Chromosome Relationships Between Pinus species

By LEON A. PEDERICK*)

School of Forestry,

University of Melbourne, Victoria, Australia

General Introduction

In many groups of plants and animals the karyotype (i. e. the basic chromosome set of a species, characterised by the number, size and form of the chromosomes) can be used as a definitive species character. Cytology often has become a useful aid to the taxonomist, providing a basic genetic observation to add to other morphological criteria used to define species relationships. In fact, determination of relationships within natural groups of species can scarcely be considered complete in an evolutionary sense without good cytotaxonomic data to reinforce conclusions based on morphological criteria.

Like all systematic characters, the karyotype is subject to variation. In some cases closely related species may have distinctive karyotype differences, as in the herbaceous genus Crepis, with species containing either 3, 4, 5, 6, 7 or 11 chromosomes, and with total chromosome relative lengths ranging from 21 to 100 (BABCOCK 1947). In other cases a single karyotype may be representative of a whole genus. The fly genus Drosophila has been particularly suitable for such study with its special salivary gland chromosomes which permit precise studies of pairing and band sequence in hybrids. Karyotype differences between species in this genus have been found to have arisen as a result of centric fusions, translocations, inversions, and losses or gains in amount of heterochromatin. The number, form and size of chromosomes also varies widely between species in grasshoppers (WHITE 1954).

In contrast to these species there are certain genera of the Liliaceae and North American species of Tradescantia in which very little karyotype variation can be determined, and the same karyotype is employed as representative of a group of species (SWANSON 1960). Conifer genera could well be included in this category.

Review of Pinus chromosomes

Since the work of SAX and SAX (1933) it has been known that the karyotype of many Pinus species comprises twelve long chromosomes, eleven of which are metacentric and with so little difference in length that it is most difficult to distinguish any particular one. Only the twelfth, being shorter and with a submedian centromere, can be regularly separated from the others. A short twelfth chromosome of similar relative size is present in the karytype of all Pinus species reported to date, and it would appear to be a characteristic of the genus. In fact, on its morphology, it could be considered to be the same basic chromosome, that is, it has been inherited by each species, perhaps with some minor change, from some progenitor of the genus Pinus.

With one exception the relative uniformity of the other eleven chromosomes has limited the progress of cytological analysis. SAYLOR (1964) studied the pine Group Lariciones (according to the classification of SHAW 1914) and found that the eleventh chromosome could be distinguished by the presence of a submedian centromere. This characteristic of the eleventh chromosome was present in all 19 of the species of the Group studied but was not found in 20 species of 7 other Pinus Groups. It was suggested that the characteristic might be useful for taxonomic purposes, particularly in the cases of P. halepensis and P. pinaster.

The general conclusion reached by SAYLOR (1964), SAX (1960), and others, regarding the evolution of Pinus is that the genus has been cytologically very stable, with very few changes in chromosome structure. Any such changes appear to have been rather small, the main source of evolutionary change towards speciation being by gene mutation.

It had become apparent that further critical work on Pinus chromosomes required the use of some additional characteristic, since number and size of chromosomes and the position of the centromere were by themselves inadequate. Secondary constrictions, observed in the chromosomes of a number of conifer species (AASS 1955, NATARAJAN *et al.* 1961, SAYLOR 1961, 1964, SIMAK 1962, MERGEN and BURLEY 1964), appeared to offer a means for positive identification of particular chromosomes, but in the usual preparations from root meristem cells these constrictions could not be found regularly enough at the same positions to provide diagnostic markers. In fact, they seemed to appear at a variety of chromosome sites.

In a recent paper (PEDERICK 1967), the author described a new technique using preparations of female gametophyte tissue from which chromosomes at the premetaphase stage of mitosis could be studied (Fig. 1). At this stage, chromosomes are longer than at metaphase and each shows a number of secondary constrictions of varying size. The more prominent of the constrictions could be readily located from cell to cell. By means of total chromosome length, arm ratio (the ratio of the lengths of the long and short arms of a chromosome), and the distribution of constrictions, each chromosome can be readily identified and characterised. The reader is referred to the earlier paper for a full description of the technique used and the nature of the secondary constrictions, an understanding of which is essential to an appreciation of Pinus cytology.

Fig. 1. — Premetaphase stage in a cell from female gametophyte tissue. ***Pinus canariensis,*** n = 12. Stained with Feulgen and acetocarmine. X 1000.

*) Present address: Forests Commission of Victoria, Melbourne 3002, Victoria, Australia.

The analysis of *Pinus* chromosomes based on premetaphase cells in female gametophyte tissue presents certain difficulties, the chief of which is the need to analyse a number of cells (5—6) of each subject in order to obtain repetitive observations of all the main constrictions of each chromosome. The work is therefore tedious and complex, but chromosome analysis can be undertaken with considerable precision.

The following account describes a study and critical comparison of the chromosomes of nine *Pinus* species.

Materials and Method of Analysis

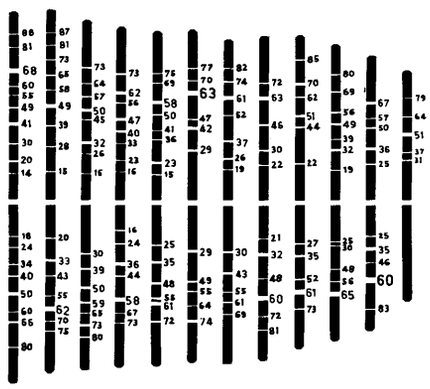
The nine species studied are listed below according to the classification of LITTLE and CRITCHFIELD (1965) which has been used as the reference for this study. The arrangement of species has been based on the classification of SHAW (1914) and the work of DUFFIELD (1952), and uses all available evidence of crossability patterns and barriers between species.

PINUS
- Subgenus STROBUS (equivalent to *Haploxylon* of SHAW) no species sampled
- Subgenus PINUS (equivalent to *Diploxylon* of SHAW)
  - Section TERNATAE
    - Subsection CANARIENSIS *P. canariensis* C. SMITH
  - Section PINUS
    - Subsection SYLVESTRES *P. nigra* ARNOLD
      P. pinaster AIT.
      P. halepensis MILLER
    - Subsection OOCARPAE *P. radiata* D. DON
      P. muricata D. DON
      P. attenuata LEMM.
      P. patula SCHIEDE and DEPPE
      P. greggii ENGELM.

The data for *P. radiata* is that presented in the earlier paper (PEDERICK 1967) and is based on preparations from four trees. Only one tree of each of the other eight species was sampled. The *P. muricata* was from a northern "blue" provenance of the species (Fort Bragg, California) and the *P. nigra* was probably of the subspecies *laricio* and from Corsica. No seed source data was available for the other species. About 10—12 suitable premetaphase plates were used from each tree. The technique of slide preparation and analysis is as described previously. The chromosome dimensions and idiograms of each species are shown in *figs. 2—10*. The standard errors of the relative lengths and arm ratios recorded in *figs. 2—10* are very similar to those for *P. radiata* recorded in the earlier paper (PEDERICK 1967).

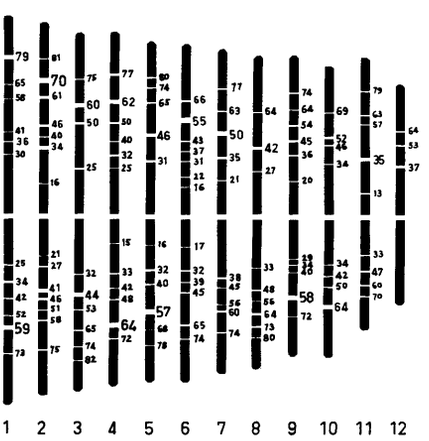
In order to compare the chromosomes of one species with those of another, enlarged idiograms were prepared, all at the same scale. In each species the relative lengths of the chromosomes had been determined as percentages of the average of the chromosome lengths. Therefore in all species the length of the average chromosome had been arbitrarily fixed at 100. Initially, comparisons of idiogram representation of individual chromosomes had to be made on the assumption that the actual total length of chromosome complement of each species would be the same, or very little different. Since very good correspondence was subsequently observed between the representations of chromosomes from species to species, it appears that the assumption has been borne out, that is, that there is very little difference, if any, in the total length of chromosome between the species studied. The good correspondence has also demonstrated a cytological affinity between the species.

Chromosomes of each species were matched on the basis of similarity in length, arm ratio, and distribution of constrictions. Similar constriction patterns frequently confirmed matches of chromosomes which would have been selected on the basis of length and arm-ratio only, when these two criteria were sufficiently distinctive. In other cases it was necessary to seek a similar constriction arrangement as the primary criterion.



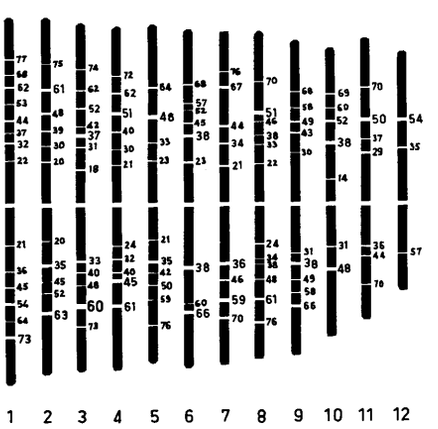
	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	116	113	109	106	105	104	101	100	98	92	86	71
Arm Ratio	1·06	1·15	1·09	1·07	1·05	1·09	1·01	1·05	1·15	1·16	1·15	1·35

Fig. 2 PINUS CANARIENSIS



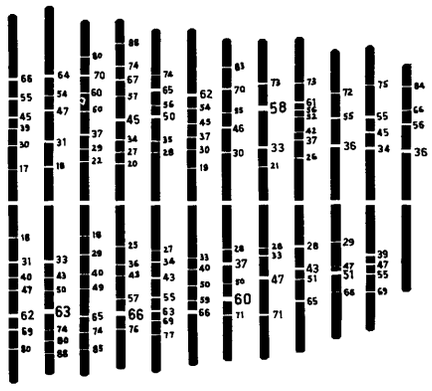
	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	121	115	111	110	105	104	100	97	94	89	84	68
Arm Ratio	1·07	1·09	1·05	1·09	1·05	1·05	1·06	1·07	1·20	1·08	1·42	1·53

Fig. 3 PINUS NIGRA



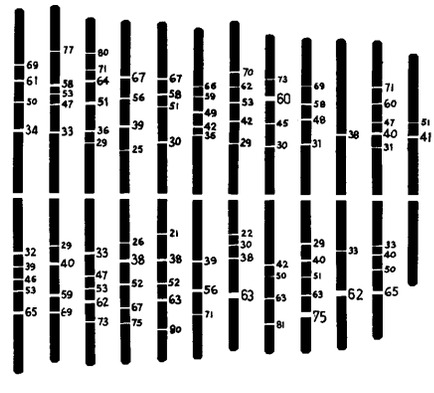
	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	115	111	108	106	105	105	103	100	97	89	87	73
Arm Ratio	1·05	1·11	1·10	1·08	1·14	1·08	1·08	1·13	1·08	1·17	1·45	1·81

Fig. 4 PINUS HALEPENSIS



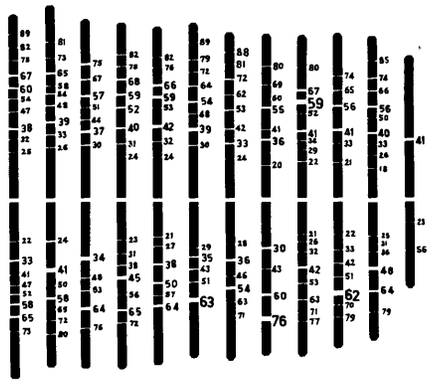
	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	115	114	110	108	106	104	100	99	97	89	88	70
Arm Ratio	1·04	1·16	1·05	1·11	1·03	1·07	1·04	1·04	1·10	1·12	1·22	1·58

Fig. 5 PINUS PINASTER



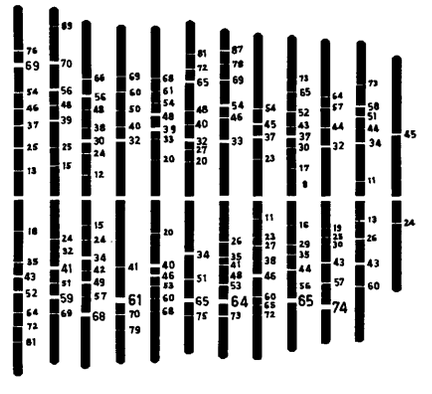
	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	112	110	107	106	105	102	102	98	97	97	92	71
Arm Ratio	1·05	1·15	1·05	1·06	1·07	1·04	1·15	1·04	1·03	1·04	1·12	1·68

Fig. 8 PINUS ATTENUATA



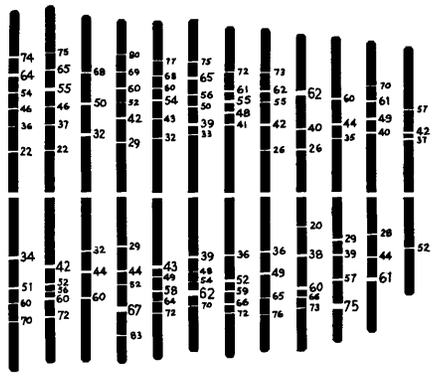
	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	112	111	107	106	104	102	101	99	98	97	93	71
Arm Ratio	1·05	1·18	1·07	1·06	1·08	1·14	1·05	1·07	1·05	1·12	1·17	1·64

Fig. 6 PINUS RADIATA



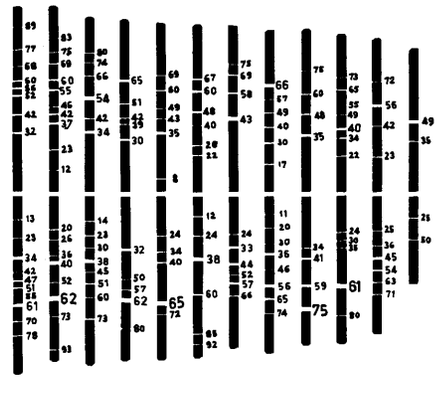
	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	114	110	108	105	104	102	102	100	97	94	93	72
Arm Ratio	1·08	1·15	1·05	1·04	1·05	1·15	1·07	1·03	1·07	1·09	1·10	1·57

Fig. 9 PINUS PATULA



	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	111	111	107	106	104	103	102	99	97	94	90	76
Arm Ratio	1·06	1·14	1·07	1·04	1·07	1·15	1·05	1·03	1·04	1·08	1·12	1·54

Fig. 7 PINUS MURICATA

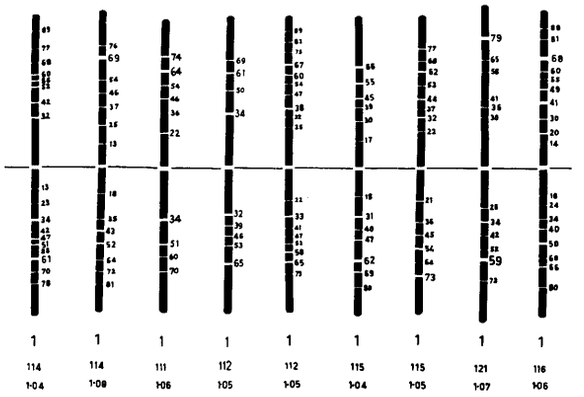


	1	2	3	4	5	6	7	8	9	10	11	12
Relative Length	114	110	107	106	104	103	101	100	98	95	91	72
Arm Ratio	1·04	1·13	1·04	1·05	1·04	1·05	1·12	1·03	1·08	1·05	1·11	1·65

Fig. 10 PINUS GREGGII

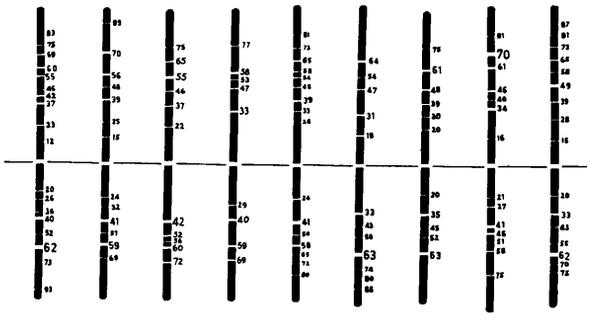
Figs. 2—10. — Chromosome idiograms of nine *Pinus* species, showing positions of constrictions. The relative prominence of each constriction is indicated by the width of gap and by the size of the number which denotes its position in the chromosome arm.

Eventually, twelve sets of chromosomes were matched, each containing one chromosome from each species, and within each set there being a general similarity of constriction arrangement. In some sets there were prominent constrictions at corresponding chromosome positions in all the species, but in others common patterns were not so apparent. However, it was usually possible to find smaller groups or pairs of chromosomes with common constriction arrangements, which could be linked through other similarities. Thus, by a process of careful selection and elimination the twelve sets of best match were obtained. In all cases each set contained chromosomes of similar rank by length from each species. No complications due to suspected translocations of chromosome arms were encountered. The



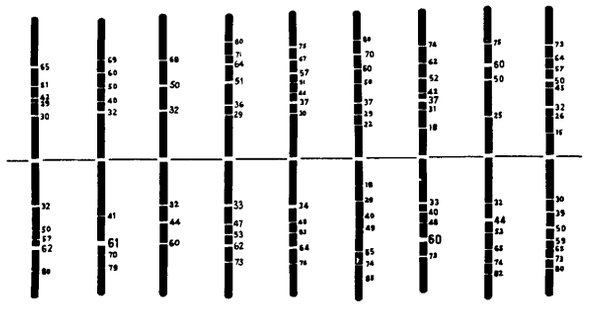
greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
1	1	1	1	1	1	1	1	1
114	114	111	112	112	115	115	121	116
1-04	1-08	1-06	1-05	1-05	1-04	1-05	1-07	1-06

Fig. 11 Chromosome set 1.



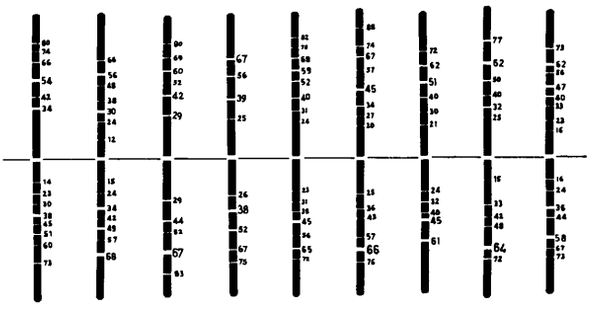
greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
2	2	2	2	2	2	2	2	2
110	110	111	110	111	114	111	115	113
1-13	1-15	1-14	1-15	1-18	1-16	1-11	1-09	1-15

Fig. 12 Chromosome set 2.



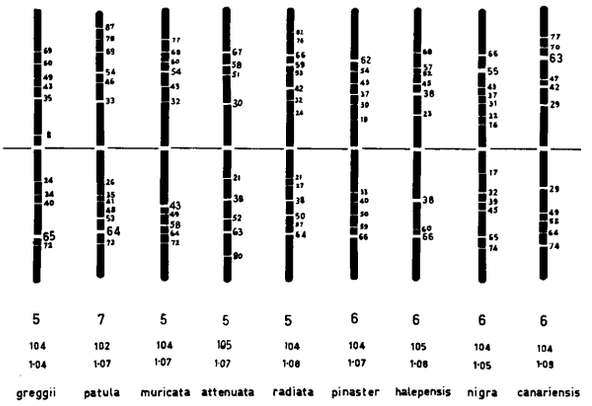
greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
4	4	3	3	3	3	3	3	3
106	105	107	107	107	110	108	111	109
1-05	1-04	1-07	1-05	1-07	1-05	1-10	1-05	1-09

Fig. 13 Chromosome set 3.



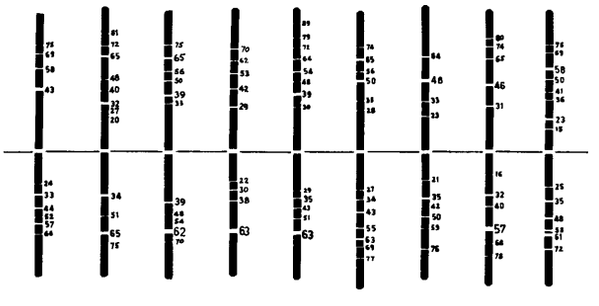
greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
3	3	4	4	4	4	4	4	4
107	108	106	106	106	108	106	110	106
1-04	1-05	1-04	1-06	1-06	1-11	1-08	1-08	1-07

Fig. 14 Chromosome set 4.



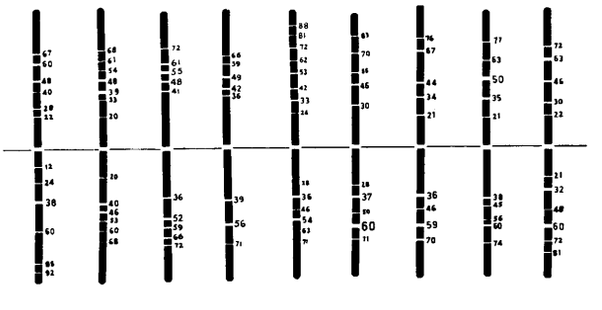
greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
5	7	5	5	5	6	6	6	6
104	102	104	105	104	104	105	104	104
1-04	1-07	1-07	1-07	1-08	1-07	1-08	1-05	1-09

Fig. 15 Chromosome set 5.



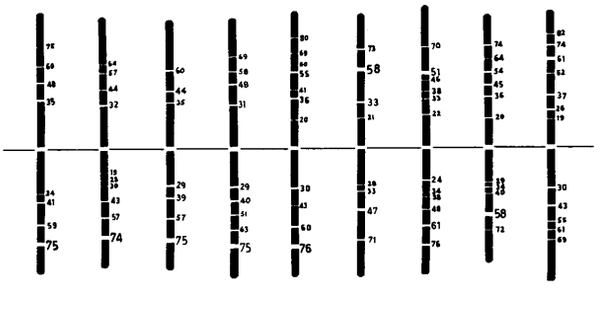
greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
7	6	6	7	6	5	5	5	5
101	102	103	102	102	106	105	105	105
1-12	1-15	1-15	1-15	1-14	1-03	1-14	1-05	1-05

Fig. 16 Chromosome set 6.



greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
6	5	7	6	7	7	7	7	8
103	104	102	102	101	100	103	100	100
1-05	1-05	1-05	1-04	1-05	1-04	1-08	1-06	1-05

Fig. 17 Chromosome set 7.



greggii	patula	muricata	attenuata	radiata	pinaster	halepensis	nigra	canariensis
9	10	10	9	8	8	8	9	7
98	94	94	97	99	99	100	94	101
1-08	1-09	1-08	1-03	1-07	1-04	1-13	1-20	1-01

Fig. 18 Chromosome set 8.

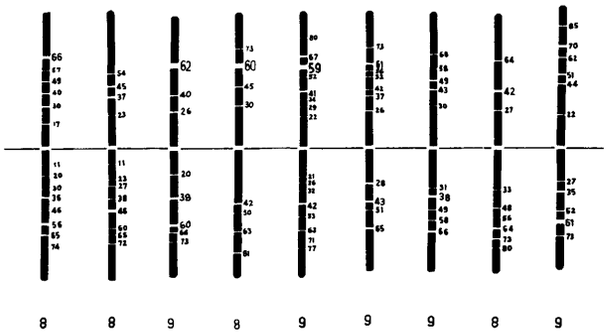


Fig. 19 Chromosome set 9.

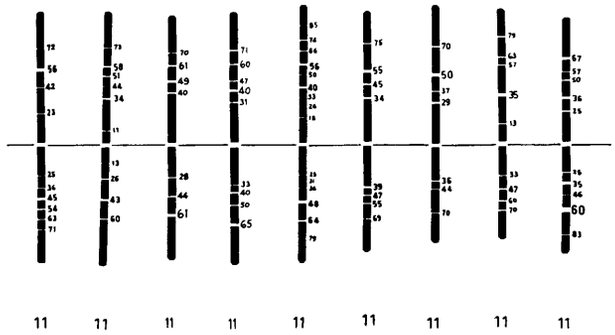


Fig. 21 Chromosome set 11.

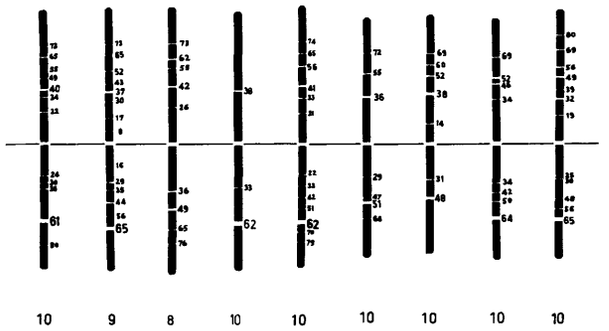


Fig. 20 Chromosome set 10.

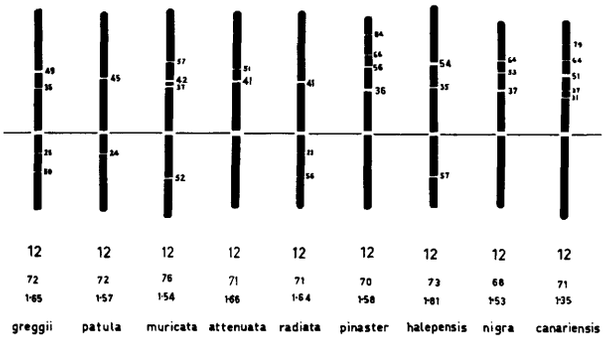


Fig. 22 Chromosome set 12.

Figs. 11—22. — Each figure comprises what are considered to be the corresponding chromosomes from each of nine *Pinus species*, all drawn to the same scale.

twelve sets of chromosomes are illustrated in *figs. 11—22*. Each set has been designated with the rank of the *P. radiata* chromosome contained in it.

Generally, it was observed that species considered to be closely related had the closest similarity of chromosome dimension and constriction arrangement. For example *P. radiata* and *P. attenuata* were very similar, as also were *P. patula* and *P. greggii*. The species of the group *Oocarpae* had many similarities which distinguished them from the others. Nevertheless, the amount of similarity encountered between all the species was remarkable.

In some chromosome sets there were greater differences in length between certain chromosomes than could be accounted for by their standard errors, thus indicating real differences in length between them. When these coincided with a difference of arm-ratio, the length difference could usually be related to a particular chromosome arm.

The statistical procedure used to compare the length of particular chromosome arms, or even portions of arms, was to refer to the original photographs, measure the section under study on each photograph, convert to relative lengths and analyse by t-test. In the following discussions, all claims of significant differences in length have been based on this type of analysis.

It was sometimes possible to suggest a specific region within a chromosome in which a difference of length between two species was localised. Thus, when the constriction distribution was similar in the proximal half of chromosomes the difference could be considered to be located in the distal part. On the other hand, a correspondence of constriction distribution was sometimes obtained only when the chromosome arms were arranged with their telomeres in line, and in these cases the difference could be considered to be near the centromere region.

Interesting differences in length and arm-ratio were observed in chromosome sets 11 and 10 in particular, also in sets 1, 6, 8 and 12. The other sets appeared uniform in length. There were also some interesting differences in constriction distribution which characterised groups of species. Only the more prominent constrictions were used as primary markers for comparison between species, because the minor constrictions were based on fewer observations. However the latter were sometimes used in conjunction with the prominent constrictions.

The chromosome sets will be discussed in numerical order with greatest emphasis on those sets containing major differences.

Conventions

The conventions used to designate chromosomes and constriction positions are: —

(1) the particular chromosome — 1 to 12. The chromosomes have been ordered by decreasing length.

(2) whether the arm is the long or short arm — L or S.

(3) the position of a constriction — the distance away from the centromere as a percentage of arm length.

Thus, 8 S 76 designates the prominent secondary constriction found on the short arm of chromosome 8 of *P. radiata* and situated 76% of the distance along that arm.

Analysis of Chromosome Sets

(i) *Chromosome set 1 (fig. 11).* This set contained the longest chromosome from each species, which except for *P. nigra,* was quite uniform in length and arm-ratio. The chromosome set was also characterised by a large number of constrictions in both arms but, unlike some others, there was no particularly prominent constriction common to all. There was evidence of close similarity between species of the *Oocarpae,* particularly between *P. radiata* and *P. attenuata,* and between *P. patula* and *P. greggii.* A constriction within the region 32 to 35 in the short arm appeared to be the most obvious one common to the *Oocarpae* species.

When the long arms were arranged with their telomeres in line there was a very noticeable correspondence between the constriction arrangement of *P. nigra* and *P. halepensis (fig. 23).* It is suggested therefore that during speciation a difference in length between the long arms has arisen near the centromeres of these related species. Because the long arm of *P. nigra* was significantly longer than that of all the other eight species it is suggested that the additional length has occurred by means of a duplication in *P. nigra.*

The short arm of *P. nigra* was also significantly longer than that of all the other species except *P. canariensis.* It was not possible, however, to suggest a difference in constriction distribution in any portion of the chromosome arm.

(ii) *Chromosome set 2 (fig. 12).* These chromosomes were readily identified in all species by their length and high arm-ratio. There was a very marked similarity of constriction distribution in the short arms of the *Oocarpae* species, particularly with prominent constrictions at 40—42 and at 58—62, with lesser ones at 50—52 and 69—73. The short arms of *P. canariensis, P. pinaster* and *P. halepensis* (but not *P. nigra)* were quite similar, particularly at 33—35 and 62—63.

Similarity between the distribution of constrictions in the long arms was less apparent.

(iii) *Chromosome set 3 (fig. 13).* These chromosomes formed a very uniform series with respect to length. There were no common prominent constrictions to form a characteristic pattern for the series, but rather, similarities in constriction distribution could be observed between groups or individual species. In the *Oocarpae,* the prominent constriction at 61—62 in the short arm of *P. patula* and *P. greggii* corresponded with one of minor prominence at the same position in the three other species. Another constriction at 32—34 was common to most of these species.

(iv) *Chromosome set 4 (fig. 14).* As in set 3, there were no significant differences in length. Similarities of constriction distribution could be found between individual species but there was no characteristic pattern common to the whole set. A very prominent constriction at 67 in the short arm of *P. muricata* corresponded only with minor constrictions in the other *Oocarpae* species, although a comparable prominent constriction was present in two of the *Sylvestres* species.

(v) *Chromosome set 5 (fig. 15).* These chromosomes formed a series with uniform dimensions, there being no significant differences in the length of any arms. The prominent constrictions at 64—65 in the short arm of the Mexican species corresponded with minor constrictions in the other *Oocarpae* species.

(vi) *Chromosome set 6 (fig. 16).* A high arm-ratio, very noticeable uniformity of length, and a major constriction in the short arm characterised the species of the *Oocarpae.*

Differences existed in the short arms of the other species. The short arms of the three *Sylvestres* species were significantly longer than that of *P. radiata.* If the prominent constriction in *P. radiata* and *P. nigra* could be regarded as corresponding, the difference in length would be located in the distal part of the *P. nigra* arm. *P. halepensis* was significantly shorter than *P. nigra* and *P. pinaster,* and the difference appeared to lie in the proximal portion (centromere region) of the arms.

There were no detectable differences in the lengths of the long arms.

(vii) *Chromosome set 7 (fig. 17).* The chromosomes of this set were very uniform in length. There were no very prominent constrictions in the *Oocarpae.* In the *Sylvestres* there was a good short arm correspondence at the more prominent constriction at 59—60 and at 36—38. In the short arm of the *Oocarpae* there also seemed to be a corresponding constriction at this same position, i. e. 36—40.

(viii) *Chromosome set 8 (fig. 18).* A very prominent nucleolar constriction was present at 74—76 in the short arm of all the *Oocarpae* species, but not in the others. This could prove to be an important cytological characteristic of taxonomic value. This constriction was most strongly developed in *P. radiata* (in which it was the most characteristic constriction of the karyotype) and was least developed in *P. muricata.* Correspondence of constrictions was very pronounced in the short arms of the *Oocarpae,* but was not very apparent in the long arms.

The short arm of *P. nigra* was significantly shorter than those of the others. When telomeres were arranged in line, the similarity of constriction arrangement with that of *P. halepensis* indicated that *P. nigra* was shorter in the proximal region.

(ix) *Chromosome set 9 (fig. 19).* This set appeared to be fairly uniform in length and no differences could be detected. In the *Oocarpae,* the Californian species were distinguished from the Mexican species by the nucleolar constriction at 59—62 in the long arm which was not present in the latter. In the *Sylvestres, P. nigra* and *P. halepensis* were quite similar but *P. pinaster* seemed to be more similar to *P. radiata. P. canariensis* was somewhat similar to *P. halepensis.*

(x) *Chromosome set 10 (fig. 20).* Within this set significant differences were analysed in the length of both arms. The long arms of the *Oocarpae* species were of similar length and were longer than those of the three *Sylvestres* species. When telomeres were lined up *(fig. 24),* particularly with *P. pinaster,* a similar constriction distribution was observed in all the species, and the length difference could be attributed to a change in the proximal region. The long arm of *P. canariensis* was significantly longer than that of *P. nigra,* but the constriction arrangement appeared to differ from that of the other species.

The short arms of the *Oocarpae* species also exceeded those of the *Sylvestres* and *P. canariensis.* The prominent short arm nucleolar constriction at 61—65 in the *Oocarpae* (very minor in *P. muricata)* seemed to correspond with one in *P. nigra* and *P. canariensis.* If these are related constrictions, the difference in length can also be attributed to a change in the proximal region.

(xi) *Chromosome set 11 (fig. 21).* The chromosomes of this set had a large arm-ratio, particularly those of the *Sylvestres.* There were also a number of interesting differences

in length. Total relative length of the *Oocarpae* species ranged from 90—95 whereas those of the other species ranged between 84—88. When the chromosomes were arranged with their centromeres in line *(fig. 21)* some correspondence of constrictions could be seen within the *Oocarpae* and within the *Sylvestres,* but not between the two groups. However, when the long and short arms were separately arranged with their telomeres in line *(fig. 25)* a much better correspondence was observed.

The short arms of *P. halepensis* and *P. nigra* were of similar length and both were significantly shorter than that of *P. pinaster.* According to the arrangement of constrictions the difference appeared to lie in the proximal part of the arm. Furthermore, the short arm of *P. pinaster* was significantly shorter than those of the *Oocarpae* species, and again the difference could be assigned to the proximal portion of arm. The length of the *P. canariensis* arm corresponded with that of *P. pinaster* but there was no similarity of constrictions. Omitting *P. canariensis,* the species can be arranged in three groups according to length of the short arm, i. .e (1) *P. halepensis* and *P. nigra,* (2) *P. pinaster,* (3) *Oocarpae* species. Since they all appear to contain a similar pattern of constriction, it is possible that these chromosomes evidence a progressive evolution of the chromosome arm in which duplications or deletions have been accumulated.

In the long arms two species differed in length. *P. halepensis* was longer than *P. pinaster* and the additional length appeared to be located in the distal region. *P. canariensis* was much shorter than the others, but it was difficult to determine in what region the difference could have originated.

(xii) *Chromosome set 12 (fig. 22).* These chromosomes are the most distinctive of the *Pinus* karyotype. The short asymmetric chromosomes appeared fairly similar when arranged together; however three significant differences were found. The short arm of *P. muricata* was longer than those of the other *Oocarpae* species, and that of *P. canariensis* was longer than those of the *Sylvestres* and four of the *Oocarpae* species. The long arms were also uniform with one exception — *P. halepensis* was significantly longer than *P. nigra* and *P. pinaster.* The region of increase in length appeared to be located in the distal portion of that arm.

There appeared to be one constriction common to the long arm of all the species. The constriction mapped at 41 in *P. radiata* is actually a combination of two constrictions, at 37 and 43, so that each of these sites corresponds with sites in other species (particularly *P. muricata).*

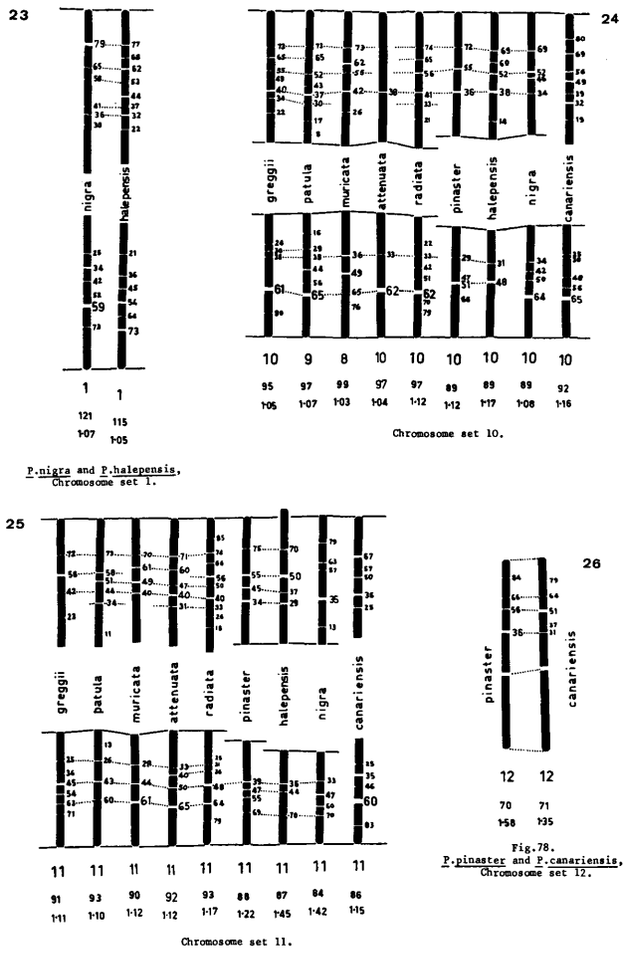
P. canarienis posed a special problem. Its total length did not differ from that of *P. pinaster* although the arm-ratio did. When the telomeres of both species were arranged in line *(fig. 26)* most constrictions appeared to correspond, but the centromeres did not. Two suggestions can be advanced to account for this difference, assuming a common ancient species origin:

(a) a short deletion in the proximal portion of the long arm of *P. canariensis,* and a duplication of similar length in the short arm, or

(b) a short slightly asymmetric pericentric inversion.

Discussion

It should be no surprise that closely related species, such as some of those studied in *Pinus,* have a very similar chromosome structure. Nevertheless it is of considerable



Figs. 23—26. — Some of the corresponding chomosomes from chromosome sets 1—12, re-arranged with their telomeres in line in order to indicate possible correspondence of constriction distribution.

interest, for studies of chromosomes in this degree of detail have never been undertaken before in conifers.

In the present study the original chromosome analyses and preparation of idiograms of each species were undertaken quite independently, and at different times. The degree of correspondence later observed in chromosome dimensions and constriction distribution, particularly between the interbreeding groups of species, was actually quite unexpected. Not only does the correspondence inspire confidence in the reliability of the method of chromosome analysis, but also it indicates that chromosome structure may be a useful aid for the study of species' relationships and the types of chromosomal change which have occurred during the evolutionary development of the genus. These two factors will be considered in turn.

While statistical analyses were undertaken on lengths of chromosomes or portions of arms, similar quantitative analyses were not possible for constrictions, and conclusions as to whether or not certain constrictions in different species corresponded have largely been based on opinion, as also was the initial selection of the particular chromosomes to be included in each chromosome set.

The comparisons therefore have been limited generally to chromosome arms containing prominent constrictions. This was necessary because the minor constrictions were based on fewer observations than the prominent ones, and also, some sites recorded as one constriction were some-

times suspected to be the average of observations relating to two constrictions. A further limitation of the analysis is due to the fact that except for *P. radiata* the data was derived from only one tree per species, (However, a study of four trees of *P. radiata* yielded only a few very minor differences). For these reasons, claims of correspondence of constriction recorded between species have tended to be conservative, and readers might consider that some examples have been over-looked.

It is interesting to relate observations of chromosome structure of species to their arrangement within classifications of *Pinus*, such as those of SHAW (1914), PILGER (1926), DUFFIELD (1952), and LITTLE and CRITCHFIELD (1965). The only similar previous attempt was that of SAYLOR (1964) (based on determinations from root squashes) who observed that the arm ratio of chromosome 11 in all species of the *Sylvestres* was much greater than in other pine species. On this basis he suggested that *P. pinaster* should be included in the *Sylvestres* (equivalent to the *Lariciones* of SHAW's classification) for SHAW had placed it in a different group. The observations of the present study have tended to confirm SAYLOR's work with respect to this characteristic in the three *Sylvestres* species studied.

There were, however, a number of differences in length between some corresponding chromosomes of these *Sylvestres* species. For example, four significant differences in chromosome length were found between *P. nigra* and *P. halepensis*, although, in their constriction arrangement, these two species had many similarities. Of the two, *P. halepensis* showed the greater number of similarities with *P. pinaster*. Although *P. pinaster* has been placed in the *Sylvestres*, it also shows some similarity with *P. canariensis*. This order of affinity is interesting because it corresponds to the arrangement of their present-day natural distribution. Of those four species, the one most similar in constriction arrangement to *P. radiata* was *P. pinaster*.

The five *Oocarpae* species, however, were more uniform, and only one length difference could be detected. In chromosome set 12 the short arm of *P. muricata* was longer than those of the other four species. The *Oocarpae* species were distinguished from the other species studied by a number of important characteristics, the principal ones being the nucleolar constrictions at about 8 S 75, and length differences in chromosomes 10 and 11.

The *Oocarpae* could be divided into two groups, the Californian and Mexican species, which are distinguished by certain nucleolar constrictions. Thus, the former have one at about 9 L 60 and the latter have ones at 3 S 61 and 5 S 64, not possessed by the others. Hybridisation between these two groups of pines appears to be much more difficult than within the groups. There is only one recorded successful inter-group cross, that between *P. patula* and *P. radiata*, of which there is only one surviving plant (CRITCHFIELD 1967).

Probably the greatest similarity of chromosomes between species in this study was observed between the two Mexican pines, *P. patula* and *P. greggii*. No length differences were found, the major constrictions corresponded, and differences were observed only at the medium and minor constrictions. This result is not surprising since the two species are quite interfertile (FIELDING and NICHOLSON 1956), although they have different ecological requirements.

The California closed-cone pines form a group noted for its interfertility. *P. attenuata* × *radiata* is a very easily produced *Pinus* hybrid. The only differences observed between the chromosome sets of these two species were at some minor constrictions. *P. muricata* (northern provenance) differed from the other two species in the length of the short arm of chromosome 12, as well as at two nucleolar constrictions — it contained one at 4 S 67 and did not have one at 10 S 62. *P. muricata* of southern provenance crosses readily with the other two species, whereas the northern provenance does not, nor does it cross readily with the southern form (BROWN 1966, CRITCHFIELD 1967). Unfortunately, a tree of southern origin was not included in this study for it would be interesting to determine whether or not it contained the same chromosome characteristics as the northern form. Observations of this type would be very helpful in determining to what extent differences in chromosome length, and constrictions are likely to be indicative of the degree of genic differentiation. Of course, constrictions are heterochromatic, and, at the present time are thought to contain few or no genes.

The number of species studied here has not been sufficient to suggest any criteria of similarity of chromosome structure which may be used to define species' relationships in *Pinus*. The chromosomes of five species in the *Oocarpae* were very uniform except for one chromosome in *P. muricata*, and otherwise were only distinguished by constriction differences. Therefore the grouping of these species on morphological grounds is confirmed on cytological grounds. However, in the *Sylvestres* there are quite a number of differences in length, although small, as well as in constriction distribution. No record of successful hybridisation between the two most similar species of those studied in this group, *P. nigra* and *P. halepensis*, is known to the writer. In order to obtain further evidence of the chromosomal differences that can exist between interfertile species it would be interesting if chromosome analyses of *P. sylvestris*, *P. resinosa* and *P. densiflora* were undertaken and compared with *P. nigra*. All these species have been crossed successfully with *P. nigra* (WRIGHT 1962). In the examples of interfertile species so far studied, all in the *Oocarpae*, only differences in constriction distribution were observed.

The pines are a very ancient group of plants and some *Pinus* fossils have been reported from deposits of the Triassic Age i. e. about 180—190 million years (CHAMBERLAIN 1935). One can only guess at the period of time which has elapsed since the ancestral forms of the species studied in this report became differentiated from each other, but it is probably well over 100 million years ago. For example, an ancestral form of the *Oocarpae* probably differentiated from a forerunner of the *Sylvestres* as a result of geographic isolation, and evolution of the modern species followed afterwards. Speciation of the California closed-cone pines may be of relatively recent origin (MASON 1932). However, the fact that a similarity can still be traced between the chromosomes of species classified in different subsections, such as *P. radiata* and *P. canariensis* indicates that there has been a remarkable selection against structural changes.

It is therefore apparent that the main source of evolutionary change has been one of gene mutation. Mutations of constrictions also have occurred, resulting in changes in the prominence of some, as well as the appearance or disappearance of others. The origin of these changes is not known.

Although chromosome lengths were remarkably constant from species to species, significant differences were detected in the lengths of corresponding arms of certain species. It is suggested that these could be caused by duplications or deficiencies. There is reason to consider that

duplications are the more likely, since there is considerable evidence from genetic studies that deficiency mutations, although viable in the heterozygote condition, are lethal when homozygous (SWANSON 1960). The likelihood of deficiencies contributing to speciation may therefore be minimal, unless the piece of chromosome is heterochromatic, which in *Pinus* would be at the constrictions only.

Duplications formed by unequal crossing-over have been detected in *Drosophila* by the presence of identical repeats of the banded structure in the giant salivary gland chromosomes, e. g. the "Bar" phenotype is related to a tandem repeat of seven bands. The "Bar" repeat only exists in laboratories because of its adverse phenotypic effect, but many other repeats are now known to be incorporated in the species complement, (see SWANSON 1960). Duplication of loci offers the means for a species to acquire new genes, which, though identical at first, may diverge by mutation so that eventually they may control different and separate functions. A number of pseudoallelic gene series in *Drosophila* e. g. "vermillion" and "beadex" have been analysed by cytological and crossing-over studies as duplications in which the genes have diverged somewhat in function. The "bithorax" pseudoallele series is of a triplicated nature, while the "Bar" locus has been repeated up to eight times in tandem sequence in some individuals. It remains doubtful whether a duplicated gene can provide an unlimited source of variation by mutation, because of its molecular structure, so it is doubtful how far such genes can diverge to control completely different functions. It seems that they may be limited to very large pseudoallelic series or perhaps to separate steps in one function. Thus, it can be seen that repeated duplications could gradually increase the length of a chromosome region as well as the number of genes.

In the comparisons between the *Pinus* species an attempt was made, using constriction arrangements, to relate the differences in chromosome length between species to particular portions of chromosome arms. In fourteen cases studied, seven appeared to be located in the proximal region, three in the distal region, and in the remaining four it was not possible to suggest any particular chromosome regions. The sizes of the duplications were usually of about 3—4% of the length of the chromosome involved. In the evolution of certain *Pinus* species it therefore appears that, if duplications are of the type observed in *Drosophila*, numbers of them have accumulated in particular chromosome regions prone to these changes. Significant differences between species would only be detectable when a sufficiently large number of these changes had accumulated. It seems reasonable to suggest that numbers of short duplications have also occurred at other points on the chromosomes, but they have been too short to detect with the present technique. However, they would cause small differences in the distance between corresponding pairs of constrictions, which would be attributed to experimental error. Also, it should not be overlooked that small differences of this type could be present as cytological variation within a species.

Other types of structural rearrangement of the chromosomes do not appear to have contributed to the length differences observed. Paracentric inversions are common between individuals within a species as well as at the species level (SAYLOR and SMITH 1966, PEDERICK 1968). However this type of rearrangement makes no difference to chromosome length. Since inverted sections in *Pinus* appear to be very short their location can not be detected by the technique described in this paper. No evidence of possible translocations was obtained.

Mention has been made that the main evolutionary change in pines has probably been one of gene mutation. The similarity of chromosome structure between species would suggest that in many cases, particularly between closely related species, similar genes are present in the same places on corresponding chromosomes, but the particular alleles present may be different. However, this will only be confirmed if and when genes are identified and located on the chromosomes. This concept should have useful implications for tree breeders since it suggests that for many species tree characteristics are probably under the control of similar combinations of genes.

Conclusions

1. Considerable similarity exists between the structure of the chromosomes of *Pinus* species which must have been isolated reproductively for many millions of years.
2. Arm length of corresponding chromosomes is so very similar between species that selection against change in length must be very strong.
3. Small but significant differences in length found in the corresponding chromosome arms of certain species have been attributed to the gradual accumulation of duplications. This process is regarded as providing the main means of change, despite an apparently high selection against change in length of chromosomes.
4. Very similar arrangements of constrictions were observed in the corresponding chromosomes of closely related species, and the arrangements were much less alike between species in different taxonomic groups. A very prominent constriction present in the short arm of chromosome set 8 of five species of the *Oocarpae* could be a cytological characteristic of that Subsection.
5. Chromosome structure therefore offers additional evidence for the study of species relationships.
6. Evolution of *Pinus* species seems to have been mainly by gene mutation.
7. Insufficient data was obtained to indicate what extent of chromosome differentiation between species is associated with limitation of their interfertility.

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Summary

Chromosome idiograms based on premetaphase chromosomes from female gametophyte tissue were prepared at the same relative scale from nine *Pinus* species. Sufficient similarity of chromosome dimensions and constriction distribution was observed to enable a confident selection of twelve chromosome sets, each having one chromosome from each species.

Greatest similarity in length and constriction distribution was observed between interfertile species but some similarities could be found between species classified morphologically in quite different groups of the genus. Within certain chromosome sets, differences in length of some

chromosome arms were found to be significant, and structural change by gradual accumulation of small duplications has been suggested as an evolutionary process in *Pinus*, despite the extremely strong selection against change of length which seems to have prevailed for many millions of years.

Chromosome structure therefore appears to offer a further useful criterion for determining species relationships.

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Multinodality, Branching, and Forking in Lodgepole Pine (Pinus contorta var. murrayana Engelm.)[1])

By E. C. Franklin and R. Z. Callaham[2])

Pacific Southwest Forest and Range Experiment Station, Forest Service, U. S. Department of Agriculture, Berkeley, California 94701

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Introduction

Environmental sources of variation in multinodality, branching, and forking have long been recognized (*cf.* Doak 1935; Stone and Stone 1943). Some earlier investigators postulated that inherent differences in these traits may exist among trees (*cf.* Shaw 1914; Downs 1949). Recent reports have shown this to be the case (Franklin 1965).

Multinodality, branching, and forking were studied in 6 wind- and 9 control-pollinated 6-year-old families of lodgepole pine (*Pinus contorta* var. *murrayana* Engelm.). The objectives were: (1) to observe the frequency and distribution of branch whorls in young trees; (2) to describe the branching and forking habits in young trees; and (3) to determine the relative importance of genetic, developmental, and environmental influences on multinodality, branching, and forking. Because of the relatively small number of families and the juvenility of the material, the results of the study can be applied only to the population actually measured.

Materials and Methods

Parent Trees

Six trees were selected to represent extreme and intermediate phenotypes in branching and forking characteristics. All grew in the Lake Tahoe Basin, at an elevation of 6,500 feet, near Meyers, California. There were two straight, unforked trees with light, flat-angled branches; one tree with three forks, and light, moderately flat-angled branches; two trees with four forks each, and moderately heavy flat-angled branches; and one tree with 10 forks, and extremely heavy, steep-angled branches. For more detailed information on the parent trees, see Franklin (1965), Table 1, pp. 11.

Breeding and Nursery Procedures

Breeding procedures followed those described by Cumming and Righter (1948). Of the 30 possible controlled crosses, all except selfs were attempted in 1957. Control- and wind-pollinated cones were collected in 1958. Sufficient numbers of seed for the study were obtained from nine of the 30 attempted controlled crosses.

Sowing was done in 1959 in the nursery at the U. S. Forest Service's Institute of Forest Genetics near Placerville,

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[1]) Based on a thesis submitted by the senior author in partial fulfillment of the Degree of Master of Science in Forestry at the University of California.

[2]) Currently Principal Plant Geneticist, Naval Stores and Timber Production Laboratory, Southeastern Forest Experiment Station, Forest Service, U. S. Department of Agriculture, Olustee, Florida; and Assistant to the Deputy Chief for Research, Forest Service, U. S. Department of Agriculture, Washington, D. C., respectively.

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