lariciones*.

Pinus pinaster is another species whose taxonomic position is questionable. Shaw considered this pine to be more closely related to species of *Insignes*, while Pilger and

[4]) Pilger's Sections are taxonomically equivalent to the Groups of Shaw and Duffield.

DUFFIELD placed it in the *Lariciones*. The karyological data for this species are not so easily interpreted as are the others, because the average value of the short-arm : long-arm ratios for the eleventh chromosome is slightly greater (0.754) than the upper limit of the submedian category; four of the ratios are just below 0.750 and six are slightly above. However, karyotypes for 20 species from seven other Groups have been determined, and only two of these species have a ratio below 0.800 for the eleventh chromosome *(Table 4)*. The value for *P. pinaster* is so close to the arbitrary limit of the submedian category that it resembles the species of *Lariciones* more than those in any of the other Groups studied, including *Insignes*.

Summary

Karyotypes are described for 19 species of pine of the Group *Lariciones*. All species had a similar but distinct karyotype characteristic of the Group. The haploid *Lariciones* karyotype differs from those of other Groups by containing two heterobrachial chromosomes instead of one.

Karyological evidence supports the inclusion of *Pinus halepensis, P. brutia, P. pityusa,* and *P. pinaster* in the Group *Lariciones*. The karyotypes of these species are very similar to those of the generally accepted species of *Lariciones,* because they possess the heterobrachial eleventh chromosome characteristic of this group.

Résumé

Titre de l'article: *Analyse des karyotypes des pins du groupe Lariciones.*

L'auteur décrit les karyotypes de 19 espèces de pin du groupe *Lariciones*. Toutes les espèces présentent le karyotype caractéristique du groupe; chaque karyotype est cependant distinct. Le karyotype haploïde des *Lariciones* diffère de ceux des autres groupes: il comporte deux chromosomes hétérobrachiaux au lieu d'un.

L'analyse karyologique permet d'inclure dans le groupe *Lariciones* les espèces suivantes: *Pinus halepensis, P. brutia, P. pityusa* et *P. pinaster*. Ces espèces sont très semblables à ceux des espèces généralement considérées comme *Lariciones*, parce qu'elles ont le onzième chromosome hétérobrachial.

Zusammenfassung

Titel der Arbeit: *Karyotypen-Analyse der Kiefern-Gruppe der Lariciones.*

Für 19 Kiefernarten der Gruppe der *Lariciones* sind die Karyotypen beschrieben worden. Sie waren alle ähnlich, aber characteristisch für diese Gruppe. Der haploide *Lariciones*-Karyotyp unterscheidet sich durch das Vorkommen von 2 heterobrachialen Chromosomen, anstatt einem solchen bei anderen Kiefern-Gruppen.

Der karyologische Befund verlangt die Zuordnung von *Pinus halepensis, P. brutia, P. pityusa* und *P. pinaster* zur Gruppe der *Lariciones*. Die Karyotypen dieser Arten sind denen der bisherigen *Lariciones* sehr ähnlich, weil sie das für diese Gruppe charakteristische heterobrachiale 11. Chromosom besitzen.

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Effects of Inbreeding in Red Pine, Pinus resinosa Ait.[1])

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(Received for publication May 1, 1964)

Introduction

"It would seem unwise to conclude that red pine has existed as a specific entity since the Cretaceous, for its Tertiary record is unknown, but the morphological evidence is temptingly suggestive." (PIERCE, 1957.)

Deposits assigned to the early Upper Cretaceous Dakota series have yielded cones and needles (CHANEY, 1954) and pollen (PIERCE, 1957) which are almost identical to those of contemporary *Pinus resinosa*. If these Cretaceous species *(Pinus clementsii* CHANEY and/or *Pinus resinosipites* PIERCE) are in fact red pine or close relatives of red pine, then the ancestry of this species extends back at least a hundred million years.

Red pine is the only North American species of the *Lariciones* group. *Pinus tropicalis* MOR., which occupies a limited range in Cuba and on the Isle of Pines, is the only other American representative of this group which is largely confined to Europe and Asia (SHAW, 1914).

Attempts to hybridize red pine with other species in the *Lariciones* group have, until recently, been unsuccessful. DUFFIELD and SNYDER (1958) reported a successful cross of *Pinus nigra* ARN. × *Pinus resinosa*, but thus far, attempts to repeat this cross have been unsuccessful.

The natural range of red pine is presented in *Figure I* and is based primarily on the map of RUDOLF (1957), with a few changes for Ontario and Manitoba. The range map is somewhat deceiving in that it indicates a relatively continuous distribution. Along the range limits, the distribution of red pine is actually disjunct and, in some cases, distances of 100 miles or more may separate individual locations. Outlying populations are found in West Virginia and northern Illinois (RUDOLF, 1957) as well as in northern Ontario (HADDOW, 1948), and Manitoba (VAARTAJA, 1962).

Cool to warm summers, cold winters, moderate rainfall, sandy soils and undulating topography are characteristic of the red pine habitat. This species occurs in areas of acid

[1]) Contribution 64 — 5. Ontario Department of Lands and Forests. This paper is the first of a series of papers based on a dissertation submitted to the Graduate School of Yale University as partial fulfillment of the requirements of the PhD degree in 1963.

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