Recently, we have begun experiments with Cosmos bipinnatus which is known to have a sporophytic incompatibility system (Crowe 1954) with most individuals being self incompatible, and have achieved considerable success in using recognition pollen from compatible plants to carry out self-pollinations. Since Pinus species are known to have extracellular pollen-wall proteins (Knox and Heslop-Harrison, 1970) the use of this technique needs investigation in Pinus where incompatibility systems exist, and large pollen samples can be readily obtained.

### Acknowledgements

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### Summary

The recognition pollen method is described, and has enabled successful hybridization of the black poplar, P. deltoides and the white poplar, P. alba. In these species, hybridization is normally prevented by incompatibility barriers. For use, the *recognition* pollen (killed pollen of the compatible type produced by freeze-thawing, gamma-irradiation or by treatment with methanol or ether) is mixed with fresh incompatible pollen and the mix dusted on the stigmas. All the progeny were hybrid. The determinative role played by pollen grain recognition substances in the control of interspecific incompatibility reactions on the stigma is demonstrated by these experiments. The *recognition* pollen method appears to have widespread applications in plant improvement.

### Zusammenfassung

Es wird eine Hilfspollen-Methode beschrieben, die eine erfolgreiche Kreuzung von Populus deltoides und P. alba ermöglichte. Beide Arten sind normalerweise unverträglich. Hilfspollen war auf verschiedene Weise getöteter Pollen der Art, die als Mutter benutzt werden sollte. Dieser wurde zur Bestäubung mit dem sonst unverträglichen Pollen der anderen Art 1 : 1 gemischt. Alle danach entstandenen Nachkommen waren Bastarde. Von dem toten Hilfspollen abgegebene Stoffe an die Narben müssen diese Kreuzungen zum Erfolg geführt haben.

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# Multivariate analysis of variation in needles among provenances of Pinus kesiya Royle ex Gordon (syn. P. khasya Royle; P. insularis Endlicher)

By Jeffery Burley[1]) and Peter M. Burrows[2])

(Received March 1971)

### Introduction

Pinus kesiya Royle ex Gordon (syn. P. khasya Royle; P. insularis Endlicher) is currently the major exotic species used for afforestation in Zambia (Cooling and Endean, 1966; Jones, 1968), and it is important or promising for many other tropical countries. It is high on the list of priorities for action by the FAO Panel of Experts on Forest Gene Resources and an international provenance trial is in progress with 19 seed lots collected in the Philippines by Turnbull[3]) during 1969 (Burley and Turnbull, 1970). It is a wide-ranging species in southeast Asia occurring naturally from Assam, where it is commonly called P. khasya, to the Philippines, where it is known as P. insularis (Figure 1). Limited evidence from studies in the natural habitat and in exotic plantations suggests that these are geographic races of the same species.

The nomenclatural situation is confused because various recognised authorities have adopted different names, e. g. P. khasya (Dallimore and Jackson, 1948); P. *kesiya* (Dallimore, Jackson and Harrison, 1966). Until an authoritative ruling has been given on the historical priority, these two names must be accepted as equivalent (see Burley, 1971).

The present study was designed to examine further the relationships among several provenances of this species, grown as exotics under relatively uniform conditions, using anatomical and morphological characteristics of needles

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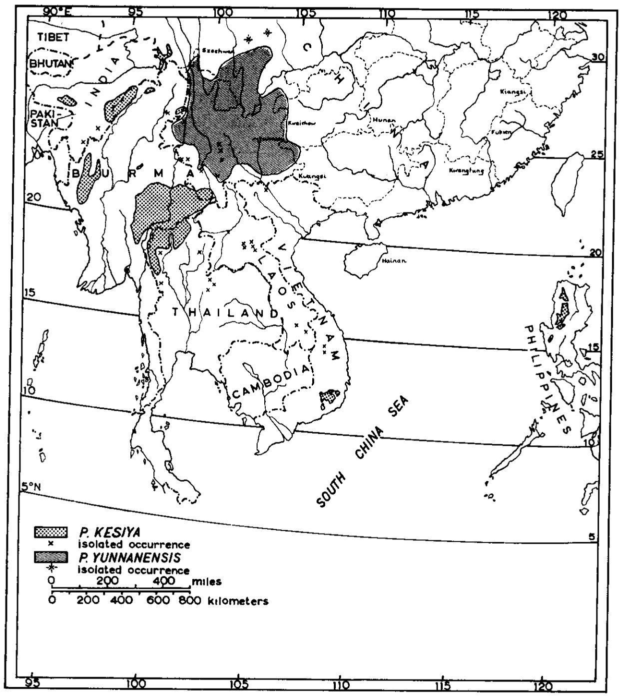

*Figure 1.* — Natural distribution of *P. kesiya* (modified by Shelbourne from Critchfield and Little, 1966). C. J. A. Shelbourne, Forest Research Institute, Rotorua, New Zealand.

that have been demonstrated to vary with provenance in other species. It was also intended to examine the potential of canonical variate analysis for provenance studies.

*P. kesiya* is generally considered to be a three-needled pine throughout its range (Champsoloix, 1958; Din, 1958; Weck, 1958; Premrasmi and Smitinand, 1960; Dallimore, Jackson and Harrison, 1966). Gaussen (1960) found occasional two-needled fascicles but there have been few studies of other needle characteristics. Savory (1962) examined seven traits in material from several African trial plots of Assam and Philippines origin; there were no important differences between the sources in number of needles per fascicle (predominantly 3, with some 2 and 4), length (12—20 mm) and colour of sheath, type of marginal serration, length of mature needles (15—23 cm), and the dimensions of the vascular bundle. The volatile constituents of oil extracted from needles of Assam and Philippines provenance grown in Zambia did exhibit qualitative differences but these were not considered large enough to separate two species.

Oils in needles do not necessarily have the same composition as oleoresin in stems but Zavarin, Mirov, Cooling, Snajberk and Costello (1968), using oleoresin from trees of four provenances grown in Zambia, also considered the Philippines material *(P. insularis)* "decidedly a part of the southern strain of *P. khasya*".

Preliminary work by Burley, Burrows and Waters (1967) indicated that the number of needles per fascicle (predominantly 3, some 2 and 4) did not vary significantly between 11 provenances grown in Zambia; length of needles was affected by site and by provenance independently with a range of 17.2—20.2 cm. Such small differences did not permit the identification of two seed lots of imperfectly known source. This suggested the use of multivariate techniques which form a main subject of the present report.

Knowledge of variation in morphology and anatomy of needles is desirable for several complementary reasons. It is desirable to determine the relative contributions of heredity, internal environment (physiology), and external environment (site), to this variation. It would be extremely valuable to construct a discriminant function to facilitate the identification of unknown or questionable provenances. If genetic differentiation has occurred between natural populations it is desirable to relate this to the variability in environmental factors that occurs in the natural range. Further, it is desirable to relate patterns of variation in morphological traits to those in productive characteristics.

Characteristics of needles that are controlled by one or two genes may be useful as diagnostic indicators in genetic studies. They can provide 'markers' for studies of inbreeding in seed orchards or of the degree of isolation of natural populations. Characteristics that are essentially metric traits (quantitative information) may be useful as indicators of health of trees (site fertility and nutrient deficiencies, pathological conditions); often their value is small in genetic studies because of their polygenic inheritance and apparently low heritability.

In spite of this, both qualitative and quantitative characteristics have been and continue to be important in taxonomic sudies. However, except in some numerical taxonomic studies that have been primarily phenetic in nature, the sample sizes required to cope with the large within-tree and between-tree variability of quantitative traits are entirely a function of the taxonomist's arbitrary specification of the differences he requires between taxa. This approach, which is the basis of the present research, is completely utilitarian; it is a method of labelling source populations unequivocally but without necessarily indicating their phylogenetic relationships.

## Materials and Methods

In December, 1963, staff of the Zambia Forest Research Division planted a trial of 11 provenances on two site types at Chati and Chichele Forest Reserves, some 30 miles west and east of Kitwe respectively. Details of the seed sources are given in *Table 1*. The collection of many of the seed lots was undertaken or arranged by Cooling[4] but two seed lots (provenance codes 7 and 11) were supplied commercially and no details of the source locations were available. Seed for "provenance" 11 was collected from plantations in Madagascar but the original source was believed to be Viet Nam. The field design on both sites comprised five

*Table 1.* — Details of seed sources for 11 provenances.

| Provenance code | Origin | Altitude (ft) | Latitude (°N) | Longitude (°E) |
|---|---|---|---|---|
| 1 | Line 5, Boboc,<br>Mountain Prov.Philippines | 5-6,000 | 16½ | 121 |
| 2 | Line 14, Stn. 1. Boboc,<br>Mountain Prov. Philippines | 6,600 | 16½ | 121 |
| 3 | Between Line 4 & Kayapa,<br>Boboc, Mountain Prov.<br>Philippines | 5,500 | 16½ | 121 |
| 4 | Line 7, Boboc<br>Mountain Prov. Philippines | 6-7,000 | 16½ | 121 |
| 5 | Daclan, Bokod,<br>Mountain Prov. Philippines | 6-7,000 | 16½ | 121 |
| 6 | Cato, nr. Masinloc,<br>Zambales Prov. Philippines | 2,000 | 15¼ | 120¼ |
| 7 * | Thailand (no other details) | ? | 12½-<br>20 | 96-<br>110 |
| 8 | Dalat, Viet Nam | approx.<br>5,000 | 12 | 108½ |
| 9 | Shillong, Assam | 3-6,000 | 25½ | 92 |
| 10 | Kalaw Township, Southern Shan States, Burma | 3,500 | 20½ | 96 |
| 11 ** | Fianarantsoa, Madagascar | 5,000 | 21½°S | 46 |

* Probably latitude 17½°, longitude 102°

** The original source of this material is not known precisely but it was believed to be Viet Nam with details identical to 8.

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[4] E. N. G. Cooling, formerly Chief Forest Research Officer, Zambia.

replications of all provenances in complete randomised blocks with inner assessment plots of 49 trees.

A preliminary sampling study indicated that the major part of variation due to random effects was attributable to differences between individual trees within plots with little attributable to differences between branches in whorls. Within a branch there were considerable differences in characteristics of needles between successive years' growth flushes, but the effect of flush was considered a fixed effect and only one annual flush was examined in the main study. Similarly only one whorl of branches was examined although there were important differences between whorls (taken as fixed effects).

In the main study three replications were selected randomly at each site during four consecutive days in May, 1966, when the trees were 2½ years old and not exceeding 10 ft in height. Within each plot three trees were selected at random; on each tree one branch was chosen randomly from the whorl nearest half height. The segment of branch corresponding to the previous year's flush of growth in each branch was removed and stored in a plastic bag until the number and length of needles were determined. Ten fascicles per branch were then stored in formalin — acetic acid—alcohol fixative. This sampling procedure was calculated to provide adequate precision for estimation of means of provenances in all traits; provenances were therefore not sampled differentially within the provenance trial. The parental materials for the provenance trial were not likely to be proportional representatives of the natural variability, but the original seed was collected from large numbers of trees in natural stands or, in the case of provenance 11, from a Madagascar plantation.

The average length of the needles in each fascicle was measured to the nearest millimetre but fascicle sheaths were too frayed to permit measurement.

A single segment 5 ± 0.01 mm long was cut from the centre of the longest needle in each of the five fascicles and subjected to the following examinations under a dissecting microscope, using reflected light.

The width of needles was determined with a stage micrometer measuring in multiples of 0.154 mm. The number of rows of stomates was recorded for the abaxial surface and incomplete rows were accorded full value. The numbers of stomates were determined in the two outer complete rows on one side of the segment; incomplete rows were rejected and the next interior complete row was utilised. Serrations were counted along one side of the segment.

Thin transverse sections were cut with a hand razor and the following features examined under a microscope using transmitted light. Resin canals and vascular bundles were counted on a section from each end of the segment. If the numbers disagreed further sections were evaluated.

## Analyses, Results and Discussion

### *(a) Single variate analysis*

*Length and number of needles: —*

Data were available for the following array of samples: — 2 sites × 11 provenances × 3 replications × 3 trees × 2 branches × 10 fascicles. The following variates were analysed on the basis of branch means with a total of 395 degrees of freedom: —

1. Mean number of needles per fascicle.
2. Percentage of fascicles with three needles.
3. Arcsin transformation of variate 2.
4. Product of mean number of needles per fascicle and mean length of needles per fascicle = needle quantity index.
5. Logarithmic transformation of variance in length of needles between 10 fascicles within each branch.

A sixth variate, length of needles, was analysed on the basis of single fascicles with 3959 degrees of freedom. Variate 4 was included to examine whether the variation in number of needles was balanced by variation in length of needles, i. e. whether or not mean "needle quantity" varied between provenances. Variate 5 was analysed to provide a test of homogeneity of variance in length of needles.

The underlying linear model is represented by

$$y_{ijklmn} = \mu + p_i + s_j + ps_{ij} + r_{jk} + pr_{ijk} + t_{ijkl} + b_{ijklm} + f_{ijklmn}$$

where

$y$ = measured value on the nth fascicle on the mth branch in the lth tree in the kth replication at the jth site for provenance i
$\mu$ = general mean
$p_i$ = effect of the ith provenance, with fixed effect and variance component represented by $\phi_p$
$s_j$ = effect of the jth site, with fixed effect and variance component represented by $\phi_s$
$ps_{ij}$ = interaction effect of the ith provenance and jth site, with fixed effect and variance component represented by $\phi_{ps}$
$r_{jk}$ = effect of kth replicate in jth site, with random effect and variance component represented by $V_r$

Analogously

$pr_{ijk}$ = provenance × replication in site effect; $V_{pr}$
$t_{ijkl}$ = tree in plot effect; $V_t$
$b_{ijklm}$ = branch in tree effect; $V_b$
$f$ = fascicle in branch effect; $V_f$

The mean squares, provenance means and standard errors for six traits, and the expectation mean squares, are given in *Table 2*.

Mean number of needles (variate 1) did not vary significantly among provenances. The predominant number of needles per fascicle was 3, supporting earlier observations on this species in its natural range, as cited above. Two-needled and four-needled fascicles have been reported (Gaussen, 1960; Savory, 1962) and in the present material 1.5% of the fascicles examined were two-needled and 2.0% were four-needled. The significant effects attributed to site and individual trees for variates 1 and 2 were largely due to one tree in a plot of Philippines provenance at Chichele in which 15 out of 20 fascicles were four-needled. Approximately one third of the trees studied were exclusively three-needled.

Variance in length of needles (variate 5) was homogeneous over all sites and provenances but mean length of needles (variate 6) was affected by sites and provenances independently. At Chati (the more favourable site) mean length of needles was 20.42 cm compared with 16.41 cm at Chichele (standard error of difference ±0.38 cm). Duncan multiple range tests of provenance means indicated that material from Madagascar had significantly longer needles than all but the Burmese stock.

Significant effects were attributed to individual trees for all traits and to branches and fascicles for length of needles. Within plots 41% of the variation in length of needles was attributed to trees, 26% to fascicles, and 33% to branches. This emphasises the necessity of adequate samp-

Table 2. — Mean squares and provenance means for six morphological characteristics.

| Entry | Source of variation | d.f. | Mean squares for Variate 1 | 2 | 3 | 4 | 5 | 6 | Expectation mean squares | Test against entry |
|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Provenances | 10 | 0.01052 | 165.15 | 160.45 | 205.19 ** | 0.6380 | 223.810 ** | $Ve + 10 V_b + 20 V_t + 60 V_{pr} + 360 \emptyset_p$ | 5 |
| 2 | Sites | 1 | 0.04050 * | 64.70 | 82.40 | 13581.70 *** | 1.2847 | 15870.900 *** | $Ve + 10 V_b + 20 V_t + 660 V_r + 1980 \emptyset_s$ | 4 |
| 3 | Provenances x Sites | 10 | 0.01263 | 145.77 | 204.01 | 107.77 | 1.2974 | 100.260 | $Ve + 10 V_b + 20 V_t + 60 V_{pr} + 180 \emptyset_{ps}$ | 5 |
| 4 | Replications in Sites | 4 | 0.00312 | 127.78 | 181.83 | 50.20 | 6.2294 *** | 50.500 | $Ve + 10 V_b + 20 V_t + 660 V_r$ | 6 |
| 5 | Provs x Reps in Sites | 40 | 0.02304 | 183.35 | 201.58 | 71.89 | 1.0329 | 71.435 | $Ve + 10 V_b + 20 V_t + 60 V_{pr}$ | 6 |
| 6 | Trees in plots | 132 | 0.02095 *** | 183.33 *** | 187.11 *** | 120.08 *** | 1.2238 *** | 113.911 *** | $Ve + 10 V_b + 20 V_t$ | 7 |
| 7 | Branches in trees | 198 | 0.00949 | 83.84 | 91.57 | 30.39 | 0.6838 | 25.912 *** | $Ve + 10 V_b$ | 8 |
| 8 | Fascicles in branches | 3564 | - | - | - | - | - | 1.712 | Ve | |
| Grand Mean | | | 3.01 | 95.45 | 84.17 | 55.52 | 0.040 | 18.42 | | |
| Provenance Code | | | Provenance means (average over both sites) | | | | | | | |
| 1 | | | 3.00 | 98.06 | 86.42 | 51.58 | -0.109 | 17.17 | | |
| 2 | | | 3.00 | 94.44 | 82.73 | 55.14 | +0.171 | 18.36 | | |
| 3 | | | 3.00 | 97.78 | 86.19 | 54.53 | -0.066 | 18.17 | | |
| 4 | | | 3.03 | 94.44 | 82.23 | 54.69 | -0.200 | 18.04 | | |
| 5 | | | 3.01 | 98.06 | 87.03 | 56.18 | +0.106 | 18.68 | | |
| 6 | | | 3.03 | 94.44 | 84.39 | 54.95 | +0.247 | 18.15 | | |
| 7 | | | 3.01 | 97.78 | 86.48 | 54.10 | +0.078 | 17.98 | | |
| 8 | | | 3.05 | 93.61 | 82.23 | 56.65 | +0.170 | 18.61 | | |
| 9 | | | 3.00 | 95.00 | 83.87 | 54.02 | +0.003 | 18.00 | | |
| 10 | | | 3.03 | 91.67 | 80.72 | 58.23 | +0.049 | 19.22 | | |
| 11 | | | 2.99 | 94.72 | 83.56 | 60.62 | -0.008 | 20.23 | | |
| S.E./provenance mean (±) | | | 0.025 | 2.26 | 2.36 | 1.41 | 0.169 | 1.17 | | |

*** Significant at 0.001 probability level
** Significant at 0.01 probability level
* Significant at 0.05 probability level

Variate 1 = number of needles per fascicle
Variate 2 = % 3-needled fascicles
Variate 3 = arcsin transformation of % 3-needled fascicles
Variate 4 = needle quantity index
Variate 5 = longarithmic transformation of variance in needle length
Variate 6 = length of needles

ling within and between trees when attempting to characterise provenances on the basis of these characteristics.

Because there were no real differences in number of needles, the pattern of variation in "needle quantity index" (variate 4) followed that of length of needles with significantly greater values at Chati. The Madagascar material had a significantly larger quantity of foliar material than other provenances.

Length of needles is clearly influenced most strongly by environment. The small differences between provenances in this and in number of needles do not assist in pin-pointing the sources of provenances 7 (Thailand) or 11 (Madagascar). The extreme values observed for the Madagascar material may indicate that environmental preconditioning occurred during seed development in Madagascar or, more likely, that this population was genetically different because of selection during thinning or because of isolation in the original source (believed to be Viet Nam).

*Needle anatomy: —*

The following variates were measured on one needle from each of five fascicles per branch: —

- A. Width of needles, recorded in microscope units which were multiples of 0.154 mm
- B. Number of rows of stomates
- C. Number of stomates per 5 mm length of row, recorded for two rows separately
- D. Number of serrations per 5 mm length of needle, counted on one edge
- E. Number of resin canals
- F. Number of vascular bundles

Variate E was almost constant at two resin canals per needle. The number of vascular bundles (variate F) was equally distributed between one and two, not fixed at one as stated by Burley, Pattinson and Waters (1966). This species belongs to the Diploxylon group (Shaw, 1914) which is normally believed to possess two vascular bundles per needle but it was often difficult to distinguish the number as the two bundles frequently coalesced within one needle. However, these numbers could be observed no matter what

Table 3. — Site and provenance means, and provenance rankings for four anatomical characteristics.

A. Site and provenance means

| MEAN | VARIATE A (Width,mm) | VARIATE B (Rows, no) | VARIATE C (Stomates, no) | VARIATE D (Serrations, no) |
|---|---|---|---|---|
| Provenance 1 (Philippines) | 1.656 | 5.11 | 53.56 | 21.82 |
| 2 ( " ) | 1.643 | 4.80 | 52.85 | 21.22 |
| 3 ( " ) | 1.679 | 4.94 | 53.97 | 22.31 |
| 4 ( " ) | 1.662 | 4.84 | 54.69 | 21.43 |
| 5 ( " ) | 1.677 | 5.13 | 52.14 | 20.18 |
| 6 ( " ) | 1.712 | 5.24 | 51.57 | 21.95 |
| 7 (Thailand) | 1.586 | 4.62 | 52.95 | 20.93 |
| 8 (Viet Nam) | 1.665 | 4.78 | 52.98 | 21.34 |
| 9 (Assam) | 1.572 | 4.21 | 57.69 | 21.00 |
| 10 (Burma) | 1.585 | 4.36 | 54.27 | 20.57 |
| 11 (Madagascar) | 1.634 | 4.88 | 50.73 | 20.41 |
| Standard error (±) | 0.0315 | 0.120 | 0.917 | 0.581 |
| Site 1 (Chati) | 1.634 | 4.90 | 53.24 | 19.69 |
| 2 (Chichele) | 1.651 | 4.72 | 53.56 | 22.70 |
| Standard error (±) | 0.0237 | 0.080 | 0.579 | 0.363 |
| Grand mean | 1.644 | 4.81 | 53.40 | 21.20 |

B. Provenance rankings

| VARIATE A (Width, mm) | VARIATE B (Rows, no) | VARIATE C (Stomates, no) | VARIATE D (Serrations, no) |
|---|---|---|---|
| 9 | 9 | 11 | 5 |
| 10 | 10 | 6 | 11 |
| 7 | 7 | 5 | 10 |
| 11 | 8 | 2 | 7 |
| 2 | 2 | 7 | 9 |
| 1 | 4 | 8 | 2 |
| 4 | 11 | 1 | 8 |
| 8 | 3 | 3 | 4 |
| 5 | 1 | 10 | 1 |
| 3 | 5 | 4 | 6 |
| 6 | 6 | 9 | 3 |

N.B. Provenances not bracketed by a vertical line are significantly different at the [redacted] 0.05 probability level. For needle width there were no significant differences.

sample sizes were adopted; variates E and F were thus omitted from all further analyses.

The data for variates A—D were subjected to analysis of variance and Duncan's multiple range test. Means for sites and provenances, with rankings at 0.05 level of probability, are shown in *Table 3*.

Throughout the analyses, the largest source of variance was that attributed to individual trees within plots. The effect of sites was significant (at the 0.001 level of probability) only on the number of serrations (variate D). At Chati there were 19.64 serrations per 5 mm segment compared with 22.70 at Chichele, but this was balanced by the significant difference between sites in needle length (20.42 and 16.41 cm); this suggests that the number of serrations formed on a mature needle is constant, regardless of the length attained by the needle.

There were significant differences between provenances in number of rows of stomates and number of stomates per row (variates B and C — 0.001 level of probability). The rankings for variates A and B were similar and the ranking for variate C was effectively an inversion of these; this indicates that the number of rows of stomates and the number of stomates per row were mutually compensating, *i. e.* there were more stomates per row in provenances with fewer rows. This was supported by the ranking of the product of variates B and C which represents the total number of stomates per 5 mm segment; this ranking was identical with that for width of needles.

It appears that individual stomatal traits permit the best discrimination of provenances on a univariate basis. There are several salient features about the rankings of the provenances. Seed lots from Assam, Burma and Thailand (9, 10, 7) appeared to be more like each other than like the remainder. Among these three provenances, Assam and Burma exhibited affinity. In the distribution of stomates, Thailand (7) was similar to Viet Nam (8) and these two tended to differ from the remainder. The Zambales provenance of the Philippines (2,000 ft altitude) differed markedly from the other five Philippines sources (5—7,000 ft).

These general features are typified by the distribution of number of rows of stomates in relation to latitude of seed source (*Figure 2*). Within the four known continental provenances there appeared to be a linear decrease in number of rows of stomates with increasing latitude north of the equator. The Philippines sources differed but insufficient source data were available to relate this variation to differences in altitude in the five seed lots from the Mountain Province. However, the most extreme value recorded was that for the low altitude source at Zambales, approximately one degree further south.

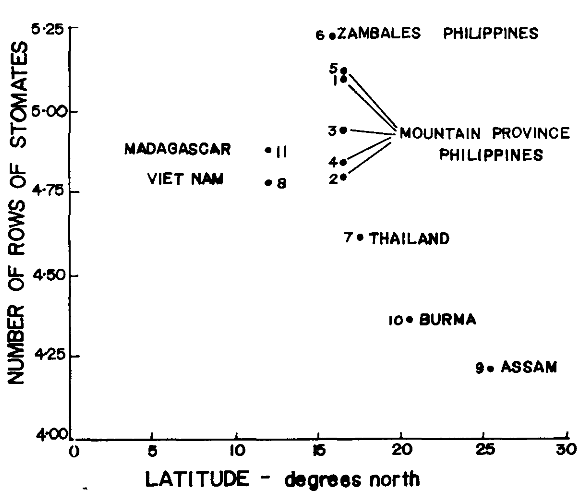

*Figure 2.* — Relationship between number of rows of stomates and latitude of seed source. (Madagascar plantation material was believed to originate in Viet Nam).

The data on number of rows of stomates (*Figure 2*) do not assist in determining the origin of the Madagascar material unequivocally; its mean value occurs exactly between that of Viet Nam and the average for the five mountain Philippines sources. Preliminary data on productive traits such as stem height, diameter, form and crown characters in the Zambian provenance trial indicates that the Madagascar material is most similar to the Thailand or Viet Nam stock (personal communication[5]).

The differences between provenances in the 12 variates examined here were not large enough to suggest that the two taxa *P. kesiya* and *P. insularis* are truly different species. If anything, the Assam and Burma group tend to be distinguishable from the remainder, and may be shown later to be of subspecific rank, within the species *P. kesiya*, but the general trend is of gradual variation throughout the range. More samples would be required within each source country to establish whether the variation is clinal or ecotypic but the nature of the natural distribution, with several wide disjunctions, suggests that ecotypic differentiation may exist.

### *(b) Multivariate analysis*

Since the development of the discriminant function (Fisher, 1936) and generalised distance (Mahalanobis, 1963), many techniques of multivariate analysis have been used in biosystematics (see *e. g.* Sokal and Sneath, 1963; Seal, 1964; Jeffers, 1967; Pearce, 1969). Discriminant analysis and principal component analysis have been used for taxonomy of hybrids and study of provenances in tree species (Hopp, 1941; Clifford and Binet, 1954; Mergen and Furnival, 1960; Gardiner and Jeffers, 1962; Jeffers and Black, 1963; Mergen, Lester, Furnival and Burley, 1966; Namkoong, 1967; Ledig, Wilson, Duffield and Maxwell, 1969) but there have been few reports of the use of canonical analysis (Namkoong, 1967).

The objects of canonical variate analysis are similar to those of principal component analysis but the applied weighting is intended to aid the discrimination of previously assigned taxa; more weight is given to variates which are relatively uniform within taxa. The multivariate analysis used here involved three stages: —

(i) tests of homogeneity among provenances in respect of multivariate means
(ii) canonical analysis
(iii) principal component analysis.

*Variates:* —

The following six variates were examined using branch means for two branches in each of three trees per plot: —

$x_1$ = length of needles (cm); mean of 10 fascicles per branch
$x_2$ = number of needles per fascicle; mean of 10 fascicles per branch
$x_3$ = width of needles (microscope units of 0.154 mm); mean of 5 needles per branch
$x_4$ = number of rows of stomates; mean of 5 needles per branch

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$x_5$ = number of stomates per unit length of needle; mean of 2 rows in each of 5 needles per branch

$x_6$ = number of serrations per unit length of needle; mean of 5 needles per branch

For analytical purposes $x_1$, $x_2$, $x_5$ and $x_6$ were scaled by $^1/_{20}$, $^1/_2$, $^1/_{10}$ and $^1/_{10}$ respectively.

The underlying analysis of variance structure derived from *Table 2* included the following matrices of sums of squares and products: —

| Source of variation | Degrees of freedom | Matrix of s.s and s.p. |
|---|---|---|
| Provenances | $b = 10$ | $S_B$ |
| Sites | 1 | |
| Provenances × Sites | 10 | |
| Replications in Sites | 4 | |
| Provenances × Replications in Sites | $w = 40$ | $S_W$ |

Between provenances variance — covariance matrix $B = {}^1/_{10} \cdot S_B$

Error variance — covariance matrix $W = {}^1/_{40} \cdot S_W$

*Homogeneity of multivariate means:* —

To test homogeneity of multivariate means several criteria are available: — T1, largest root criterion; T2, sum of roots criterion; T3, likelihood ratio criterion. T1 is calculated from only one of the latent roots of a matrix (as described below) whereas T2 and T3 are calculated from all the roots.

It is sometimes argued that T1 can not be as powerful as T2 and T3 because it does not use all available information; however, T1 uses the largest root and this effectively uses the information that all other roots are smaller. SCHATZOFF (1966 a) examined the choice of criteria and concluded that T1 is preferable if there is a single dominant root; otherwise T2 and T3 are equally good. Although these test criteria are well formulated a practical problem is that tables and charts for significance levels on the basis of exact distributions are only now becoming available.

For T1 (largest root criterion) tabulations or charts of percentage points have been compiled by several workers for various numbers of variates including $p = 6$ (see PILLAI, 1965), but not for T2 (sum of roots criterion).

For T3 (likelihood ratio criterion) exact results were given by SCHATZOFF (1966 b) for a large number of treatments (provenances) and variates (p). However, the simplest method is BARTLETT's (1947) approximation using the $\chi^2$ distribution and the present material was suitable for this method.

The latent roots of $W^{-1}B$ and their cumulative contributions were given by: —

| Root | Value | Cumulative % |
|---|---|---|
| $d_1$ | 12.7297 | 58.18 |
| $d_2$ | 6.7801 | 89.17 |
| $d_3$ | 1.3013 | 95.12 |
| $d_4$ | 0.6564 | 98.12 |
| $d_5$ | 0.3452 | 99.70 |
| $d_6$ | 0.0666 | 100.00 |

For the likelihood ratio criterion of homogeneity of multivariate means, $T_3 = m \sum_{i=1}^{4} \log_e (1 + \frac{1}{4} d_i)$

$$\text{with } m = \tfrac{1}{2} (2w + b - p - 1) = 41\tfrac{1}{2}$$

and $T_3$ referred to $\chi^2$ on $pb = 60$ degrees of freedom. Critical values for $T_3$ are 79.082, 88.379 and 99.607 for probability levels $p < 0.05$, 0.01 and 0.001 respectively. Here $T_3 = 122.622$, thus rejecting the hypothesis of homogeneity of provenance multivariate means at the 0.001 level of probability.

*Canonical analysis:* —

Canonical variables are linear functions of the p observed traits and are defined as the principle components of the "between provenances" variance — covariance matrix in the p — space obtained after standardisation for the "error" variance — covariance matrix. The first canonical variable corresponds to the best linear discriminant function (in FISHER's sense); the second canonical variable corresponds to the next best discriminant function, etc.

Arithmetically the first canonical variable is defined by the latent vector associated with the largest latent root of the determinantal matrix $W^{-1}B$, where B is the estimated "between provenances" variance — covariance matrix and W is the estimated "error" variance — covariance matrix. The second canonical variable is defined by the latent vector associated with the next largest latent root, etc.

The main computational problem with canonical analysis is the determination of the latent roots and vectors of $W^{-1}B$; this matrix is asymmetric whereas the standard JACOBI algorithm for latent roots and vectors applies to symmetric matrices. The following computational procedure was adopted:

Step 1. Determine the $(p \times p)$ matrix D and the $(p \times p)$ matrix H such that
$WH' = H'D$ and $H'H = I$ the unit matrix, where D is a diagonal matrix containing the latent roots of W on its leading diagonal and where H is a matrix whose rows contain the corresponding latent vectors.

Step 2. Find $V = H' D^{-1/2}$
where V is such that $VV' = W^{-1}$

Step 3. Form $C = V'BV$
This corresponds to expressing the "between provenances" variance — covariance matrix in the standardised space referred to earlier. Moreover, the latent roots of C are also those of $W^{-1}B$ that are required; the difference is that C is symmetric.

Step 4. Find the $(p \times p)$ matrix L and the $(p \times p)$ matrix J such that
$CJ' = J'L$ and $J'J = I$ the unit matrix, where L is a diagonal matrix containing the latent roots of C on its leading diagonal and J is a matrix whose rows contain the corresponding latent vector.

Step 5. The required latent roots of $W^{-1}B$ are contained in the diagonal of L and the corresponding latent vectors are contained in the rows of the matrix K given by $K = JV'$

Step 6. Suppose the original variates are $x_1, x_2, \ldots x_p$ and suppose the row of K corresponding to the ith largest latent root of C is $K_1, K_2, \ldots K_p$; then the ith canonical variable is defined by

$$Z_i = K_1x_1 + K_2x_2 + \ldots\ldots + K_px_p$$

With this computational procedure the sample variances of the canonical variable values calculated for the original data units are all unity. In the present study there were five plot replications of each provenance. Provenance totals (over all replications) were scaled by $^1/_5$ before

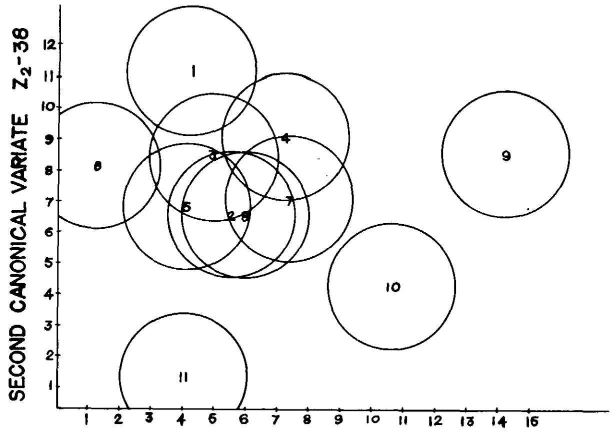

*Figure 3.* — Relationship between first and second canonical variates for 11 provenances.

transformation by K to yield provenance mean canonical variable values with unit variance.

As shown by the latent roots of $W^{-1}B$ tabulated above, the use of two canonical variables ($Z_1$ and $Z_2$, corresponding to latent roots $d_1$ and $d_2$) achieved a worthwhile reduction in the number of variables to consider. The coefficients for $Z_1$ and $Z_2$ were: —

| Trait | $Z_1$ coefficient | $Z_2$ coefficient |
|---|---|---|
| $x_1$ | 1.27 | —17.75 |
| $x_2$ | 6.67 | 8.01 |
| $x_3$ | —0.91 | —0.39 |
| $x_4$ | —1.79 | 1.86 |
| $x_5$ | 4.60 | 2.97 |
| $x_6$ | —3.44 | 0.28 |

Provenance means for canonical variables $Z_1$ and $Z_2$ are shown in *Figure 3.* The circles (centred on each provenance mean intersection of $Z_1$ and $Z_2$) have radii equal to twice the standard error of a provenance mean (unity).

For both $Z_1$ and $Z_2$, the coefficients for trait $x_2$ contribute markedly but it is felt that this reflects the tree effect on number of needles per fascicle. The univariate analysis of variance indicated that needle number varied little between provenances and that most of the variation was contributed by one essentially four-needled tree. The power of the canonical analysis was thus utilised largely on discriminating between individual trees with a trait that has no taxonomic value for discrimination among provenances.

The first canonical variate was thus composed largely of the contrast between traits $x_5$ and $x_6$ with smaller contributions from variation in length of needles ($x_1$) and number of rows of stomates ($x_4$). Examination of the signs of the coefficients indicates that as the number of stomates per unit length increases, the number of rows of stomates and of serrations decrease. This implies a "packing" or "storage" index of stomates and serrations per unit length of needle.

The second canonical variate was composed almost entirely of the length of needles ($x_1$) with a minor contribution from the decrease in number of stomates per unit length ($x_5$) with increasing needle length. This implies that the number of stomates per complete row is a predetermined constant for mature needles.

The canonical variates illustrated in *Figure 3* indicate that the three seed lots 9 (Assam), 10 (Burma) and 11 (Madagascar) were significantly different from each other and from the remaining sources. The analysis does not help to identify unequivocally the original source of the material from Madagascar; this appears to be equally attributable to Viet Nam, Thailand or the Philippines.

### *Principal component analysis*

It would appear, however, that much of the discriminating power of the canonical analysis was dissipated on the Madagascar material. All of the other provenances were accepted as natural populations of precisely known origin (with the exception of provenance 7 which originated "somewhere in Thailand"), whereas the Madagascar material was of totally unknown lineage and treatment.

Further discriminating power was utilised to separate the Philippines provenances. This was because the canonical analysis sought to maximise differences between taxa in relation to variation within taxa, whereas relatively little variation would be expected among the insular provenances.

Principal component analysis makes no assumptions about the relative amounts of variance within and between taxa nor about previous assignment of taxa. Data from six traits were therefore reanalysed to compute mean principal components for all eleven provenances and for ten (excluding Madagascar). In the latter case, component values for Madagascar were computed by standardising each trait mean by the overall mean and standard deviation. Standardised values were multiplied by the respective vectors to obtain principal components for the 'unknown' provenances. These were then evaluated graphically by plotting two-dimensionally all components against all others in turn and by regressing component values against latitude of seed origin. There was no effective difference between graphs based initially on all 11 provenances and those based on ten, and the relation between the first two principal components is given in *Figure 4* for which the component values for Madagascar were computed as described above.

In the two analyses the first three principal components explained 92—94% of the total variation of six traits, and in both cases the interpretations of these components were virtually the same *(Table 4).*

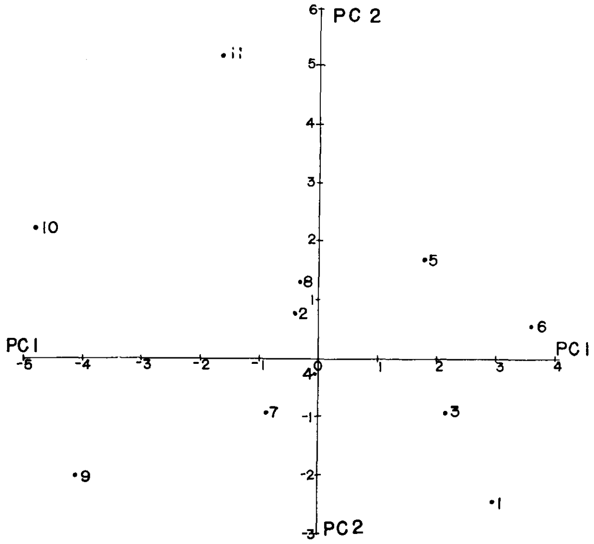

*Figure 4.* — Relationship between the first two principal components for 11 provenances.

*Table 4.* — Proportional eigenvectors for first three principal components.

| Component | Trait<br>% 3-needle fascicles (arcsin) | Needle length | Needle width | Rows stomates, no. | Stomates, no./row. | Serrations, no. |
|---|---|---|---|---|---|---|
| | A. 11 provenances | | | | | |
| 1 | 0.5834 | -0.3345 | 0.9402 | 1.0000 | -0.5994 | 0.5721 |
| 2 | -0.2514 | 1.0000 | 0.1468 | 0.2453 | -0.8489 | -0.7176 |
| 3 | 1.0000 | -0.1521 | -0.3955 | 0.0159 | -0.0676 | -0.5576 |
| | B. 10 provenances | | | | | |
| 1 | 0.5947 | -0.4725 | 0.9124 | 1.0000 | -0.7146 | 0.6091 |
| 2 | -0.4148 | 1.0000 | 0.3350 | 0.2352 | -0.7367 | -0.5712 |
| 3 | 1.0000 | -0.1243 | -0.3863 | 0.0596 | -0.2247 | -0.8557 |

The first principal component was composed largely of the number of rows of stomates and needle width while the second comprised needle length and number of stomates per row. The main contributor to the third component was the percentage of fascicles with three needles.

As illustrated by the figures, principal component analysis (*Figure 4*) emphasised differences already shown by canonical variate analysis (*Figure 3*). Samples from Assam and Burma appeared as different from each other as from the remaining sources; Madagascar appeared most like Viet Nam but these analyses can not be considered an unequivocal identification.

Values for both principal components were regressed on latitude of seed origin for the ten known provenances and there was a significant relationship for the first component at the 0.05 probability level ($r^2 = 0.49$). Narrower needles with fewer rows of stomates originated from more northerly sources.

## Conclusion

An examination of more characters (*e. g.* bark, strobili, cones, seed, crown shape) in the natural range and in provenance trials would be desirable before deciding the relative ranks of the taxa examined here; such an examination should preferably be undertaken by an experienced pine taxonomist reviewing all the southeast Asian pines, particularly *P. tabulaeformis*, *P. yunnanensis*, and the disputed *P. insularis* and *P. kesiya*.

However, on the basis of the characters examined here, there is little reason to separate the insular sources from southern continental sources; this agrees with the conclusion of Zavarin *et al.* (1968) which were based on biochemical evidence. One identity has been recommended or adopted by Gaussen (1960), Savory (1962), and Critchfield and Little (1966) who used the name *P. insularis* and Dallimore, Jackson and Harrison (1966) who used *P. kesiya.*

There would be somewhat more justification for recognising Assam and Burma provenances as separate ranks but because of the large number of samples required to characterise provenance means precisely it is felt that the several sources examined here are merely geographic ecotypes within an overall clinal pattern of variation from north to south within one species. The natural distribution of the species shows large disjunctions that lend themselves to ecotypic variation.

The material derived from Madagascar plantations differed from all other sources in most needle characters and in many growth characteristics; this may be attributable to random sampling in either Madagascar or in the original natural source, or to environmental pre-conditioning in Madagascar, but it is more likely to be the result of changes in the genetic constitution of the population caused by two or three silvicultural thinnings in Madagascar. The greatest similarity was with the Viet Nam material.

More intensive sampling within the natural range is necessary to characterise the clinal or ecotypic nature of the variation pattern, and more morphological characters should be assessed by a skilled pine taxonomist to determine the taxonomic rank of such variations.

The use of multivariate analysis drew attention to relationships between characteristics and between provenances that were not easily detected in univariate analysis. Two canonical variates or two principal components explained approximately 90% of total variation and the biological interpretation of these was relatively simple with the characteristics examined here, although it could become a problem if additional, dissimilar traits were assessed.

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## Summary

Multivariate analysis (including analysis of canonical variates and principal components) of twelve morphological and anatomical characteristics of needles of *Pinus kesiya* Royle ex Gordon (syn. *P. khasya* Royle; *P. insularis* Endlicher) failed to detect important differences among plots of ten natural provenances and one plantation source (Madagascar) grown in a replicated provenance trial in Zambia. Statistically significant differences were attributed to material of Assam and Burma origin and of Madagascar provenance but the general pattern of variation indicated a latitudinal cline with some ecotypic variations. Material from a low altitude in the Philippines (Zambales) differed from five seed lots collected at high altitudes (Mountain Province). Even with a high sampling intensity and the use of multivariate analysis, needle characteristics were not powerful discriminators among provenances; it was not possible to locate the exact origin of a questionable seed lot.

## Zusammenfassung

Trotz Anwendung aller variationsanalytischen Methoden waren unter Heranziehung von 12 morphologischen und anatomischen Nadelmerkmalen bei *Pinus kesiya* keine wesentlichen Unterschiede zwischen 10 Provenienzen und einer Bestandesabsaat aus Madagaskar auf den Versuchsflächen (mit Wiederholungen) in Zambia zu erkennen. Statistisch signifikante Unterschiede zwischen den Herkünften aus Assam und Burma auf der einen Seite und der Absaat aus Madagaskar werden einem Breitengrad-Klin einer Ökotypen-Variation zugeschrieben. Material aus niederen Lagen der Philippinen unterscheidet sich von den Herkunftsproben aus Hochlagen. Es war aber nicht möglich, die Herkunft einer fraglichen Samenprobe eindeutig zu lokalisieren.

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# Parallel and Divergent Evolution in Rocky Mountain Trees

By Fan Hao Kung and Jonathan W. Wright[1])

The Rocky Mountains of western United States are the home of several tree species which grow well in the north central states. Some are planted commonly as ornamentals, Christmas trees or for timber production. Because there is an active demand for provenance information, a series of provenance tests was undertaken in Michigan as part of the NC-51 regional tree improvement project.

The tests were designed to yield practical information, but theory and practice can not be divorced in such work. Equal emphasis was placed on the study of the factors involved in the evolution of genetic differences. The tests covered four tree species inhabiting somewhat the same geographic ranges. Each species has been the subject of a separate detailed report. It is the purpose of the present paper to place the evolutionary data from all four species in one common focus.

The provenance tests on which the present paper is based have been described in greater detail in a series of previous papers each dealing with one species: ponderosa pine *(23, 24, 28)*, Douglas-fir *(8, 26)*, white fir *(5, 9, 29)*, limber and southwestern white pines *(21, 27)*.

### Biological Subdivisions of the Four Species

PONDEROSA PINE, *Pinus ponderosa* Laws. — Three taxonomic varieties have been recognized:

Var. *ponderosa* of the Pacific Coast states, Idaho and Montana.

Var. *scopulorum* Engelm. from the southern Rocky Mountains to central Montana and the Black Hills of South Dakota

Var. *arizonica* (Engelm.) Shaw from SW New Mexico, SE Arizona and N Mexico; not included in the present experiments.

A related species, *P. washoensis* Mason and Stockwell, occurs on the east side of Mt. Rose, western Nevada.

Haller (1957, cited in *23, 24*) on the basis of field study, recognized races from California, the Pacific Northwest south to the Shasta Valley of N California, S Arizona-New Mexico, the high plateau of N Arizona-New Mexico and S Utah-Colorado and the northeastern quarter of the range.

Five extensive-range provenance studies which have been conducted in other regions have been summarized in papers by Squillace and Silen *(20)*, Wells *(23, 24)* and Wang *(22)*. These studies were a 20-origin 1-site experiment in Idaho, a 10-origin 6-site experiment in the Pacific Northwest, a 13-origin 1-site experiment in New Zealand, a small nursery experiment in Arizona and a 126-origin nursery experiment in California. From these studies the following races were recognized as reasonably distinct: California, North Plateau (= Pacific Northwest), Arizona-New Mexico (= southern Rockies), central and northern Rockies-Black Hills.

Callaham and Liddicoet *(3)* described an elevational cline in California from an elevational transect study.

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