Karyotype Analysis of the genus Pinus – Subgenus Pinus')

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Early studies showed that the karyotypes of pine species are very similar in chromosome number, chromosome length and centromere location. All species studied had a haploid complement of 12 chromosomes; the smallest chromosome was heterobrachial (i.e. submedian centromere location) while the remaining eleven had median centromeres.

More recently differences between karyotypes have been found that indicate a complete investigation of the genus is warranted. For example, group (designated subsections[3]) in this paper) differences based on centromere location were reported by Saylor (1964), and clear interspecific differences based on the location and size of secondary constrictions were found by Pederick (1967 and 1970)[4]).

Karyotypes are described in this paper for all species of the subgenus Pinus except those of the subsection Sylvestres (Lariciones) reported on earlier (Saylor, 1964). The descriptions are based primarily on chromosome arm lengths and centromere location. They represent over half of the information obtained by the author from a karyological survey of the genus.

Material and Methods

Karyotype data were obtained 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 12⁰ C, fixed in 3 : 1 ethyl alcohol-acetic acid for 1—4 hours, hydrolyzed in 1N HCl for 10—15 minutes at 60⁰ C, and stained in aceto-carmine.

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 utilized in making species comparisons included:

(1) Patterns caused by divergence of the long (b) arm lengths from a descending order comparable to that for the short (a) arms. (See Table 1, P. leiophylla chromosomes 5, 7 and 10.)

(2) Places where the greatest differences in the lengths of the short (a) arms occur between adjacent chromosomes other than between chromosomes 11 and 12 whose a arms are always the most different. (See Table 1, difference in a arm lengths between chromosomes 1—2 and 10—11 for P. leiophylla.)

(3) Location of primary and secondary constrictions.

It is recognized that this method of evaluation is subject to criticism because of the similarity in a arm lengths of chromosomes in adjacent positions and the resulting opportunity for reversals in the order of the chromosomes. This problem has been described by Matern and Simak (1968) and should be kept in mind in all analyses of the data. The karyotype patterns presented here are simply the best interpretation of the data from a sample judged adequate according to the agreement of data from different seedlings. The patterns described were present in more than 50 percent of the plates obtained for a given species, and they were obtained easily by minor adjustments for most of the other plates. Only the karyotype features particularly characteristic of the subsections (and on occasion for individual species or groups of related species) are discussed in any detail.

Results

As with all reports thus far, the karyotypes of the 46 species included in this study were in general agreement with the basic karyotype originally described by Sax and Sax (1933) for the genus. Except for the smallest chromosome, which was always heterobrachial and noticeably different in size, the other 11 chromosomes usually possessed median centromeres and were generally similar in total length. Although it was not possible to illustrate each species studied, the photomicrographs in Figures 1—3 are representative of each subsection and group of related species.

The values in Table 1 are mean arm lengths, and do not provide critical information about interspecific difference in chromosome lengths. The apparent differences are most likely the result of unequal pretreatment periods, or differential reaction of the chromosomes to the pretreatment, or a combination of the two.

The location of secondary constrictions is included in the karyotype descriptions. As indicated previously (Saylor, 1961 and 1964), however, the reliability of these data must be considered marginal, because these constrictions usually do not appear consistently using the above techniques. Considerable variation existed in the ability to detect secondary constrictions among the various species. Those cases in which they were especially easy to determine are noted later in the discussion; for the others, they 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 Pederick (1967 and 1970) in studying the haploid female gametophyte chromosomes.

Leiophyllae

Although grouped together in the same subsection, P. leiophylla (Figure 1a) and P. lumholtzii differ conspicuously in several morphological traits. The karyotypes of these two species also differ in several features. The b arm patterns are completely different and there is no agreement in secondary constriction location (Tables 1 and 2). The karyotypes are most similar in the place where major differences occcur in lengths of a arms of adjacent chromosomes; in both species the greatest differences in a arm

Table 1. — Mean arm lengths in millimeters of chromosomes magnified 2000×. (Horizontal lines denote b arm lengths that break descending sequence of values — See text. Vertical lines denote location of greatest difference in a arm lengths between adjacent chromosomes other than between chromosomes 11 and 12 — See text. x, *, and z denote secondary constrictions in the distal, medial, and proximal portion of the arms, respectively.)

Chromosome	P. leiophylla a	b	a/b	P. lumholtzii a	b	a/b	P. canariensis a	b	a/b
1	14.0	15.2	.921	13.4	14.5	.924	15.3	16.4	.932
2	13.5	14.0	.964	12.8	13.4*	.955	14.6	14.9	.979
3	13.2	13.5	.977	12.3*	12.9	.953	14.0	15.9	.880
4	12.9	13.3	.969	12.1	12.7	.952	13.8	14.3*	.965
5	12.8x	14.4	.888	11.7	12.2	.959	13.5	15.4	.876
6	12.6*	13.1	.961	11.5	13.0	.884	13.3	14.2	.936
7	12.4	13.9	.892	11.3	12.1	.933	13.1	13.9	.942
8	12.2*	13.1	.931	11.1	12.0	.925	12.9	14.4	.895
9	11.9	12.6	.944	10.9	13.1	.832	12.5*	13.3	.939
10	11.6	13.3	.872	10.5*	11.7	.897	11.8	12.8	.921
11	11.1	12.2	.909	9.9	11.4	.868	10.3*	13.8	.746
12	7.0	11.6	.603	6.3	10.8	.583	7.1	11.7	.606

Chromosome	P. roxburghii a	b	a/b	P. pinea a	b	a/b	P. palustris a	b	a/b
1	15.0*	15.9	.943	15.8	17.1*	.923	13.5	14.3	.944
2	14.5	15.4*	.941	15.3	15.7	.974	13.0	13.7	.948
3	14.3	14.8	.966	14.9	16.8	.886	12.8	13.1	.977
4	14.0	14.3	.979	14.5	14.9	.973	12.6	13.9*	.906
5	13.8	16.5	.836	14.2	15.2	.934	12.4	12.8	.968
6	13.6	14.4	.944	14.0	14.4	.972	12.0	13.4*	.895
7	13.2	13.8	.956	13.6	13.8	.985	11.8	12.4	.951
8	12.6	14.8*	.851	13.4	16.1	.832	11.5	13.2	.871
9	12.4	13.8	.898	13.0	14.3	.909	11.3	11.7	.965
10	11.7*	12.4	.943	12.6	13.4	.940	10.9	12.5	.872
11	11.0	14.5	.758	10.9*	12.9	.844	10.4	11.3	.920
12	7.3	12.4	.588	7.2	12.7	.566	6.8	11.2	.607

Chromosome	P. taeda a	b	a/b	P. echinata a	b	a/b	P. pungens a	b	a/b
1	14.2	15.4	.922	13.5	14.7	.918	13.4*	14.3x	.937
2	13.6	14.7	.925	12.9*	13.6	.948	12.8	13.5	.948
3	13.2*	13.7*	.963	12.4	12.9	.961	12.4	12.8	.968
4	13.0	14.6	.890	12.0	13.6	.882	12.1	13.3	.909
5	12.7	13.5*	.940	11.8	12.5	.944	11.8	12.2	.967
6	12.5	12.8	.976	11.6*	11.9	.974	11.6	13.1	.885
7	12.2	13.5	.903	11.4*	12.7	.897	11.3*	11.9*	.949
8	11.7*	12.7	.921	11.2	11.5	.973	11.1	12.4x	.895
9	11.4	12.1	.942	10.7	12.3	.869	10.8	11.3	.955
10	11.2	13.6	.823	10.1	11.5	.878	10.6	12.4	.854
11	10.5	11.9	.882	9.5	10.9	.872	10.0	11.3	.884
12	7.7	11.7	.658	7.1	10.8	.657	7.1	10.9	.651

Chromosome	P. jeffreyi a	b	a/b	P. ponderosa a	b	a/b	P. washoensis a	b	a/b
1	15.7	17.7	.887	14.3	15.3	.934	14.5	15.6	.929
2	15.4	15.9	.968	13.8*	14.7	.938	14.0*	14.7	.952
3	14.9	15.3	.973	13.5	13.9	.971	13.6	14.0	.971
4	14.7	16.7	.880	13.3	14.6	.910	13.4	14.7	.911
5	14.4	14.7*	.979	13.1	13.5	.970	13.2	13.9*	.949
6	14.2	15.9*	.893	12.9*	14.5	.889	13.0	13.4	.970
7	14.0*	14.6	.958	12.7	13.0	.976	12.7*	14.6	.869
8	13.7	14.1	.971	12.4	14.0	.885	12.4	13.6	.911
9	13.4	15.2	.881	11.9*	13.1	.908	12.2	12.9	.945
10	12.4	13.6	.911	10.8	11.8	.915	11.2	12.3	.910
11	11.1	14.7	.755	10.2	12.4	.822	10.4	12.8	.812
12	7.9	12.9	.612	7.5	12.1	.619	7.0	11.4	.614

Chromosome	P. arizonica a	b	a/b	P. englemannii a	b	a/b	P. durangensis a	b	a/b
1	14.6	15.0	.973	12.6	13.5	.933	13.4	14.4	.930
2	14.1	14.4*	.979	12.2	12.4	.983	12.9	13.3	.969
3	13.8	14.9	.926	11.8	12.9x	.914	12.6*	12.9	.976
4	13.5	14.4	.937	11.7	12.0	.975	12.4	14.0	.885
5	13.3*	13.7	.970	11.4	11.8	.966	12.2	12.9	.945
6	13.1	14.6	.897	11.3	12.1	.933	12.1	12.5	.968
7	12.9	13.7	.941	11.1	11.6	.956	11.7	12.2	.959
8	12.7	14.9	.852	10.9	12.1	.900	11.4	13.6	.838
9	12.3	13.3	.924	10.5*	11.4	.921	11.0	11.9	.924
10	11.1	12.2	.909	9.7	10.9	.890	10.1	11.1	.909
11	10.2	12.8	.796	9.0	11.4	.789	9.5	11.8	.805
12	7.5	12.2*	.614	6.5	10.5	.619	6.5	11.2*	.580

Chromosome	P. cooperi a	b	a/b	P. montezumae a	b	a/b	P. michoacana a	b	a/b
1	14.1	15.0	.940	15.6	16.4	.951	14.4	15.2*	.947
2	13.4	13.8	.971	14.9	15.2	.980	13.7	14.0	.978
3	13.1	14.6	.897	14.3	15.0	.953	13.4	14.6*	.917
4	12.8*	13.7	.934	14.1	16.3	.865	13.2	14.1	.936
5	12.3	12.7	.968	13.9	14.2	.978	13.0	14.9	.872
6	12.1	13.7*	.883	13.5	15.3*	.882	12.8	13.6	.941
7	12.0	12.3	.975	13.2	13.8	.956	12.6	13.0	.969
8	11.7	13.1	.893	13.0	14.5	.896	12.4	14.1	.879
9	11.4	12.3	.926	12.4*	13.6	.911	11.8	12.8	.921
10	10.5	11.1	.945	11.5	12.6	.912	10.9	11.7	.940
11	9.8	12.0	.816	10.6	13.1	.809	10.0	12.6	.793
12	6.6	11.4	.578	7.7	12.9	.596	7.0	11.5	.608

Chromosome	P. rigida a	b	a/b	P. serotina a	b	a/b	P. glabra a	b	a/b
1	14.6	15.2	.961	14.2	14.9	.953	12.8	13.7	.934
2	14.0	14.5	.965	13.5	13.8*	.978	12.3	12.9*	.953
3	13.7	15.7*	.873	13.0*	13.4z*	.970	11.8	12.4	.951
4	13.5	14.2	.951	12.7	14.1	.900	11.6	13.3	.872
5	13.1	13.5	.970	12.5	12.8	.977	11.4	11.7	.974
6	12.9	14.4*	.896	12.3	13.6	.904	11.2	12.0	.933
7	12.6	13.2	.955	12.0	12.7	.944	11.0	11.5	.956
8	12.4	14.0	.886	11.6	13.2	.878	10.7*	12.3	.869
9	12.0	12.6	.952	11.3	12.2	.926	10.3*	11.1	.927
10	11.4x	13.1	.870	11.1*	12.6	.880	10.1	12.1	.834
11	10.7	12.1	.884	10.6	11.8	.898	9.6	10.7	.897
12	7.7	12.0	.642	7.3	11.7	.623	7.2	10.2	.705

Chromosome	P. elliottii a	b	a/b	P. caribaea a	b	a/b	P. occidentalis a	b	a/b
1	13.9	14.8	.939	13.9*	14.6	.952	14.2	15.6	.910
2	13.3*	14.2	.936	13.3	13.7	.970	13.5	13.9	.971
3	12.9	13.1x	.984	12.8	13.1*	.977	13.1	13.3*	.984
4	12.7*	13.8	.920	12.6	13.8*	.913	12.9	14.1	.914
5	12.5	12.8	.976	12.3	12.6	.976	12.6*	13.4	.940
6	12.3	13.6	.904	12.1	13.2	.916	12.4	14.6	.849
7	12.1	13.0	.930	11.9	12.3	.967	12.1	12.5	.968
8	11.8	13.6	.867	11.6	13.5	.859	11.7	14.2*	.823
9	11.6	12.8	.906	11.4*	12.3	.926	11.5*	12.9	.891
10	11.0	11.8	.932	11.1	11.6	.956	11.3	12.3	.918
11	10.4	11.6	.896	10.6	12.5	.848	10.8	12.9	.837
12	7.3	11.5	.634	7.3	11.2	.651	6.9	11.3	.610

Chromosome	P. sabiniana a	b	a/b	P. torreyana a	b	a/b	P. coulteri a	b	a/b
1	15.4	17.2	.895	16.6	18.0	.922	15.2	16.4	.926
2	15.1	16.2	.932	15.6	16.9	.923	14.7	15.1	.973
3	14.8	15.4	.961	15.1	15.8	.955	14.3	16.0*	.893
4	14.5	14.9	.973	14.4	14.8	.972	14.0	15.0	.933
5	14.2	15.7	.904	14.1*	15.7	.898	13.8	14.1	.978
6	14.0	15.2	.921	13.9	14.6	.952	13.4	14.9	.899
7	13.7*	14.0	.978	13.7*	16.5	.830	13.2	14.0	.942
8	13.1	15.3	.856	13.5	14.3	.944	13.0*	15.5	.838
9	12.8	13.8	.927	13.2z	14.6	.904	12.7	14.0*	.907
10	12.3	13.0	.946	12.9	13.9	.928	12.2	13.1	.931
11	11.7z	14.2	.823	12.4z	15.0	.827	11.6	13.9	.834
12	7.7	13.1	.587	7.6	12.7	.598	7.7	12.7	.606

Chromosome	P. hartwegii a	b	a/b	P. rudis a	b	a/b	P. pseudostrobus a	b	a/b
1	14.4*	15.3	.941	15.1	16.4	.920	15.3*	15.9	.962
2	13.7	14.1	.971	14.6*	15.7*	.929	14.5	15.4*	.941
3	13.3	13.5	.985	14.3	15.0	.953	14.1*	14.7	.959
4	13.1	14.5	.903	14.0	16.0	.875	13.6	15.5	.877
5	12.8	13.3	.962	13.6	14.8	.918	13.3	14.1	.943
6	12.5	12.9	.968	13.3	13.8	.963	13.0	13.6	.955
7	12.3	14.1	.872	12.9	15.8	.816	12.6	14.3	.881
8	12.4	13.0	.944	12.4	13.0	.953	12.4	13.0	.960
9	11.4	13.3	.857	12.1	13.6	.889	11.7	14.0	.835
10	10.6*	11.9	.890	10.8	12.8	.843	10.8*	12.3	.878
11	9.8	11.3	.867	9.9	11.7	.846	9.6	13.2	.727
12	7.0	12.2	.573	7.4	12.8	.578	7.0	12.2	.573

Chromosome	P. oaxacana a	b	a/b	P. tenuifolia a	b	a/b	P. douglasiana a	b	a/b
1	13.5	14.2	.950	13.3	14.3	.930	14.2*	15.4	.922
2	12.9	13.3*	.969	13.1	13.4*	.977	13.7	14.6	.938
3	12.7	12.9	.984	12.8	14.2	.901	13.5	14.0	.964
4	12.3	13.5	.911	12.6	13.2	.954	13.3	13.6	.977
5	12.1	13.1	.923	12.3	12.8	.960	13.0	14.3	.909
6	11.8	12.2	.967	12.1	13.4*	.902	12.7	13.4	.947
7	11.5	13.6	.845	11.7	12.4	.943	12.3	13.6	.904
8	11.3	12.4	.911	11.5	12.3	.934	12.1	12.6	.960
9	11.1	11.9	.932	11.2	13.2	.848	11.7	13.4*	.873
10	10.4	11.1*	.936	10.2	11.2	.910	11.0	12.1	.909
11	9.6	12.1	.793	9.6	12.0	.800	10.0	12.6	.793
12	6.6	11.1	.594	6.8	11.1	.612	7.0	11.1	.630

Chromosome	P. teocote a	b	a/b	P. lawsoni a	b	a/b	P. contorta a	b	a/b
1	14.3	14.9	.959	13.6	14.3	.951	12.7	14.0	.907
2	13.4*	14.1*	.950	13.1	13.8	.949	12.3	13.1	.938
3	12.8	13.4	.955	12.8	13.4*	.955	12.0	12.5	.960
4	12.6*	13.0	.969	12.4	13.0	.953	11.8	12.0	.983
5	12.4	14.1	.879	12.2	13.8	.884	11.6	12.7x	.913
6	12.2	12.9	.945	12.0	12.4*	.967	11.3	11.5	.982
7	12.1	13.5	.896	11.8	13.3	.887	11.1	12.5	.888
8	11.9	12.7	.937	11.6	12.5	.928	10.9	11.6	.939
9	11.4	11.9	.957	11.5	11.9	.966	10.5	12.1*	.868
10	11.0	13.3	.827	11.2	12.5	.896	10.1	10.7	.943
11	10.1	11.3	.893	10.7*	11.6	.922	9.3	10.5	.885
12	6.7	11.3	.592	6.9	10.9	.633	6.3	10.0	.630

lengths are between chromosomes 1—2 and chromosomes 10—11.

Canarienses

The karyotypes of *P. canariensis* and *P. roxburghii (Figure 1 b)* were two of the easiest determined. This includes location of secondary constrictions, which proved to be exceptionally reliable for these species. The b arm sequences (3, 5, 8, 11) are quite similar, with differences occurring only between chromosomes 3 and 5. For *P. roxburghii*, the b arm of chromosome 3 does not break the sequence, and the b arm of 5 is the longest of all 12 chromosomes which is a rather unusual feature *(Table 1)*. The greatest difference in *a* arm lengths is between chromosomes 10 and 11 for both species.

Pineae

Although *P. pinea (Figure 1 c)* is the only species in this subsection, there is nothing particularly distinctive about the karyotype.

Sylvestres

Karyotypes of the species in this subsection were described in an earlier paper (SAYLOR, 1964). The most distinctive karyotypic feature discovered thus far is associated with this group. In addition to the smallest chromosome having a submedian centromere (which is general throughout the genus) the second smallest chromosome is also heterobrachial. The most common b arm sequence is 4, 6, 8, 11. Major differences in *a* arm lengths are present between chromosomes 10 and 11 and between 9 and 10, respectively, which is quite unusual also.

Australes

Eight of the ten species in the subsection have a b arm sequence of either 4, 6, 8, 10 or a sequence that deviates from this by only one position. In addition the b arms of chromosomes 11 and 12 are nearly identical for several species *(Table 1)*. Major differences in *a* arm lengths occur between chromosomes 1 and 2 for nearly all of the species; comparable differences also frequently are present between chromosomes 10 and 11.

The karyotype of *P. echinata* differs the most from the general pattern; it is most similar to *P. taeda*. The data now presented for *P. palustris* and *P. taeda* differ somewhat from an earlier report (SAYLOR, 1961), but are considered to be more correct because of the greater number of plates used and because of refinements in drawing techniques and interpretation of the data.

Because of its use in other special studies, *P. rigida (Figure 1 d)* was the most thoroughly studied species included in this report, and thus the karyotype data are considered very reliable. This species, like *P. roxburghii*, has a distinguishing feature in which the b arm of a chromosome other than number one is the longest. In 31 of the 40 plates analyzed, the b arm of chromosome 3 was longer than any other *(Table 1)*. Although this feature was not observed in every plate and although it was not included in the description by YIM (1963), it is considered reliable enough to be included in this karyotype description.

No differences were detected between the two varieties of *P. elliottii*. However, a very noticeable difference was found between the b arm sequence of the closely related *P. caribaea* and *P. elliottii*. Chromosome 11 of *P. caribaea (Figure 1 e)* always disrupted the sequence, and this occurred for a wide variety of samples including those from the Bahama and Caicos Islands, Cuba, and Honduras. A similar pattern was found for *P. occidentalis (Figure 1 f)*, which some consider very closely related to *P. caribaea*.

Pinus cubensis is the only species in the subgenus *Pinus* that was not evaluated. All efforts to obtain seed failed.

Sabinianae

No general b arm pattern was evident for this subsection except for position 11. However, the karyological data for the species of *Sabinianae* are sufficiently different from those of the *Ponderosae* to indicate subsectional status is warranted, as proposed by SHAW (1914) and CRITCHFIELD and LITTLE (1966).

Of the three species in the subsection, *P. sabiniana* and *P. torreyana (Figure 2 a)* are the most crossable (CRITCHFIELD, 1966), and they possess the most similar karyotypes. The reliability of secondary constriction locations is greater for these species than for any others studied. The identical location of constrictions in arms 7 a and 11 a of *P. sabiniana*

(Continued from Table 1)

Chromo-some	Arms a	Arms b	a/b	Arms a	Arms b	a/b	Arms a	Arms b	a/b
	P. banksiana			*P. virginiana*			*P. clausa*		
1	13.4	14.1	.950	12.9	13.5ˣ	.955	12.7	13.6	.933
2	12.6	13.3*	.947	12.4*	13.1*	.946	12.1	12.8	.945
3	12.3	12.9	.953	12.0	12.7	.944	11.7*	12.4*	.943
4	12.0	12.6*	.952	11.8	12.1	.975	11.5	11.8	.974
5	11.8ˣ	13.7	.861	11.6	12.8	.906	11.3	12.4	.911
6	11.7	12.2	.959	11.4	11.8	.966	11.2*	11.4	.982
7	11.5	13.2	.871	11.3	12.8	.882	11.0	12.0	.916
8	11.3	11.7	.965	11.1	11.6	.957	10.8	11.6	.931
9	11.0	12.4	.887	10.8	12.4	.870	10.6ˣ	11.1	.954
10	10.6	11.3	.938	10.4*	11.3	.920	10.3	12.0	.858
11	9.7	10.9	.889	9.9	10.5	.942	9.7	10.1	.960
12	6.8	10.4	.653	6.7	9.9	.676	6.6	10.0	.660
	P. radiata			*P. muricata*			*P. attenuata*		
1	15.1*	16.5	.915	12.3	13.2	.931	14.0*	14.3*	.979
2	14.7	15.0	.980	11.9	12.2	.975	13.4	13.7	.978
3	14.4	16.0	.900	11.7	12.8	.914	13.0	14.0	.928
4	14.0	14.6	.958	11.5	11.8	.974	12.9*	13.5	.955
5	13.8	16.6	.831	11.2	13.2	.848	12.6	14.8	.851
6	13.6	14.4	.944	11.1	11.6	.956	12.3	13.2	.931
7	13.4	14.0	.957	11.0	11.2	.982	12.1	12.8	.945
8	13.2*	15.1	.874	10.8*	11.9	.907	11.9	13.7	.868
9	12.9	13.8	.934	10.6	11.1	.954	11.7	12.5	.936
10	12.7	14.5	.875	10.2*	11.7	.871	11.4	13.1	.870
11	12.2	13.7	.890	9.7	10.5	.923	10.7	12.2	.877
12	7.7	12.2	.631	6.4	10.3	.621	7.2	11.6	.620

Chromo-some	Arms a	Arms b	a/b	Arms a	Arms b	a/b
	P. greggii			*P. patula*		
1	13.6	14.1	.964	13.0	13.8	.942
2	13.2	13.8	.956	12.5	13.4*	.932
3	13.0	14.8*	.878	12.2	12.5*	.976
4	12.7	13.0	.976	12.1ˣ	13.0	.930
5	12.4	12.8	.968	11.8	12.2	.960
6	12.2	14.0	.871	11.6	13.4	.865
7	12.0*	12.8	.937	11.5*	12.0	.958
8	11.8	12.5	.944	11.3	12.5	.904
9	11.5	13.2	.871	11.1	11.8	.940
10	11.2	12.2	.918	10.9	12.5	.872
11	10.6	11.7	.905	10.3	11.4	.903
12	7.2	10.7	.672	6.5	10.5	.619
	P. pringlei			*P. oocarpa*		
1	14.2	15.0	.946	14.1	15.6	.904
2	13.7	14.0	.978	13.6*	14.4	.944
3	13.2	13.8*	.956	13.3	13.7	.970
4	12.9	14.4	.895	13.0*	14.3	.909
5	12.7	13.3	.954	12.8	13.2	.969
6	12.5	14.1	.886	12.6	14.0	.900
7	12.3	12.7	.968	12.3	13.1	.938
8	12.1*	13.1	.952	12.1	12.7	.952
9	11.8	13.5	.874	11.7	13.6	.860
10	11.5	12.6	.912	11.3*	12.5	.904
11	10.8	11.9	.907	10.8	11.5	.939
12	7.4	12.2	.606	7.2	11.4	.631

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 b (longer) arm lengths	Chromosomes with the highest a/b ratios
Leiophyllae				
P. leiophylla SCHIEDE & DEPPE	21	8	5, 7, 10	2, 3, 4
P. lumholtzii ROBINS. & FERN	11	2	6, 9	2, 3, 4
Canarienses				
P. canariensis C. SMITH	22	12	3, 5, 8, 11	2, 4, 7
P. roxburghii SARG.	26	14	5, 8, 11	3, 4, 7
Pineae				
P. pinea L.	13	6	3, 5, 8	2, 4, 7
Australes				
P. palustris MILL.	19	6	4, 6, 8, 10	3, 5, 9
P. taeda L.	23	8	4, 7, 10	3, 6, 9
P. echinata MILL.	20	11	4, 7, 9	3, 6, 8
P. pungens LAMB.	18	8	4, 6, 8, 10	3, 5, 9
P. rigida MILL.	40	20	3, 6, 8, 10	1, 2, 5
P. serotina MICHX.	21	6	4, 6, 8, 10	2, 3, 5
P. glabra WALT.	9	6	4, 6, 8, 10	2, 5, 7
P. elliottii ENGELM.	16	14	4, 6, 8	1, 3, 5
P. caribaea MORELET	20	14	4, 6, 8, 11	2, 3, 5
P. occidentalis Sw.	16	5	4, 6, 8, 11	2, 3, 7
Sabinianae				
P. sabiniana DOUGL.	22	4	5, 8, 11	3, 4, 7
P. torreyana PARRY	15	5	5, 7, 9, 11	3, 4, 6
P. coulteri D. DON	17	9	3, 6, 8, 11	2, 5, 7
Ponderosae				
P. jeffreyi GREV. & BALF.	27	9	4, 6, 9, 11	3, 5, 8
P. ponderosa LAWS	28	18	4, 6, 8, 11	3, 5, 7
P. washoensis MASON & STOCKWELL	14	7	4, 7, 11	2, 3, 6
P. arizonica ENGELM.	20	6	3, 6, 8, 11	1, 2, 5
P. engelmannii CARR	23	12	3, 6, 8, 11	2, 4, 5
P. durangensis MARTINEZ	16	9	4, 8, 11	2, 3, 6
P. cooperi C. E. BLANCO	10	5	3, 6, 8, 11	2, 5, 7
P. montezumae LAMB.	12	6	4, 6, 8, 11	2, 5, 7
P. michoacana MARTINEZ	9	5	3, 5, 8, 11	1, 2, 7
P. hartwegii LINDL.	19	9	4, 7, 9, 12	2, 3, 6
P. rudis ENDL.	18	11	4, 7, 9, 12	3, 6, 8
P. pseudostrobus LINDL.	21	8	4, 7, 9, 11	1, 3, 8
P. oaxacana MIROV	10	6	4, 7, 11	2, 3, 6
P. tenuifolia BENTH	20	9	3, 6, 9, 11	2, 4, 5
P. douglasiana MARTINEZ	17	4	5, 7, 9, 11	3, 4, 8
P. teocote SCHIEDE & DEPPE	24	11	5, 7, 10	1, 4, 9
P. lawsoni ROEZL	14	4	5, 7, 10	3, 6, 9
Contortae				
P. contorta DOUGL.	15	5	5, 7, 9	3, 4, 6
P. banksiana LAMB.	20	8	5, 7, 9	4, 6, 8
P. virginiana MILL	18	4	5, 7, 9	4, 6, 8
P. clausa (CHAPM.) VASEY	28	13	5, 7, 10	4, 6, 11
Oocarpae				
P. radiata D. DON	18	7	3, 5, 8, 10	2, 4, 7
P. muricata D. DON	22	9	3, 5, 8, 10	2, 4, 7
P. attenuata LEMM.	15	6	3, 5, 8, 10	1, 2, 4
P. greggii ENGELM.	18	6	3, 6, 9	1, 4, 5
P. patula SCHIEDE & DEPPE	18	7	4, 6, 8, 10	2, 3, 7
P. pringlei SHAW	17	11	4, 6, 8—9, 12	3, 5, 7
P. oocarpa SCHIEDE	15	10	4, 6, 9	3, 5, 8

and *P. torreyana* is especially meaningful. The proximal location of the constriction in 11 a is of special importance because constrictions are so infrequent in this chromosome and in this region of the arms *(Table 1)*.

Ponderosae

Although subgroups of closely related species in this subsection tend to have similar *b* arm sequences, no general pattern exists for the entire subsection. Chromosome 11 is involved in most of the *b* arm sequences, however *(Table 2)*.

The most diagnostic feature for this subsection involves the location of major differences in *a* arm lengths. For 14 of the 17 species, the greatest difference occurs between chromosomes 9 and 10 and between 10 and 11 in that order *(Table 1)*. The atypical species include three of four species

in one subgroup (P. pseudostrobus — P. douglasiana) for which the situation is reversed, i. e. the greatest differences are between chromosomes 10 and 11 and between 9 and 10, respectively. Such a situation involving chromosomes 9—11 does not exist for any other subgenus except Sylvestres whose species can be recognized by other diagnostic traits (DUFFIELD, 1952; SHAW, 1914).

A general b arm pattern involving chromosomes 4, 6, 8, and 11 exists for ponderosa pine and related species. Of the nine species that might be included in this complex (Table 1 — P. jeffreyi to P. michoacana), only P. washoensis and P. michoacana (Figure 3 a) deviate from the above sequence by more than one position. Major differences in a arm lengths are present between chromosomes 9—10 and 10—11 for all of these species.

Pinus jeffreyi has been a taxonomic problem historically because of characteristics that associate it with species in two subsections. As CRITCHFIELD (1966) pointed out, it shows affinity to species of Sabinianae through its similar oleoresin makeup (primarily paraffin hydrocarbon content) and its ability to cross with P. coulteri. On the other hand it has numerous morphological features similar to P. ponderosa (of the Ponderosae) with which it crosses equally as well as with P. coulteri. In its karyotype, P. jeffreyi has certain features that resemble both P. coulteri and P. ponderosa. However, there is a slight tendency to favor the latter.

Pinus arizonica, P. engelmannii (Figure 2 c), and P. cooperi show remarkable similarity in their karyotypes. A unique feature of two species in this group, P. arizonica and P. durangensis, is the obvious location of a major secondary constriction in the b arm of chromosome 12 (Table 1); to date this has not been observed for any other species in the survey. It is of interest that in karyotype features P. montezumae more closely resembles P. ponderosa and relatives than it does P. pseudostrobus, with which it often grows.

Pinus hartwegii (Figure 2 f) and the closely related P. rudis are two of four species that differ strikingly from the others in this subsection. Both species have a b arm sequence of 4, 7, 9, 12 which is unique because of the involvement of chromosome 12 (Table 2); thus far this has been observed in only one other species (P. pringlei of the Oocarpae).

Pinus pseudostrobus, P. oaxacana, P. tenuifolia (Figure 2 d) and P. douglasiana also form a somewhat related group taxonomically, and certain features of their karyotypes reflect a general similarity. A b arm sequence of 4, 7, 9, 11 is the most common sequence for these species, with P. tenuifolia diverging the most from the basic pattern. The sequence of major differences in a arm lengths for this group is just the reverse of that for the ponderosa pine complex. The largest difference is between chromosomes 10 and 11, with the next largest difference is between 9 and 10 (Table 1).

The two other species that are noticeably different from the others in this subsection are P. teocote and P. lawsoni (Figure 2 e). Their b arm sequence of 5, 7, 10 is totally different from the other patterns as is the location of greatest differences between a arms which occurs between chromosomes 1—2 and 10—11. These results provide additional evidence on the distinctness of these two species. As CRITCHFIELD (1967) pointed out, they differ from other species of Ponderosae in several respects such as cone size and location of leaf resin canals. The only difference between the karyotypes of the two species involves chromosomes

11 and 12. In P. teocote the b arms of these chromosomes are essentially identical, while in P. lawsoni the b arm of 12 is much shorter than that of 11.

Contortae

Characteristics of the karyotypes for the species in this subsection are very similar. All species have a b arm pattern of 5, 7, 9 except P. clausa which differs at position 10. Because chromosome 10 is fairly easy to identify and because it consistently disrupted the b arm sequence in P. clausa, this is considered to be a real divergence from the basic sequence. The sequence presented for P. virginiana (Figure 3 b) differs slightly from that published earlier (SAYLOR, 1961), but for the reasons given before it is now considered to be more reliable.

The greatest difference in a arm lengths is between chromosomes 10 and 11 in each species. Major differences also occur between chromosomes 1 and 2 for three of the species.

It is of interest that the members of this subsection are not unified on the basis of crossing data. Although P. contorta and P. banksiana cross rather easily and crosses have been made between P. virginiana and P. clausa, hybrids have not been made between either of the former with the latter two species. Also, P. clausa has been crossed with P. elliottii in one of the few successful intersubsectional crosses (SAYLOR and KOENIG, 1967). Although this indicates some tie exists between P. clausa and the subsection Australes, the karyological data support retention of P. clausa and P. virginiana in the Contortae subsection.

Oocarpae

Two different b arm patterns exist for the species in this subsection. The California closed cone pines (P. radiata, P. muricata, P. attenuata) all have a sequence of 3, 5, 8, 10. The remaining Latin American pines do not show such agreement, although patterns involving chromosomes 4, 6, and 9 are most common in these pines. The pattern of this karyotypic feature is similar, therefore, to other taxonomic traits such as location of needle resin canals, cone shape and serotiny in which significant differences have been found between the California and Latin American closed cone pines. In addition it has been found that the California pines of the subsection cross more or less readily among themselves as do the Latin American members, but crosses between species in the two groups are more difficult to make (CRITCHFIELD, 1967).

The one karyological feature for which there is complete agreement for all species in this subsection involves differences in a arm lengths. The greatest difference is between chromosomes 10 and 11 and the second largest difference between chromosomes 1 and 2.

The b arm sequence presented here for P. radiata (Figure 3 c) differs slightly from a pattern that can be constructed from the information presented by PEDERICK (1967). The differences (i. e. reversal of sequence at positions 2—3 and 7—8) very possibly reflect problems involved in reproducing the idiogram (which was remeasured to obtain chromosome data for comparison) to represent exactly the actual chromosome lengths.

Pinus greggii (Figure 3 d) is the third species in the subgenus Pinus in which the b arm of a chromosome other than number 1 is the longest (Table 1). In every plate (18) studied of this species, the b arm of chromosome 3 was the longest. Secondary constrictions in arms 3 b and 7 a were
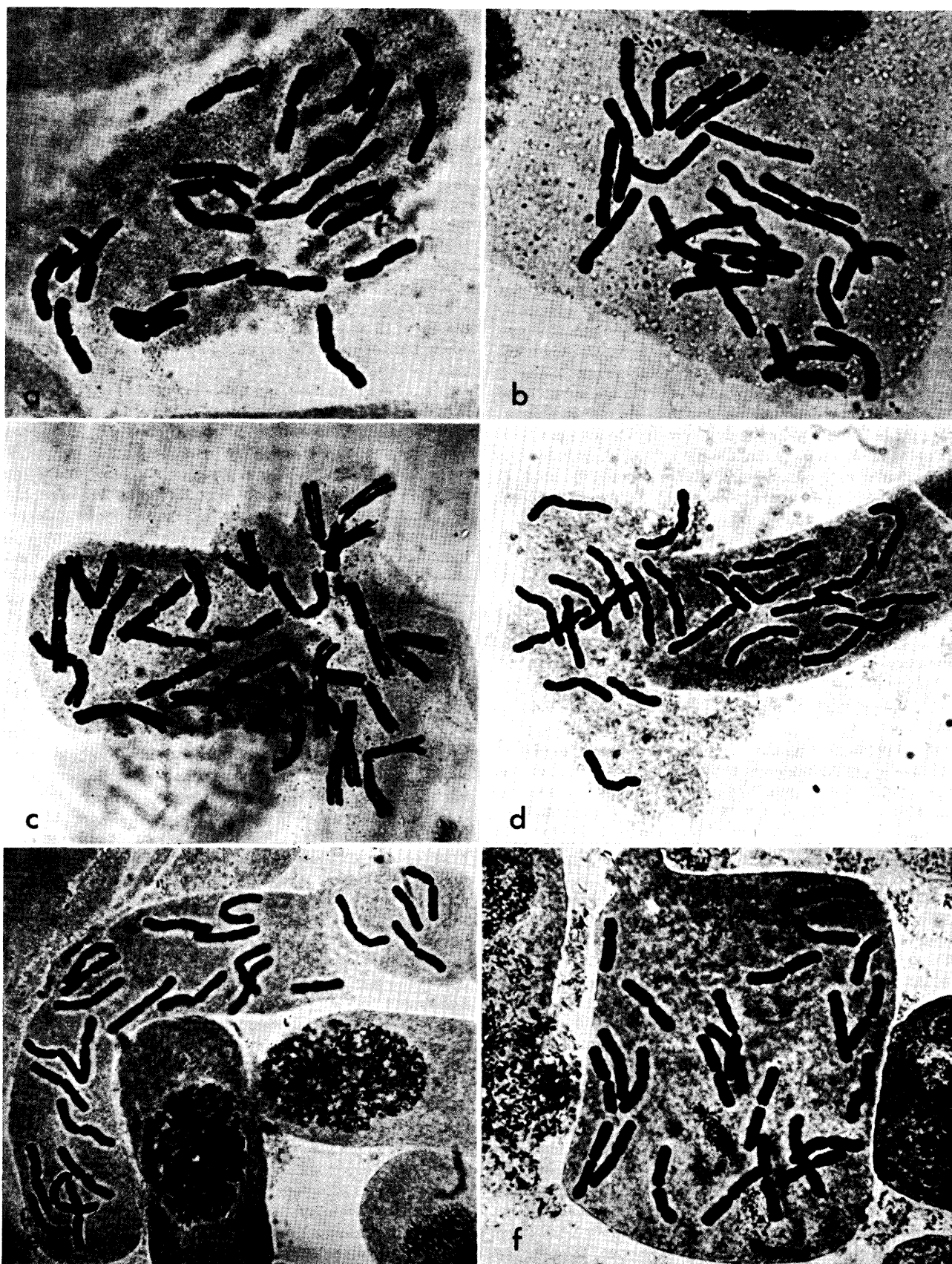
Figure 1. — Metaphase chromosomes from aceto-carmine preparations of root-tip meristems (2n = 24). (a) *P. leiophylla (1225×)*, (b) *P. roxburghii (1225×)*, (c) *P. pinea (1125×)*, (d) *P. rigida (1075×)*, (e) *P. caribaea (970×)*, (f) *P. occidentalis (1225×)*.
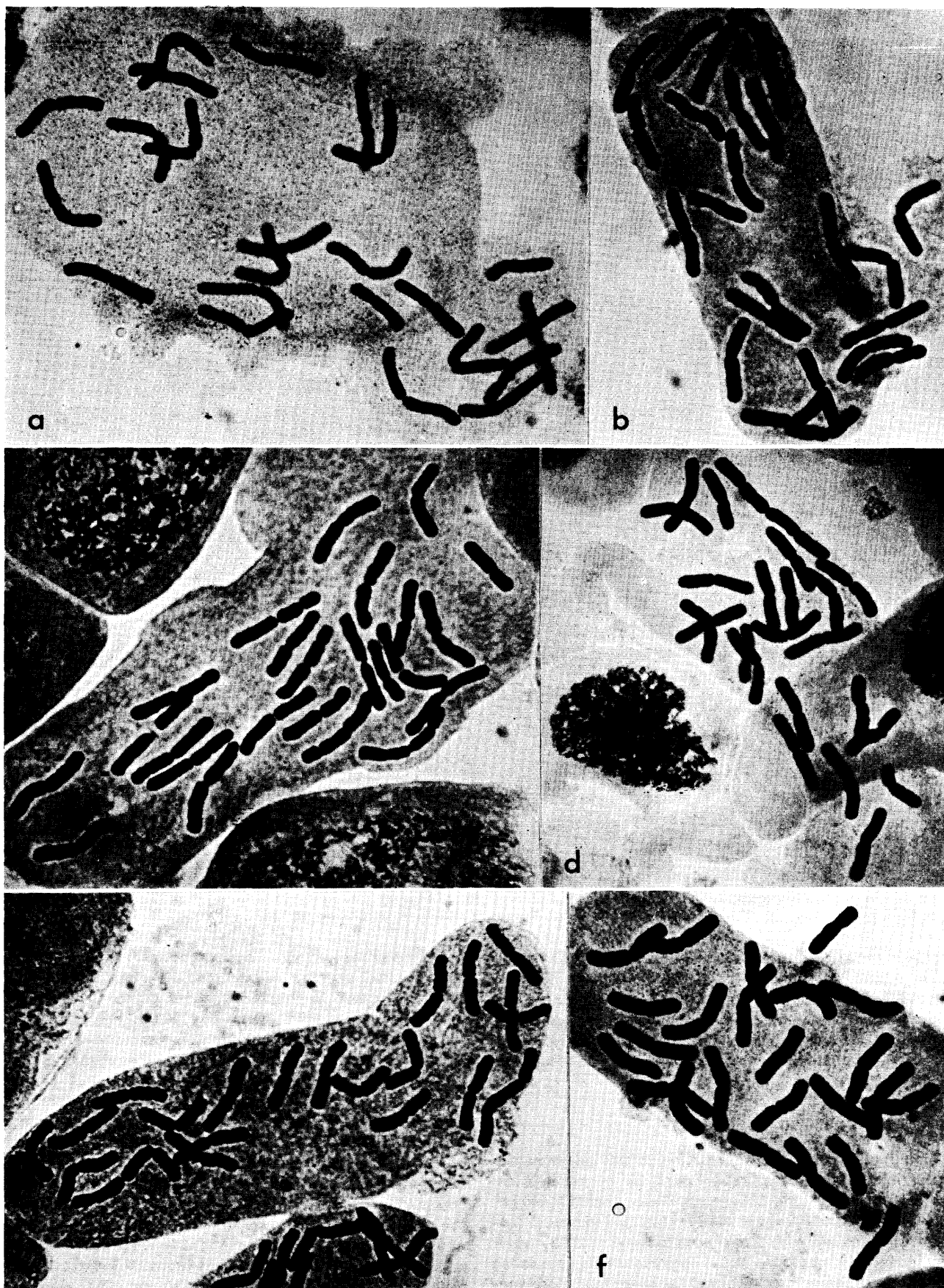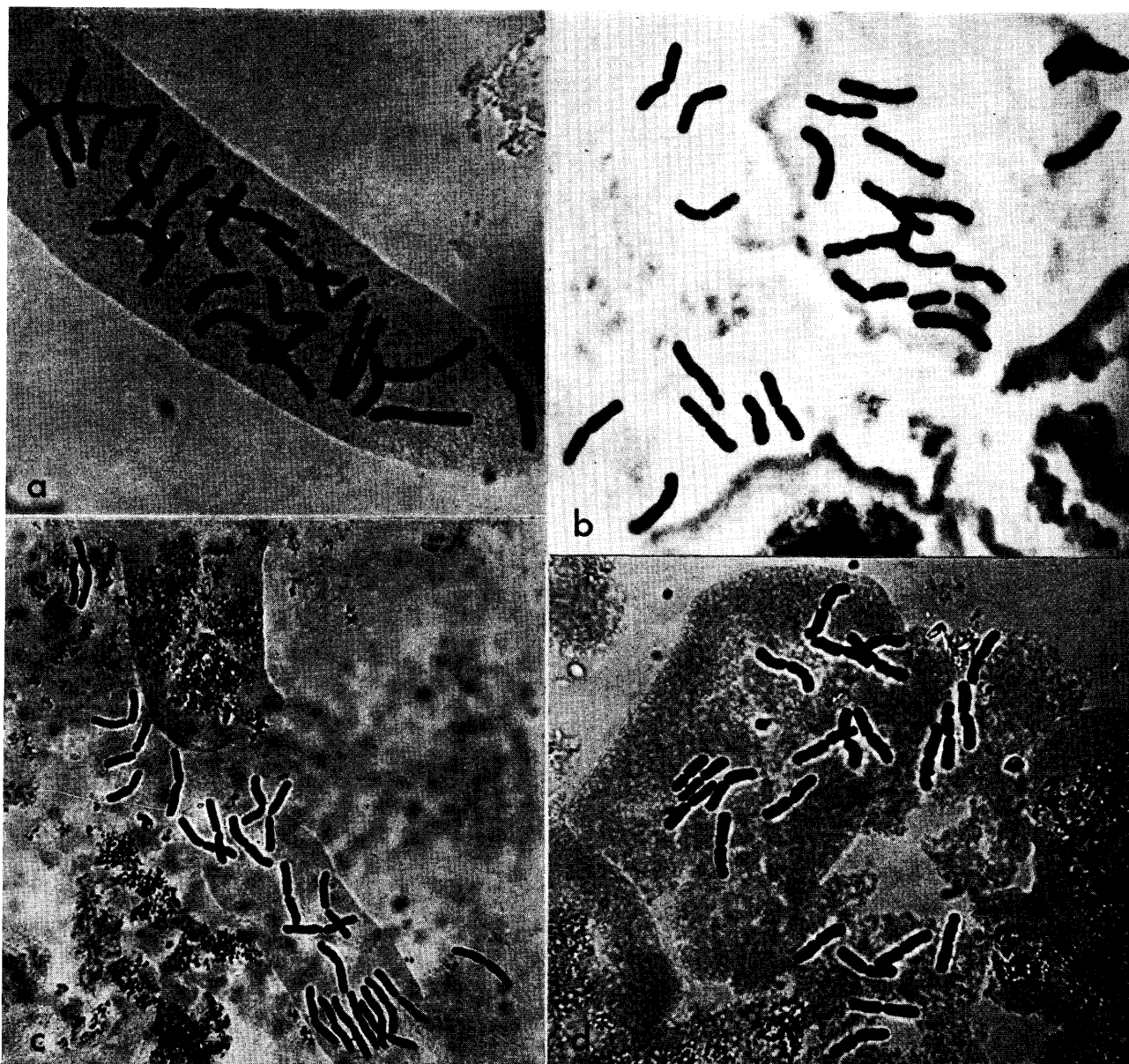
Figure 2. — Metaphase chromosomes from aceto-carmine preparations of root-tip meristems (2n = 24). (a) *P. torreyana* (1225×), (b) *P. ponderosa* (1225×), (c) *P. engelmannii* (1440×), (d) *P. tenuifolia* (1125×), (e) *P. lawsonii* (1125×), (f) *P. hartwegii* (1225×).

Figure 3. — Metaphase chromosomes from aceto-carmine preparations of root-tip meristems (2n = 24). (a) *P. michoacana* (1125×), (b) *P. virginiana* (950×), (c) *P. radiata* (760×), (d) *P. greggii* (930×).

especially conspicuous in this species and also in the closely related *P. patula.*

The *b* arm patterns of *P. pringlei* and *P. oocarpa* are quite similar. The most significant difference between them involves *P. pringlei* which is one of three species in the subgenus in which the *b* arm of chromosome 12 breaks the sequence *(Table 2).*

Discussion

It is apparent from the information in this paper, which constitutes the most comprehensive body of karyological data ever presented for the genus *Pinus,* that the karyotypes of species of pine are similar but not identical. Although the differences detected are frequently subtle rather than strikingly obvious, their importance is becoming clearer as information is obtained for more and more species.

As with most organisms, the patterns of species relationships obtained from karyological data tend to be correlated with those based on other characteristics. Several examples can be cited: (1) The subsection *Sylvestres* is one of the most distinctive in the genus based on various morphological and anatomical traits, and the karyotypes of these species are also the most distinctive encountered. (2) Species of *Contortae* form a rather unified subsection morphologically and the karyological data support this arrangement. (3) The subgroups in the *Ponderosae* and the *Oocarpae* subsections established on the basis of karyological affinities are closely correlated with groupings based on other traits. Evidence of individual species differences is the most difficult to obtain, but unequivocal results were obtained even at this level in some subsections. Examples are species such as *P. roxburghii, P. rigida,* and *P. greggii,* all of which have the longest *b* arm in their karyotypes associated with a chromosome other than the longest one (i. e. number 1). *Pinus hartwegii, P. rudis,* and *P. pringlei* all have special features involving the shortest chromosome (number 12) in the karyotype that distinguish them from others in their subsections. Similarly distinguishing fea-

tures exist for *P. teocote* and *P. lawsoni* that set them apart from other species in their subsection.

The results of this study along with those from meiotic analyses (e. g. SAYLOR and SMITH, 1966) indicate that structural changes of various types have occurred in pine chromosomes. It is felt that the degree of chromosomal differentiation is correlated with evolutionary relationships and that it is great enough to be of importance to taxonomists and breeders.

Summary

Karyotype descriptions based primarily on chromosome arm lengths and centromere location are presented for 46 species of the subgenus *Pinus*. Although all species have karyotypes that are basically similar, distinct karyological features were found for certain subsections, groups of related species and occasionally for individual species.

In general the karyological data support other taxonomic evidence regarding species relationships. Important features of the subsections include:

Leiophyllae — Karyotypes of *P. leiophylla* and *P. lumholtzii* show a level of distinctiveness comparable to that found for several other taxonomic traits.

Canariensis — Karyotypes of *P. canariensis* and *P. roxburghii*, two of the easiest determined, were rather similar.

Pineae — Nothing distinctive was found for the single species in this subsection.

Sylvestres — This is the most distinct group of species in the subgenus and possibly the genus; all species differ from those in other subsections in having two heterobrachial chromosomes instead of one.

Australes — Eight of ten species have similar karyotype patterns. Significant differences were found between *P. elliottii* and *P. caribaea*, but no intraspecific variation was found in either species.

Sabinianae — No basic pattern was found, but the evidence supports subsectional status for this group of species. There was exceptional agreement in the location of secondary constrictions for *P. sabiniana* and *P. torreyana*.

Ponderosae — No basic pattern exists except within subgroups of related species. These include:

(1) *P. jeffreyi, P. ponderosa, P. arizonica, P. engelmannii, P. durangensis, P. cooperi, P. montezumae,* and *P. michoacana.*

(2) *P. hartwegii* and *P. rudis* (these pines differ strikingly from all others).

(3) *P. pseudostrobus, P. oaxacana, P. tenuifolia* and *P. douglasiana.*

(4) *P. teocote* and *P. lawsoni* (these species are also greatly different from the others).

Contortae — All four species have very similar karyotypes, with *P. clausa* being the most different.

Oocarpae — Two different patterns exist in this subsection. The California closed cone pines form one subgroup, while the remaining Latin American pines form another, although less unified, group.

Zusammenfassung

Für 46 Arten der Untergattung *Pinus* werden Karyotypen-Beschreibungen mitgeteilt, die vorwiegend auf Untersuchungen der Chromosomen-Armlängen und der Lage der Centromeren beruhen. Obwohl alle Arten Karyotypen besitzen, die grundsätzlich ähnlich sind, ließen sich doch distinkte karyologische Merkmale für bestimmte Subsektionen, Gruppen verwandter Arten und manchmal auch für einzelne Arten finden. — Im allgemeinen unterstützen die karyologischen Daten andere taxonomische Hinweise über die Verwandtschaft der Arten. Wichtige Merkmale werden besprochen.

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