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Karyotype Analysis of the Genus *Pinus* – Subgenus *Strobus*¹⁾

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Summary

Descriptions of karyotypes (based primarily on centromere location and arm lengths) are presented for 22 pine species of the subgenus *Strobus* (soft pines). This study concludes a comprehensive investigation of the genus *Pinus* for which karyological information now has been presented for 87 species.

Results for this group of soft pines are similar to those obtained in previous studies. The karyotypes show a remarkable degree of similarity based on general features, yet they are not always identical. Again the karyological data support in general other taxonomic evidence of species relationships, and they indicate the genus has been quite conservative in changing major features of its karyotype.

Important karyological features of the subsections are described and compared.

Key words: Karyotype analysis for 22 pine species of subgenus *Strobus*.

Zusammenfassung

Für 22 Kiefernarten der Sektion *Strobus* werden die Karyotypen erstmalig aufgrund der Lage der Centromeren sowie der Chromosomenschenkelängen beschrieben. Diese Studie schließt eine eingehende Untersuchung ab, aus der nunmehr karyotypische Informationen für insgesamt 87 Arten der Gattung *Pinus* vorliegen. Die Ergebnisse für die Sektion *Strobus* sind denen ähnlich, die in früheren Untersuchungen erzielt wurden. Die Karyotypen zeigen einen bemerkenswerten Grad an Ähnlichkeiten bei den Hauptmerkmalen, sie sind aber nicht immer identisch. Wiederum unterstützen die karyotypischen Daten im allgemeinen andere taxonomische Merkmale der Verwandtschaft zwischen den Arten und zeigen an, daß die Gattung bei der Änderung von Hauptmerkmalen der Karyotypen sehr konservativ gewesen ist. Wichtige Karyotypmerkmale der Untersektionen werden beschrieben und verglichen.

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Introduction

Although previous studies have shown that the karyotypes of pine species are in general similar, differences have been observed that are useful in understanding relationships among some of the species. Based primarily on centromere location, SAYLOR (1964, 1972) found differences between certain subsections of hard pines (subgenus *Pinus*) and occasionally between species. FEDERICK (1967, 1970) also illustrated interspecific differences in his detailed karyotypic analyses based on the size and location of secondary constrictions.

Karyotypes are described in this paper for 22 species of soft pines (subgenus *Strobus*)³⁾. This report includes data for a majority of the species in this subgenus, and concludes the comprehensive karyological study of the genus *Pinus* on which 87 species have now been reported.

Materials and Methods

Karyotype data for this and previous studies were obtained by using identical procedures. Slides were prepared from squash preparations of root-tip meristems of 2—12 month old 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 1N HCl for 10—15 minutes at 60° C, and stained in acetocarmine.

Chromosome dimensions were obtained from projection drawings as described by SAYLOR (1961). To allow comparisons, the data are presented in the same manner used in previous reports. For each karyotype, the chromosomes are arranged from 1—12 according to a descending order of lengths of the short (*a*) arms. (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 diagnostic features used in making species comparisons included:

³⁾ The taxonomic classification used in this presentation follows that of CRITCHFIELD *et al.* (1966) and LITTLE *et al.* (1969).

Table 2. — Diagnostic features of species karyotypes obtained by arranging the chromosomes in a descending order of the *a* (shorter) arm length. (See Table 1).

Species	Number of Plates Drawn	Number of plants	^{"b"} Arm Sequence Chromosomes showing exception to a descending order in <i>b</i> (longer) arm lengths	Short/Long Arm Ratios Chromosomes with	
				Largest	Smallest ^{1/}
Subsection Cembrae					
<i>P. koraiensis</i> Sieb. & Zucc.	17	8	5, 7, 9, 11	4, 3	11, 9
<i>P. pumila</i> Regel	24	11	6, 8, 11	5, 7	11, 6
<i>P. sibirica</i> Du Tour	10	3	3, 5, 7, 9, 11	4, 6	11, 9
<i>P. cembra</i> L.	15	5	4, 7, 11	2, 3	11, 7
<i>P. albicaulis</i> Engelm.	19	11	6, 9, 11	4, 5	11, 6
Subsection Strobi					
<i>P. strobus</i> L.	18	6	5, 8, 11	4, 7	11, 8
<i>P. monticola</i> Dougl.	34	8	5, 7, 9, 11	8, 4	11, 7
<i>P. lambertiana</i> Dougl.	29	13	5, 7, 9, 11	4, 6	11, 9
<i>P. flexilis</i> James	20	11	3, 5, 8, 11	4, 2	11, 8
<i>P. strobiformis</i> Engelm.	25	12	2, 5, 8, 11	4, 7	11, 2
<i>P. ayacahuite</i> Enrenb.	22	13	3, 5, 7, 10	4, 6	11, 10
<i>P. peuce</i> Griseb.	15	5	3, 6, 9, 11	2, 5	11, 9
<i>P. armandii</i> Franch.	20	11	5, 7, 10	4, 2	10, 5
<i>P. griffithii</i> McClelland	15	7	6, 8, 10	5, 1	11, 10
<i>P. parviflora</i> Sieb. & Zucc.	18	8	2, 6, 8, 11	7, 1	11, 2
Subsection Cembroides					
<i>P. cembroides</i> Zucc.	15	4	5, 8, 12	4, 3	11, 8
<i>P. edulis</i> Engelm.	30	10	5, 8, 12	1, 3	11, 8
<i>P. monophylla</i> Torr & Frém	20	8	3, 5, 8, 12	4, 1	8, 11
Subsection Gerardianae					
<i>P. gerardiana</i> Wall.	14	8	4, 9, 11	3, 7	11, 9
<i>P. bungeana</i> Zucc.	14	7	4, 8, 11	3, 1	11, 8
Subsection Balfourianae					
<i>P. balfouriana</i> Grev. & Balf.	17	8	5, 7, 9, 11	3, 6	11, 9
<i>P. aristata</i> Engelm.	26	14	4, 6, 9, 11	2, 3	11, 6

^{1/} Not including chromosome number 12

were apparent in approximately half the plates and thus are considered to be at least probable locations of major secondary constrictions. It was never possible in the analysis of these diploid root-tip figures to use location of secondary constrictions with the reliability and precision utilized by FEDERIC (1967 and 1970) in studying the haploid female gametophyte chromosomes.

Results and Discussion

Similar to previous studies, the karyotypes of the 22 species included in this report were in general agreement with the basic karyotype described originally by SAX *et al.* (1933) for the genus. All species had a haploid complement of 12 chromosomes, and except for the smallest chromosome which was always heterobrachial and noticeably different in size, the other 11 chromosomes possessed median centromeres and were rather similar in total length.

Although once again major differences in chromosome number and morphology were not observed, minor differences or special features were detected for some species (Table 1—2). Those considered to be significant are discussed below along with the karyotypic features considered to be most characteristic of the subsections.

For the subgenus *Strobus*, there was in general less similarity of karyotypic features for species of the same subsection (or closely related species within a subsection) than occurred in the subgenus *Pinus*. Reasons for this are not clear, but it should be noted that more difficulty was experienced in pairing homologous chromosomes with some species of the subgenus *Strobus* than occurred in the subgenus *Pinus*. Although it was felt this situation did not seriously effect the results, it is considered important enough to report and should be kept in mind in interpreting these data.

Cembrae

Of the five species in this subsection, four are found in Eurasia and one in North America. The range of *P. pumila*

overlaps those of both *P. koraiensis* and *P. sibirica*, but *P. cembra* is separated from these species by 2415 km. *Pinus albicaulis* is the lone North American species.

No basic karyotypic pattern of *b* arm sequences exists for this group (Table 1). However, the *b* arm of chromosome 11 breaks the descending sequence for all five species. This feature of chromosome 11 is also common in the other subsections of *Strobus* except for the species of *Cembroides*. It occurs much less frequently, however, for species of the subgenus *Pinus*. Another interesting feature of chromosome 11 for this subsection (especially *P. pumila*) is that the centromere tends to be located in a more nearly median location than for the other subsections (except *Gerardianae*).

Although *P. pumila* has generally been considered a variant of the *P. cembra* — *P. sibirica* group, some investigators consider it more closely related to *P. parviflora* (CRITCHFIELD *et al.*, 1966). Interestingly, the *b* arm pattern for *P. pumila* (Figure 1b) resembles that of *P. parviflora* much more than it does *P. cembra* (Figure 1a) or *P. sibirica*.

The *b* arm of chromosome 3 for *P. sibirica* was the longest of the complement which is rather unusual. Only two other species of *Strobus* (and six in the entire genus) have a chromosome other than number 1 with the longest *b* arm.

Strobi

More species (14) are grouped in this subsection than in any other in the subgenus *Strobus*. Their location is about evenly divided between Eurasia and North America.

The karyotypes of the 10 species studied were quite variable and no basic or common karyotype could be determined for the subsection or groups of closely related species within it. For the *b* arm patterns, chromosomes 5, 8, and 11 broke the descending sequence most frequently and could represent the basic pattern from which others evolved. As with all other species of pine in this and previous studies, the most median chromosomes were found

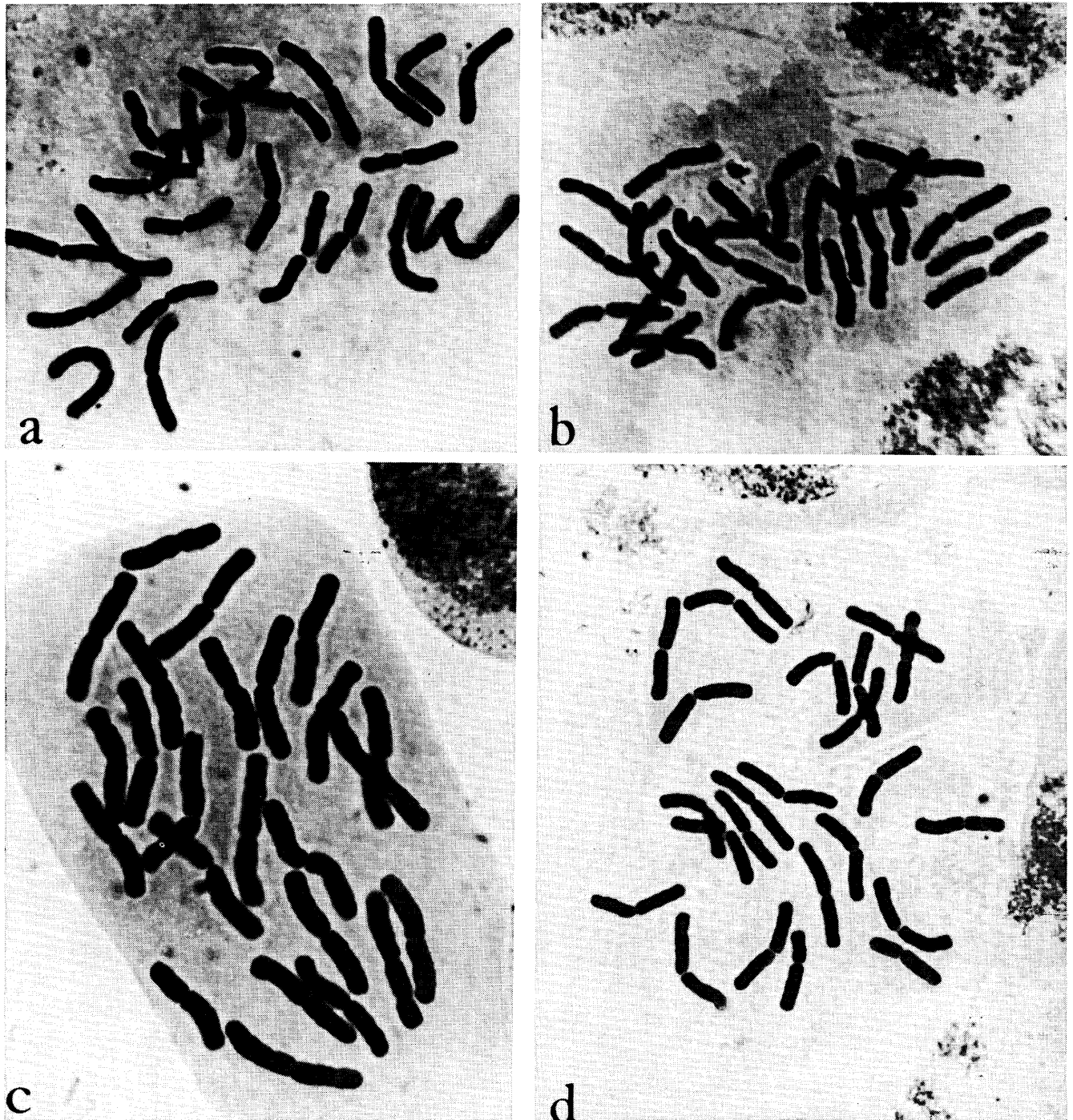


Figure 1. — Metaphase chromosomes from aceto-carmin preparations of root-tip meristems ($2n = 24$). (a) *P. cembra* (1050X), (b) *P. parviflora* (1050X), (c) *P. flexilis* (1150X) (d) *P. strobiformis* (1350X).

in positions 1—4 while the most submedian were in positions 9—12.

The degree of similarity of karyotypes among related species varied considerably. For example, *P. strobiformis* is known to form a link geographically and morphologically between *P. flexilis* and *P. ayacahuite*, but the karyotypes are not all alike. Although those for *P. flexilis* (Figure 1c) and *P. strobiformis* (Figure 1d) are rather similar, the karyotype of *P. ayacahuite* differs noticeably from the other two. Also, while there was some agreement between *P. strobus* and *P. monticola*, there were few karyotypic features in common between *P. peuce* and *P. griffithii*, two morphologically similar species.

Three species had karyotypes with somewhat special features. *Pinus parviflora* and *P. strobiformis* were the other species of soft pine to have a chromosome other than number 1 with the longest *b* arm; for both species it was number 2. For *P. ayacahuite* (Figure 2a), the secondary constriction in the *a* arm of chromosome 11 was the easiest to recognize of any species in the subgenus; it occurred in nearly every plate observed.

Cembroides

Of the eight pines of this subsection covering parts of southwestern United States and Mexico, only three were

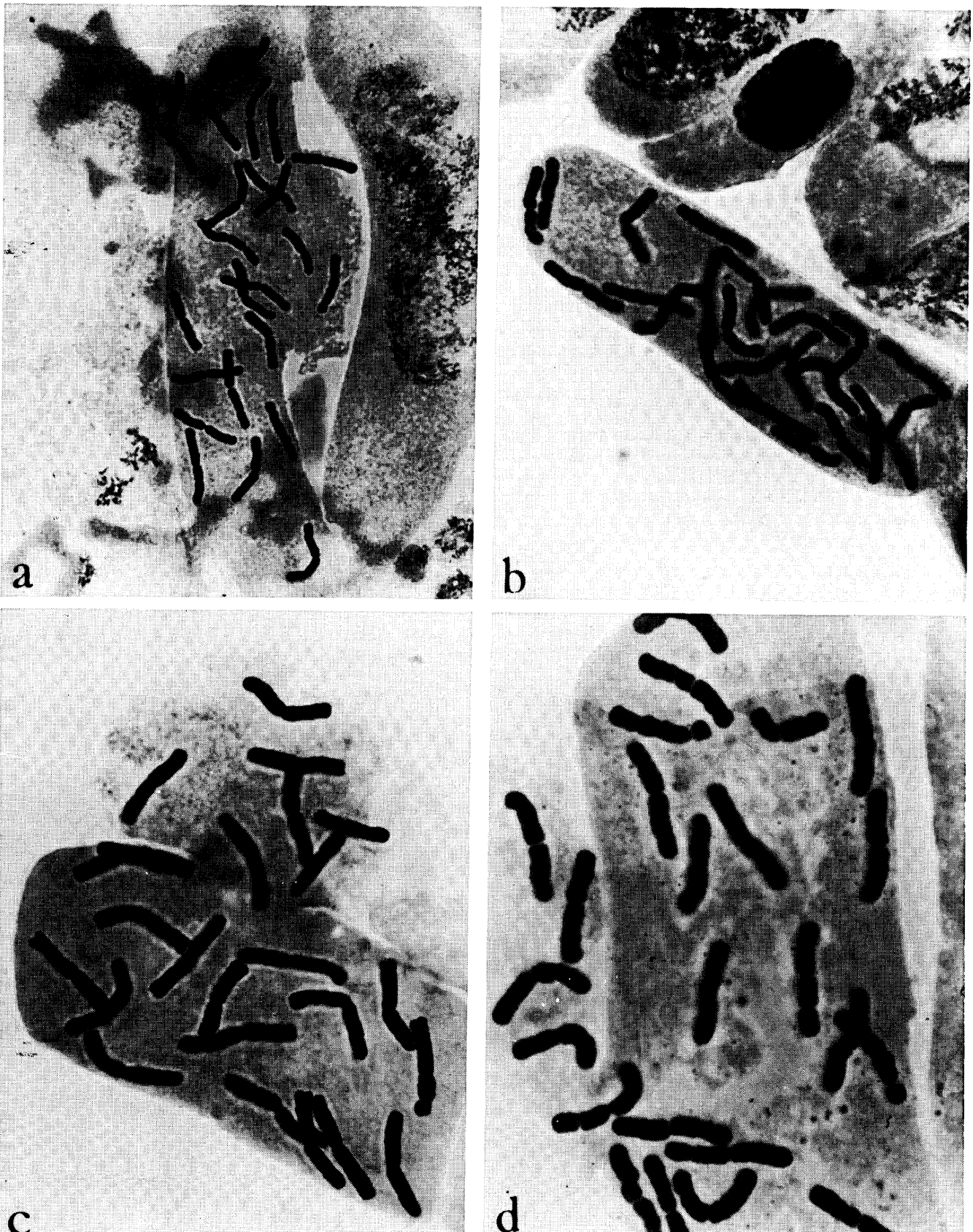


Figure 2. — Metaphase chromosomes from aceto-carmin preparations of root-tip meristems ($2n = 24$). (a) *P. ayacahuite* (920X), (b) *P. cembroides* (1050X), (c) *P. gerardiana* (1225X), (d) *P. aristata* (1350X).

studied because of problems in obtaining viable seeds of the others. However, it was easier to determine the karyotypes of those studies--*P. cembroides* (Figure 2b), *P. edu-*

lis, and *P. monophylla*--and there was closer agreement of features among these species than for any other group studied in the subgenus *Strobos*.

A basic *b* arm sequence of 5, 8, 12 was found for all three; only *P. monophylla* deviated slightly by having the *b* arm of chromosome 3 also break the sequence. Similarly, all three had chromosomes with the most median centromere located in position 1, 3, and 4. A particularly unique feature about the karyotypes of these species involved chromosome 12. The *b* arm of this chromosome broke the descending sequence rather than chromosome 11 which normally is involved. A *b* arm sequence involving chromosome 12 did not occur for any other species of soft pine, and it occurred for only three species of hard pines (*P. hartwegii*, *P. rudis*, *P. pringlei*) all of which are found in Mexico.

Gerardiana

The karyotypes of the two species of this subsection (*P. gerardiana* and *P. bungeana*) were somewhat similar. Two of the three positions breaking the *b* arm descending sequence are similar in both species. In addition, the centromere of chromosome 11 for both is located in nearly a submedian position. The occurrence of secondary constrictions was quite different, however. So few were observed for *P. gerardiana* (Figure 2c) that none were recorded for the karyotype, but for *P. bungeana* they occurred consistently and clearly for chromosome 4 and also chromosome 12 which is very unusual.

Balfouriana

The two species of this subsection (*P. balfouriana* and *P. aristata*) had only minimal agreement in karyotypes. Two positions (9 and 11) out of four breaking the *b* arm sequence were similar for both species; for the location of the most median or submedian chromosomes they agreed for one position each, number 3 and 11 respectively.

Although the location of secondary constrictions did not agree, the constrictions were very easy to determine, especially the one found in the *a* arm of chromosome 10 for *P. aristata* (Figure 2d).

Conclusions

The data from this concluding study along with those reported on previously (SAYLOR 1964, 1972) constitute the most comprehensive body of karyological information ever presented for the genus *Pinus*. When evaluated collectively they cover 87 species and provide an important overview of general karyological features of the genus.

It may be concluded from these results that indeed as reported by earlier investigators (e.g. SAX *et al.* 1933) the karyotypes of all species of pine have a remarkable degree of similarity based on general features of chromosome number, length, and centromere location. Thus it would appear that this genus, which has been in existence for millions of years and been subjected to a wide variety of

selection forces, has been quite conservative in changing major features of its karyotype. Polyploidy or even the addition or loss of a single chromosome does not exist in the genus at any level except for an occasional individual. Similarly, evolution has proceeded with quite limited involvement of chromosome translocations and inversions that include the centromere. Although it was anticipated initially that more differences would be found, it is nevertheless important to describe the basic species karyotypes and to document the similarities as well as the differences that do exist.

Careful evaluation of the data did indicate that although the karyotypes are basically similar they are not always identical. Even with the constraints listed in the methods section, it was felt that the techniques were reliable enough to provide evidence of occasional differences between subsections, groups of related species within subsections and even among individual species. Examples from this and other studies include: (1) The subsection *Sylvestres* which is delimited by the heterobrachial chromosome 11. (2) The six species (three soft pines and three hard pines) which have a unique feature involving chromosome 12. (3) Six individual species (found in five different subsections) that have the unusual condition of a chromosome other than number one with the longest *b* arm.

Karyotype studies along with those from meiotic analyses (e.g. SAYLOR *et al.* 1966) indicate that structural changes of various types have occurred in pine chromosomes. In some instances the changes are great enough and of a type that can be combined with other descriptive data for a better understanding of taxonomic relationships. The results of this and previous karyological studies (SAYLOR 1964, 1972) have in general supported findings using other characteristics. They have not provided evidence to contradict major taxonomic relationships established previously.

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