Variation in Samples of Two-year-old *Pinus attenuata*, *P. radiata* and their Hybrids

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Abstract

SAMPLES of two-year-old *Pinus attenuata* and *P. radiata* displayed many morphological differences, particularly in stature, branching, development of buds, foliage, and colour. Smaller, but still distinct, differences were noted between three samples of *P. attenuata* from widely separate places in California.

F_1 hybrids between the species were intermediate in most characters. Seed collected from a single putative hybrid in Ashley Forest, Canterbury, gave rise to a strongly segregated hybrid swarm.

Analysis by hybrid indices, using seven characters, effectively discriminated the two species, placed the F_1 hybrids half-way between the species, and suggested that the hybrid swarm was mainly the result of back-crossing to *P. radiata*.

INTRODUCTION

IN 1953 some small samples of seed were received from the Institute of Forest Genetics in California. Among them were collections from the following sources:—

(1) *Pinus attenuata* Lemmon. More than one tree at Ensenada, Baja California, Mexico.

(2) *P. attenuata*. A single tree at 4,370ft elevation, on the south slope of Mt. Shasta, about two miles NW of McCloud, California.

(3) *P. attenuata*. A single tree referred to as Eld-2-1, a few miles from the Institute of Forest Genetics, Placerville, California.

(4) *Pinus* × *attenuradiata* Stockwell and Righter. The result of artificial pollination of the one *P. attenuata* parent (Eld-2-1) with pollen from two *P. radiata* trees in the arboretum at Placerville.

During his visit to New Zealand in 1952, Dr. J. W. Duffield had noted in Ashley Forest, Canterbury, a tree which he regarded as a hybrid between *P. attenuata* and *P. radiata*, and seed from this tree was also received.

All five lots were sown in the spring of 1953. The seedlings did not attract attention at first, but in the winter of 1954 a striking contrast was noted between the *P. attenuata* from McCloud and those from Placerville. The foliage of the former was tinged a dull purple, the stems were all bent to one side near the ground, the tuft of apical leaves on each was inclined instead of erect, and the tips of these leaves were curved in the same direction; the whole appearance was lax. The P. *attenuata* from Placerville had erect stems, the foliage was greyish and only slightly coloured with purple, and the leaves were stiff and straight.

In the winter of 1955, the five lots of seedlings displayed such impressive differences that a morphological analysis seemed practicable. A sixth sample was added from adjacent *P. radiata* seedlings which had been grown from a Rotorua seed-lot under the same conditions.

The essential similarities and differences between the six samples are summarized in Table I, and representative individuals are illustrated in Plate 19. So distinct were the first five samples (i.e., all except the one from Ashley) that a competent observer, who was familiar with the differences between them, could have taken a thorough mixture of individuals and sorted them correctly into their five original groups.

TABLE I.—SHOWING GENERAL RESEMBLANCES AND DIFFERENCES BETWEEN YOUNG TREES FROM SIX DIFFERENT SEED-LOTS.

Taxonomic Status	*P. attenuata*			Interspecific F_1 Hybrids	*P. radiata*	Hybrid Swarm
Place of Origin.	ENSENADA	McCLOUD	PLACERVILLE	PLACERVILLE	ROTORUA	ASHLEY
Stems	Tall, rather crooked	Short, rather crooked	Short, rather crooked	Very tall, almost straight	Very tall, straight	Very variable
Branches (long shoots)	Medium-long	Long	Long	Medium-long	Short	Very variable
Young shoots	Yellow-brown to greenish, scarcely shining	Dark grey-brown, shining	Yellow-brown, shining	Greenish brown, rather shining	Green, not shining	Very variable
Density of dwarf-shoots	Sparse	Medium	Sparse	Dense	Dense	Very variable
Needle-leaves	Pale, dull yellow-green	Bluish, grey-green	Dull grey-green	Green	Dark green	Dark green to pale yellow-green
	Very lax	Stiff	Stiff	Lax	Rather lax	Lax, some very
	Very long, thick	Long, very thick	Long, thick	Very long, thick	Short, thin	Dimensions very variable
Scale-leaves	Brown	Brown, dark at edges	Brown, very dark at edges	Brown, dark at edges	Uniformly brown	Very variable
Purple colouring in winter	Weak	Very strong	Strong	Weak	Absent	Very variable
Stem apices	Leafy, or in imperfect buds	Mainly in buds	All in perfect buds; narrow, resinous	Mainly in buds; fat, rather soft	Leafy	Very variable

MORPHOLOGICAL ANALYSIS OF SINGLE CHARACTERS

Random samples of the six populations were taken during August. Fifty trees of *P. radiata* were lifted and recorded in the laboratory, taking care that they did not become dried and shrivelled. The other samples consisted of 10 trees each for the three *P. attenuata* lots, 10 for the F_1 hybrids, and 50 for the progeny from Ashley Forest. These trees were recorded in the nursery or in the laboratory just before they were planted out. The characters recorded were:

(1) *Stem Height*

This was measured from the level of the cotyledons to the tip of the bud or apical tuft of primary leaves. No attempt was made at statistical comparisons, but there were some obvious differences, as indicated in Table 1.

(2) *Number of Pseudo-whorls of Long Shoots on Stem*

This was usually four in all lots except the Mexican *P. attenuata,* most of which had three.

(3) *Number of Shoots in each Pseudo-whorl*

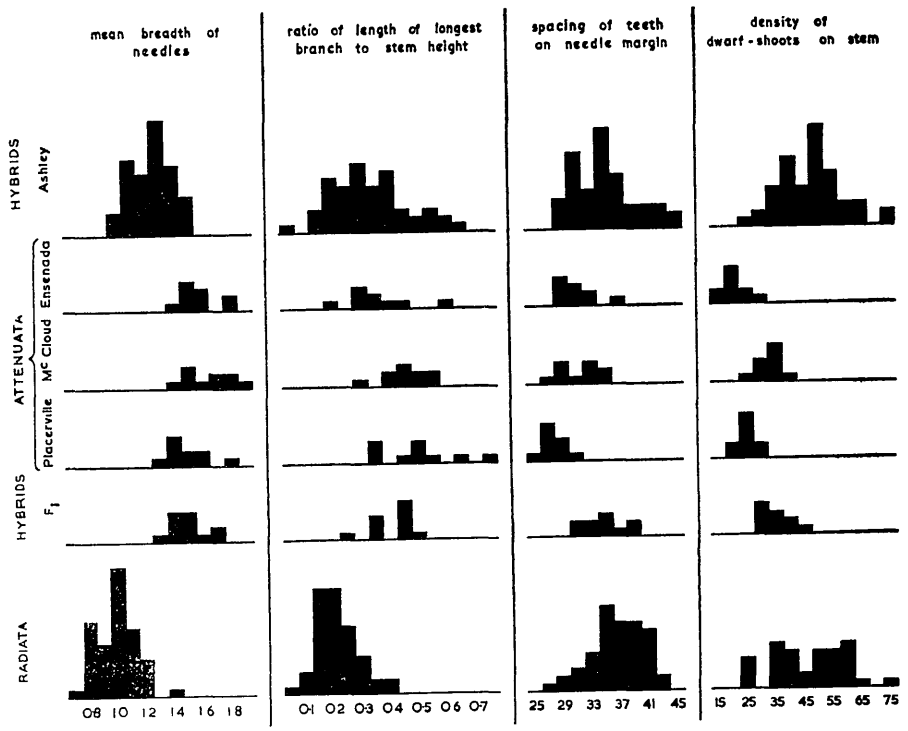
P. attenuata had, in general, rather fewer shoots in each pseudo-whorl than *P. radiata,* but the distinction was slight.

(4) *Length of Lateral Shoots in Relation to Stem Height*

By plotting the length of the longest lateral shoot against stem height for each tree, it was evident that the two species differed *P. attenuata* had relatively longer branches, although the Mexican lot differed little from *P. radiata.* The ratio *Length of longest branch/Height of stem* was therefore used as a branching index for each tree (Text-fig 1).

(5) *Proportion of Stem Bearing Dwarf-shoots*

The stem can be divided into alternating parts distinguished as those which bear dwarf-shoots and those which do not. By expressing the total length of the sections bearing dwarf-shoots as a percentage of stem height, a good contrast was found between the *P. attenuata* from Placerville and *P. radiata;* the F_1 hybrids were intermediate but resembled *P. radiata* the more closely of the two. When the other lots of *P. attenuata* were examined, however, such a distinction was no longer clear.



TEXT-FIG. 1.—Frequency distributions of four characters in samples of six different populations. Further details in text.

(6) *Density of Dwarf-shoots on Stem*

The number of dwarf-shoots on 10 cm of stem was counted for each tree (except 15 of the *P. radiata* sample). The count was made about mid-way between base and apex, and any sections devoid of dwarf-shoots were excluded. In a few cases only 7.5 cm of stem was suitable, and the count was scaled up accordingly. In *P. radiata* nearly every node of a series had an axillary dwarf-shoot, but in *P. attenuata* there were many such nodes without visible axillary structures. It was this, rather than a difference in the length of the internodes, which resulted in the general contrast between the dense foliage of one species and the sparse foliage of the other. (Text-fig. 1.)

(7) *Primary Leaves*

At first, the mean length and mean breadth of 10 leaves per tree were calculated. The Mexican *P. attenuata* appeared to have longer and broader leaves than any of the other samples, but the dimensions were excessively variable and failed to distinguish the species.

(8) *Scale-leaves*

These, the scarious homologues of the primary leaves, were usually associated with apical buds, but were also found in places further back on the stem. They were not suitable for measurement, and were not found on every tree, but they did show a sharp contrast in colour between the Placerville *P. attenuata* and the *P. radiata*. In the former, as well as in some mature specimens of *P. attenuata* in New Zealand, they were dark brown, especially at the margin and apex, which were sometimes almost black. In *P. radiata* they were paler brown and more uniformly coloured. This specific difference seemed not so strong for the McCloud sample and was even weaker for the Mexican one. The progeny from Ashley Forest showed a complete range between the extremes; there were more individuals like *P. radiata* than those like *P. attenuata*, but most were intermediate.

(9) *Number of Needle-leaves in Ten Fascicles*

For this and all the other characters of the needles, samples of 10 fascicles each were removed from the stem of each tree, taking reasonable care that each sample was representative of the cauline foliage as a whole. Samples from some trees included one or more immature fascicles; other samples contained only mature fascicles. Although it might have been better to restrict the sampling to, say, the middle third of the stem, the method proved satisfactory.

P. radiata often had 3 needles to a fascicle, very often 4, and sometimes 5 or more; P. *attenuata* had usually 3, and sometimes 4; the F_1 hybrids were intermediate; the Ashley hybrids were also intermediate but more like *P. attenuata*.

(10) *Mean Length of Ten Needles*

The ranges and means (in cm) for this character in the six samples were:—

P. radiata	7.8 – 10.2 – 12.2
P. attenuata (Placerville)	9 3 – 10 9 – 12.4
P. attenuata (McCloud)	9.1 – 11.0 – 12.6
P. attenuata (Ensenada)	11.0 – 12.5 – 13.4
P. × *attenuradiata* (F_1)	11.2 – 12.4 – 14 0
Hybrids (Ashley Forest)	8.8 – 11 1 – 13.9

This indicates that the *P. attenuata*, especially the Ensenada lot, had longer needles than P. *radiata*. The F_1 hybrids had very long needles; this was perhaps a symptom of heterosis

(11) *Mean Breadth of Ten Needles*

The greatest breadth of one needle in each fascicle was measured to the nearest 0 1 mm. This character was somewhat correlated with needle length, as one might expect, but it was the more efficient of the two for discriminating the species. (Text-fig. 1.)

(12) *Spacing of Teeth on Needle Margins*

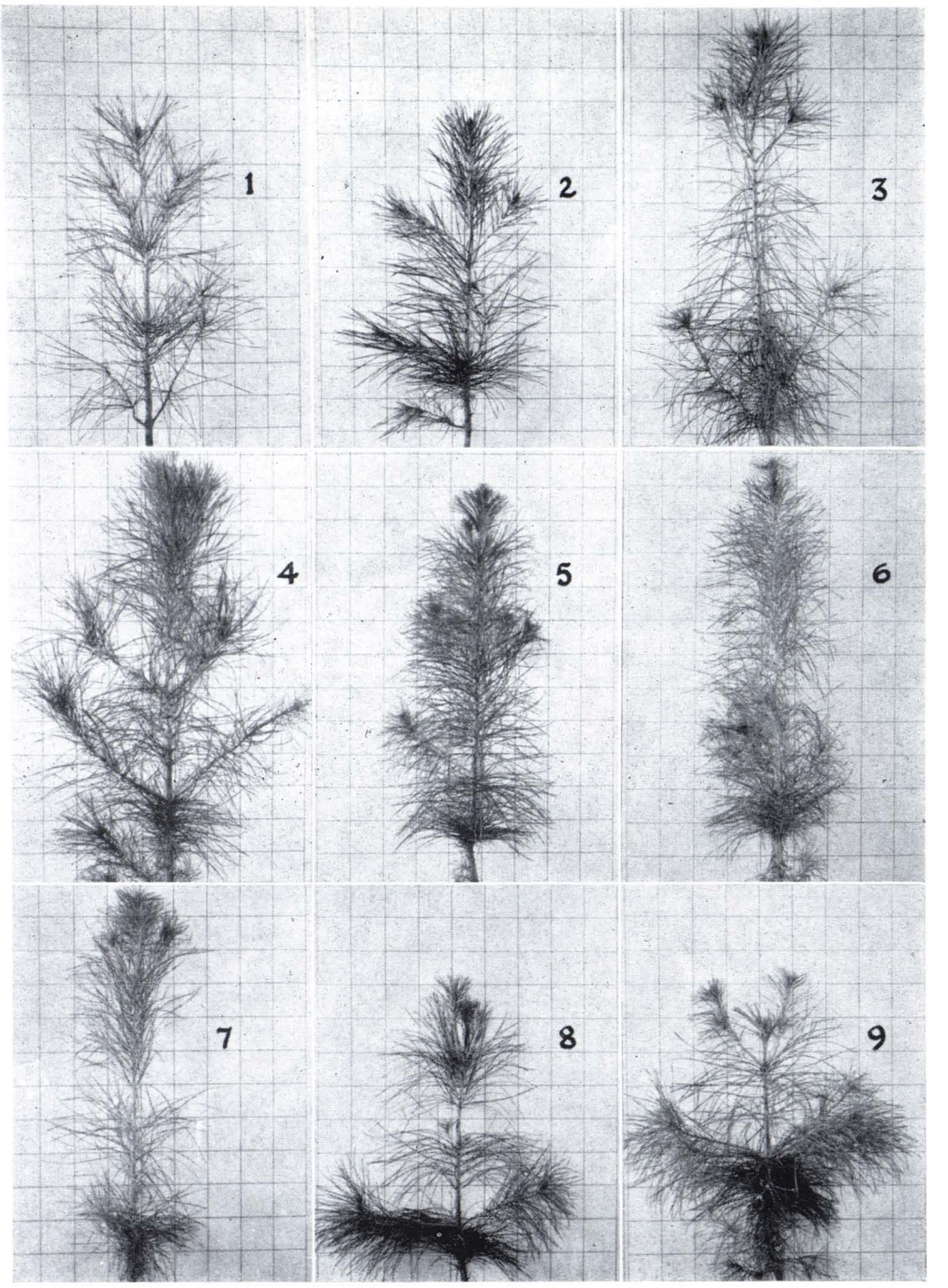
The central part of one needle of each fascicle was laid on the stage of a binocular microscope at low magnification, with one margin occupying a diameter of the field (1 12 cm). In this way counts of marginal teeth were made, and the mean calculated, of 10 needles from each tree. *P attenuata* had more widely spaced teeth than *P. radiata* (Text-fig 1). Scatter diagrams of tooth counts plotted against needle length suggested that within each species the two characters were quite independent of one another.

(13) *Colour and Appearance of Young Shoots*

For this rather composite character, individuals were scored in subjective grades according to their position in a scale ranging from "pale green, not shining" (typical *P. radiata*) to "yellow-brown, shining" (extreme *P. attenuata*) The contrast between *P. radiata* and the *P. attenuata* from McCloud and Placerville was strong, and the F_1 hybrids were intermediate The Mexican *P. attenuata*, however, was intermediate or like *P. radiata* The hybrids from Ashley Forest were mostly intermediate, but varied more towards "green, not shining" than towards the other extreme

(14) *Winter Colouring of Foliage*

This was an outstanding character, because the foliage of *P. radiata* was entirely and invariably green, whereas in every other lot there was some degree of purple tingeing. This colour appeared in autumn and disappeared in spring. It was strongest distally and usually faded out proximally in relation to individual leaves and to the shoots. From a distance, in combina-



Two-year-old individuals showing some of the differences between six populations. The background is ruled in 5 cm squares. FIGS. 1–3—*Pinus attenuata* (1, from Placerville; 2, from McCloud; 3, from Ensenada). FIG. 4—An F_1 hybrid, *P. attenuata* x *P. radiata*. FIG. 5—*P. radiata* from Rotorua. FIGS. 6-9—Four hybrids, showing extreme segregation towards *P. radiata* (6, 7) and *P. attenuata* (8, 9).

Photos: W. J. Wilson, N.Z. Forest Service.

TABLE II.—SHOWING METHOD OF ALLOTTING VALUES TO SEVEN CHARACTERS FOR CALCULATING HYBRID INDICES.

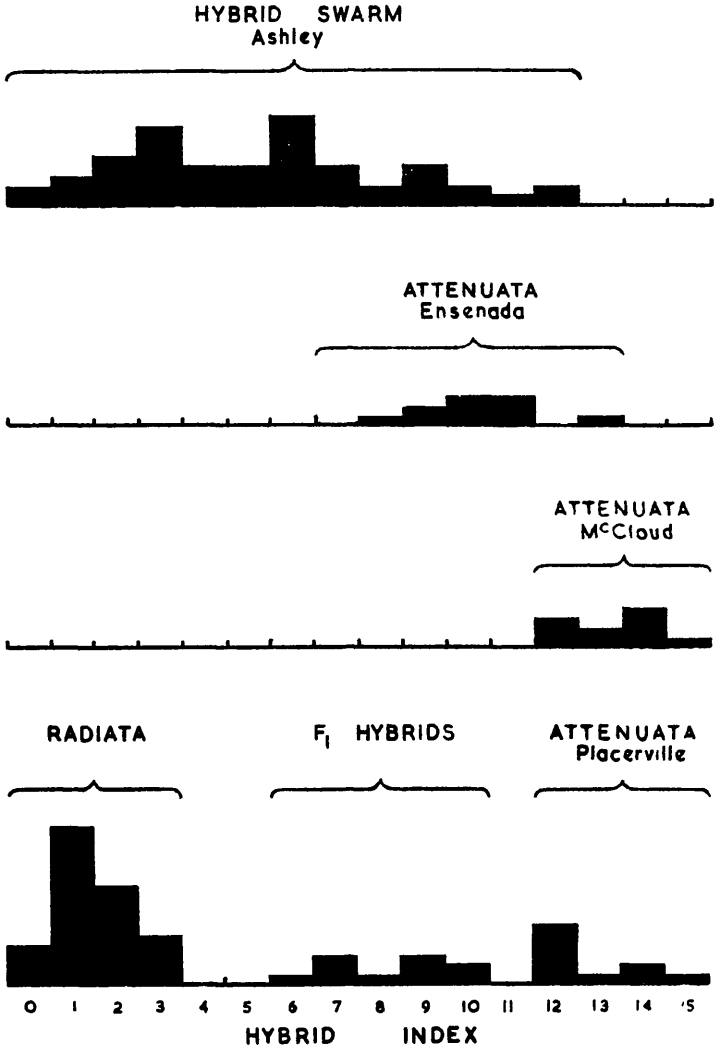
Character.	Index Value.			
	0	1	2	3
Purple colouring in winter	Absent	Faint to moderate	Strong, conspicuous	—
Development of bud on stem apex	None	Imperfect, scales loosely imbricated	Perfect	—
Mean needle breadth (mm)	0.6–1.1	1.2	1.3	1.4–1.8
Ratio of length longest branch: stem height	0.05–0.27	0.28–0.37	0.38–0.75	—
No. of dwarf-shoots on 10 cm of stem	41–80	31–40	10–30	—
Mean number of teeth on 1.12 cm of needle margin	35–45	32–34	25–31	—
Appearance of young shoots	Pale green, not shining	Intermediate	Brown, shining	—

tion with the greyish green of some *P. attenuata,* it resulted in a dull, bronze-like brown. It was strongest in the lot from McCloud; the tops of these were a distinctly reddish purple hue. In the hybrid swarm it showed most vividly against some of the pale green tufts of primary leaves, and ranged from the merest tinge of pale mauve to strong purple.

Some individuals must have been less exposed to cold than others, and this might account for some of the individual variation within each lot of *P. attenuata* or hybrids. Nevertheless, the difference between the species was striking. and the presence of purple in the hybrid lots can probably be ascribed to *P. attenuata* genes.

(15) *Degree of Bud Formation*

Three subjective grades were used. In *P. radiata* the apices were mostly surmounted by tufts of pale green primary leaves, and only a few showed the earliest stages of transition from this to the brown, imbricated scale-leaves of a fully developed bud The *P attenuata* from Placerville all had compact, narrow, cylindrical buds, whitened by encrusting resin The F_1 hybrids all had terminal buds more or less developed, but these were fatter, less compact, and had scarcely any exuded resin. The McCloud lot had similar buds, but not on every tree The Mexican lot showed very little bud formation, resembling *P. radiata* rather closely The hybrid swarm had all degrees of bud formation, but the largest single category was the one coinciding with *P. radiata.*



TEXT-FIG. 2 —Frequency distributions of hybrid indices, calculated from seven characters, for samples of six different populations. (Table II contains details.)

ANALYSIS BY HYBRID INDICES

The characters chosen, and the method of allotting index values to them, are set out in Table II. The final results of the analysis are shown in Text-fig. 2.

From this it is apparent that the characters used did, in the aggregate, discriminate the two species well enough, particularly if the Mexican *P. attenuata* sample was excluded. They also placed the F_1 sample in the intermediate position which was expected in theory.

Considering the hybrids from Ashley Forest, it may perhaps be assumed that their original *P. attenuata* parent was of Californian, not Mexican, lineage. If this be so, and if the known parent was an F_1 hybrid, the frequency distribution suggests that most of them were the result of back-crossing to *P. radiata,* which was plentiful nearby. At the same time, the tail of the histogram extending towards the *P. attenuata* end of the scale suggests that one or more of the following also occurred:

(a) back-crossing to *P. attenuata,*
(b) selfing,
(c) pollination by one or more other hybrids.

Of these possibilities, the first can almost certainly be ruled out, because, as far as is known, *P. attenuata* never existed anywhere near the known parent. The others must be accepted, because there is no known intrinsic barrier to selfing, and because there is good evidence of other hybrids in the vicinity

On the other hand, if the known parent was a hybrid of a later generation, e.g., an F_2, and such that genes from *P. attenuata* outnumbered those from *P. radiata* in its genotype, its progeny might perhaps all have arisen from back-crossing to *P. radiata.* From a study of the phenotype and circumstances of the known parent, it was concluded that this latter interpretation was nearer the truth. Even so, some of the effective pollen grains may have been of hybrid origin.

CHARACTER-COMBINATIONS IN THE HYBRID SWARM

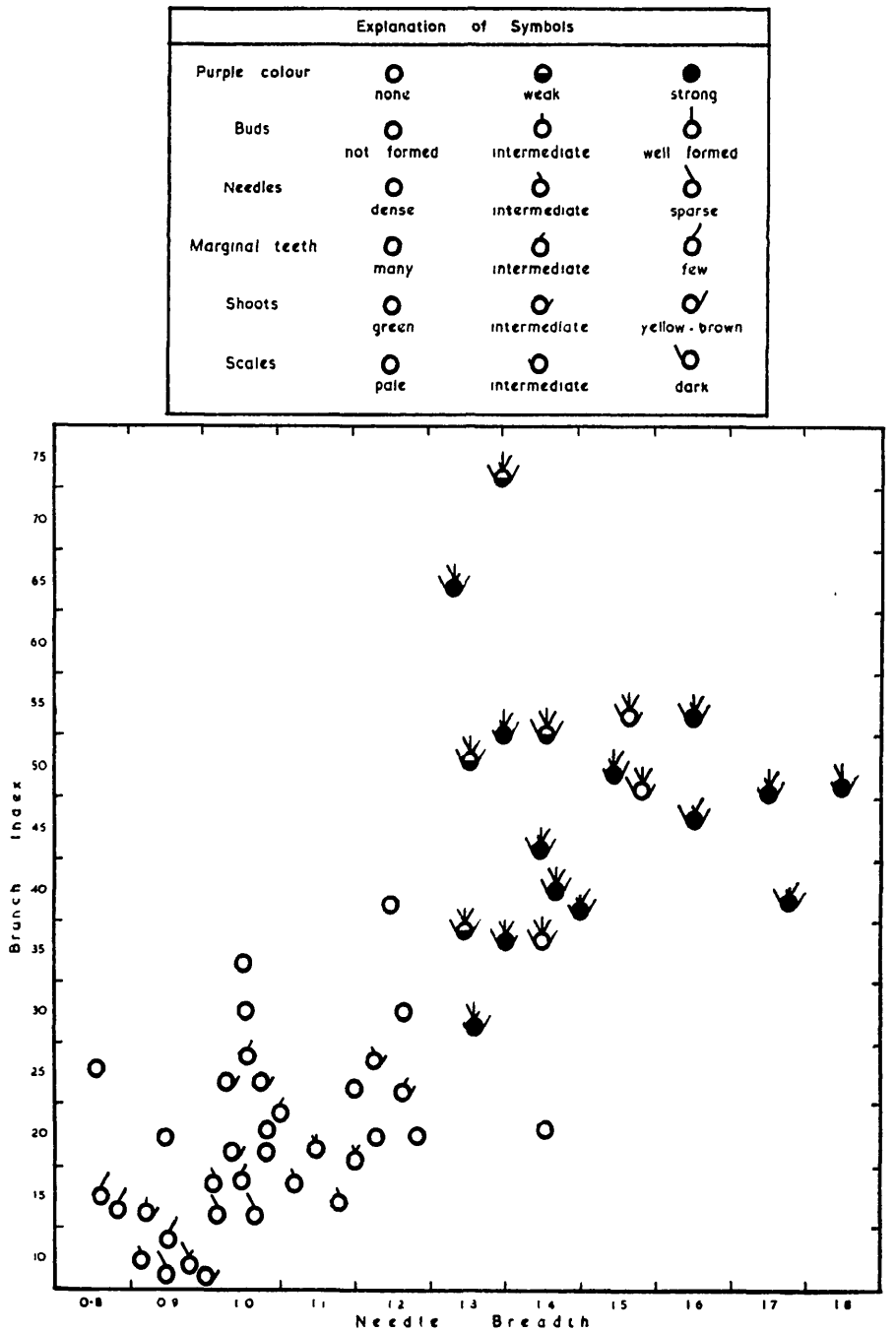
The new combinations of characters in the progeny from Ashley Forest were perhaps more diverse than might have been expected, but a general trend from the *P. radiata* complex towards the *P. attenuata* complex was discernible (Text-figs. 3 and 4).

By the principle of extrapolated correlates (Anderson, 1949: 92–101) one may visualize what the original *P. attenuata* parent would have been like at the same age, and under the same conditions of cultivation, as the others. It is pictured as similar to those shown in Plate 19, Figs. 8 and 9, with its foliage yellowish green and strongly tinged with purple, its young shoots yellow-brown and shining, its scale-leaves very dark-edged, and its stem apex encased in a perfect bud. The individual thus conceived would have clear affinities to the samples from Placerville and McCloud, but its foliage would be like that of the Ensenada sample. It would differ from all three in its very long and patent branches.

DISCUSSION

This study has its weaknesses, the worst of which probably lie in the choice of certain characters for the polygraphic analysis and for the analysis by hybrid indices. Nevertheless, it is presented for the following reasons:

(1) It shows that the application of these analytical techniques has been extended to unusual material, inasmuch as two-year-old trees are extremely young and show only some of the vegetative characters, and none of the reproductive characters, of maturity.



TEXT-FIG. 3.—Scatter-diagram of 35 individuals of *P. radiata* and 20 of *P. attenuata* (10 from Placerville and 10 from McCloud) The *P. radiata* sample forms the lower left-hand cluster; the *P attenuata* sample forms the upper right-hand cluster.

(2) It supports the recent claim by Anderson (1954. 100–01) that these methods can give good results with small samples.

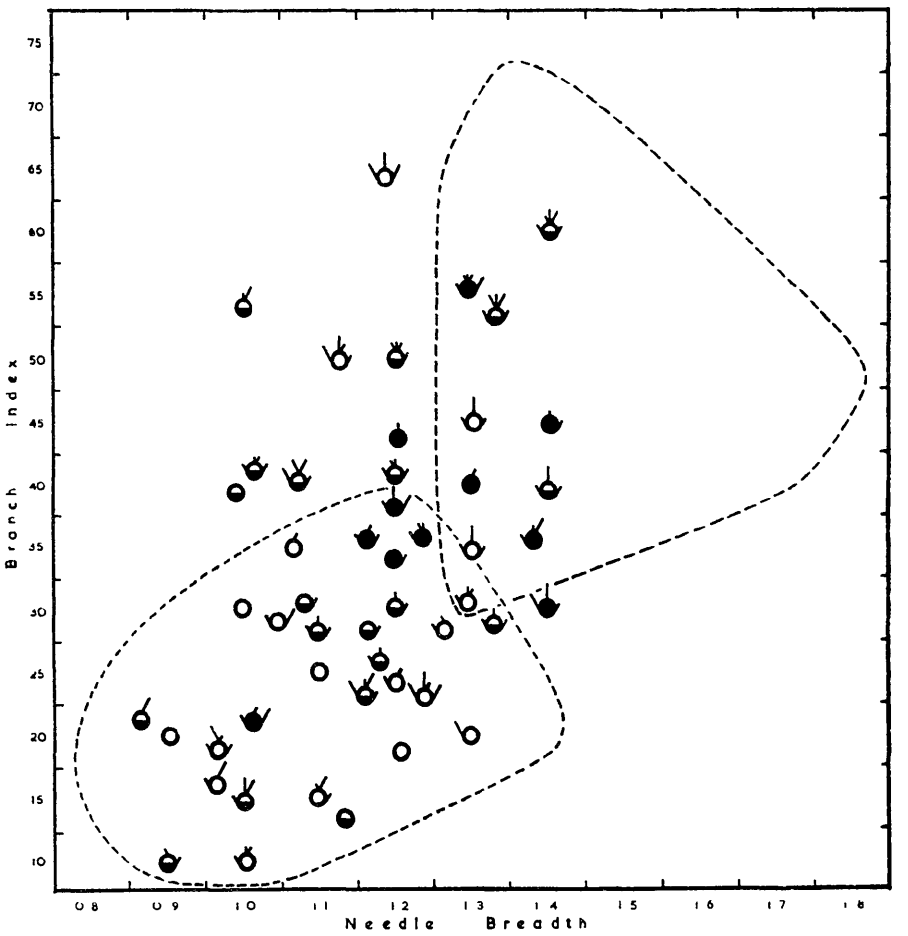
(3) It shows that differences between *P. attenuata* and *P. radiata* are manifested at a very early age.

(4) It affords satisfactory evidence that the progeny of the Ashley tree consisted of a swarm of hybrids between *P. attenuata* and *P. radiata.*

(5) It suggests that *P. attenuata,* in its natural state, probably has a morphological and genetic variation which is correlated with its wide territorial distribution. A knowledge of this variation might one day have an important application in the culture of *P. radiata.*

ACKNOWLEDGMENTS

It is a pleasure to record the kindness of Dr. J. W. Duffield, who sent the seeds from California, and of Dr. F. I Righter, who supplied more information about their origin.



TEXT-FIG 4.—Scatter-diagram of 50 individuals, which resulted from open pollination of a single hybrid The symbols were constructed in the same way as those in Text-fig 3. The dotted lines represent the limits of the samples shown in Text-fig. 3.

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