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# Leaf Peroxidase Types in *Acacia karroo* HAYNE (Acacieae, Leguminosae): a Range-Wide Study

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**Summary**

Peroxidase variation has been assessed in 63 populations (4322 individuals) of *Acacia karroo* HAYNE (Acaciae: Mimosoideae) from across its entire southern African range. The 5 bands detected in the fast zone by starch gel electrophoresis combine to form 27 different phenotypes, with between 2 and 22 phenotypes found per population. SHANNON's measure of phenotype diversity varied from 0.15 to 4.11 (mean 2.71), whilst apportionment of the diversity showed that 81% of the diversity occurred within populations. Six coastal dune populations of *A. karroo* from Zululand/Mozambique clustered together and had a low phenotypic diversity. Band M is common throughout the range of the species, but it is virtually fixed to the exclusion of the other four bands in the coastal dune populations. Band K, on the other hand, is restricted to the Karoo region of the Cape Province in South Africa with lower frequencies in the adjacent highveld region of the central and western Transvaal Province to the North; it is completely absent to the east of the Drakensberg mountains and very rare in the west and north of its range. The implications of these data for seed sampling strategies in *A. karroo* are that although the high proportion of within-population variation makes it appropriate to sample large numbers of individuals within fewer populations to sample maximum genetic diversity, it is equally important to sample populations where bands are at high frequency because of the adaptive advantage that this may represent.

*Key words: Acacia karroo,* phenotypic variation, peroxidase, Leguminosae.

*FDC:* 165.3; 165.53; 161; 176.1 *Acacia karroo.*

## Introduction

*Acacia karroo* HAYNE is one of the most widely distributed trees in southern Africa (BARNES *et al.,* 1996). It occurs from the Cape Peninsula, at the southern extremity of the continent, northwards to the southern parts of Angola, Zambia and Malawi. It is adapted to a wide range of climatic and edaphic conditions (ROSS, 1979). It will grow under summer maximum to winter maximum rainfall patterns where annual precipitation varies from 100 mm to greater than 1500 mm, and mean annual temperatures from 24 °C down to 12 °C, where daily temperatures may rise to greater than 40 °C and drop to less than – 10 °C. Over its inland range it tends to be restricted to the heavier soils and it is one of the few species that thrives on heavy black vertisols with high pH, but it will grow on deep alluvial clay-loam soils, in river valleys and even on acid soils and shales. At the other extreme, it thrives on the unconsolidated sands of the coastal dunes in Zululand and it is tolerant of extremely saline conditions (BARNES *et al*., 1996).

*Acacia karroo* has many desirable attributes and products, such as fuelwood, an edible gum, an ability to ameliorate the environment through fixing atmospheric nitrogen and utilising water and nutrients from great depths. It is resistant to drought, frost, fire and salinity. However, such traits are offset by its invasive tendency (WELLS *et al.,* 1986). As a result of the extensive geographical and ecological range of the species and its economic potential there has been considerable interest in assembling a collection of the genetic resources of *A. karroo* from across the species' natural range.

The traditional approach to the assessment of forest genetic resources has been to examine a combination of morphological and agronomic traits that, in the main, exhibit continuous variation. However, the effectiveness of this approach has been questioned by several authors (BROWN, 1979; GOTTLIEB, 1977). The application of isozyme markers has provided a powerful tool for the study of genetic diversity within and among plant populations (SOLTIS and SOLTIS, 1990).

BRAIN (1985; 1989) in a study of leaf peroxidase variation in South African populations of *Acacia karroo* revealed interesting patterns of variation among them, suggesting the existence of distinct geographic races and the correlation of isozyme phenotypes with environmental factors such as low temperature and rainfall. A similar correlation between allele distribution and environmental factors has also been shown for shikimate dehydrogenase and alcohol dehydrogenase loci (OBALLA, unpubl.).

Leaf peroxidase is a valuable and practical means of assessing genetic variation in *Acacia* since it can be measured

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*Table 1.* – Location of the 63 *Acacia karroo* populations surveyed for leaf peroxidase variation, the number of individuals surveyed per population (n), band frequencies and the phenotype diversity ($H_{pop}$). MAR is mean annual rainfall. $\bar{H}_{pop} = 1.67$; $H_{pop}/H_{sp} = 0.81$; $(H_{sp} - \bar{H}_{pop})/H_{sp} = 0.19$.

| Code | Population | n | Lat. °S | Long. °E | MAR | Alt. | Band frequency | | | | | Hpop |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | mm | m | K | L | M | N | O | |
| 1 | Munster | 32 | 30.51 | 30.22 | 1050 | 0 | 0.00 | 0.03 | 0.42 | 0.50 | 0.05 | 1.39 |
| 2 | Kloof | 65 | 29.48 | 30.5 | 1000 | 600 | 0.00 | 0.05 | 0.41 | 0.48 | 0.06 | 1.50 |
| 3 | Kingsley | 47 | 27.55 | 30.32 | 892 | 1250 | 0.00 | 0.01 | 0.42 | 0.55 | 0.02 | 1.18 |
| 4 | Hluhluwe | 58 | 28.03 | 32.03 | 1127 | 550 | 0.00 | 0.00 | 0.49 | 0.37 | 0.14 | 1.43 |
| 5 | Lusaka | 76 | 15.26 | 28.2 | 804 | 1280 | 0.00 | 0.01 | 0.52 | 0.41 | 0.05 | 1.30 |
| 6 | Nelspruit | 100 | 25.3 | 30.58 | 762 | 665 | 0.00 | 0.04 | 0.22 | 0.52 | 0.23 | 1.64 |
| 7 | Pietersburg | 38 | 23.54 | 29.23 | 476 | 1450 | 0.01 | 0.05 | 0.25 | 0.59 | 0.10 | 1.56 |
| 8 | Ashburton | 98 | 29.4 | 30.21 | 928 | 684 | 0.00 | 0.09 | 0.38 | 0.36 | 0.18 | 1.82 |
| 9 | Bulawayo | 149 | 19.58 | 28.32 | 541 | 1075 | 0.02 | 0.04 | 0.30 | 0.49 | 0.15 | 1.73 |
| 10 | Blantyre-Zomba | 25 | 14.2 | 35.05 | 1338 | 1000 | 0.02 | 0.06 | 0.38 | 0.42 | 0.12 | 1.78 |
| 11 | Gwaai | 82 | 19.25 | 28 | 625 | 1000 | 0.01 | 0.04 | 0.28 | 0.41 | 0.27 | 1.80 |
| 12 | Mt. Silinda | 40 | 20.2 | 32.43 | 1470 | 1100 | 0.02 | 0.04 | 0.28 | 0.39 | 0.26 | 1.85 |
| 13 | Lebomba | 70 | 26.3 | 32 | 865 | 600 | 0.00 | 0.05 | 0.32 | 0.48 | 0.16 | 1.67 |
| 14 | Linthipe | 44 | 14.1 | 34.08 | 905 | 1200 | 0.00 | 0.03 | 0.36 | 0.39 | 0.22 | 1.69 |
| 15 | Windhoek | 44 | 22.04 | 17.06 | 361 | 1728 | 0.00 | 0.11 | 0.32 | 0.49 | 0.08 | 1.67 |
| 16 | Hluhluwe II | 80 | 28.03 | 32.03 | 1127 | 550 | 0.00 | 0.08 | 0.31 | 0.57 | 0.04 | 1.46 |
| 17 | Rosh Pinah | 19 | 27.57 | 16.45 | 90 | 600 | 0.00 | 0.05 | 0.37 | 0.55 | 0.03 | 1.37 |
| 18 | Pomeroy | 74 | 28.34 | 30.27 | 850 | 1300 | 0.00 | 0.14 | 0.23 | 0.50 | 0.13 | 1.77 |
| 19 | Inyanga | 120 | 18.17 | 32.42 | 973 | 1500 | 0.00 | 0.15 | 0.39 | 0.36 | 0.09 | 1.78 |
| 20 | Malala | 54 | 15.36 | 35.1 | 1044 | 1000 | 0.00 | 0.13 | 0.33 | 0.42 | 0.12 | 1.80 |
| 21 | Umtata | 81 | 31.35 | 28.47 | 654 | 700 | 0.05 | 0.08 | 0.48 | 0.34 | 0.04 | 1.73 |
| 22 | Keiskammahoek | 97 | 32.41 | 27.09 | 690 | 375 | 0.03 | 0.13 | 0.47 | 0.31 | 0.06 | 1.81 |
| 23 | East London | 104 | 33 | 27.54 | 808 | 125 | 0.06 | 0.13 | 0.37 | 0.42 | 0.03 | 1.83 |
| 24 | Estcourt-Weenen | 84 | 28.5 | 30.04 | 738 | 1013 | 0.00 | 0.20 | 0.38 | 0.34 | 0.08 | 1.82 |
| 25 | Jedibe | 36 | 19.03 | 22.34 | 501 | 914 | 0.00 | 0.21 | 0.49 | 0.21 | 0.10 | 1.78 |
| 26 | Rusape | 34 | 18.2 | 32.09 | 808 | 1600 | 0.00 | 0.21 | 0.43 | 0.34 | 0.03 | 1.68 |
| 27 | Uitenhage | 58 | 33.46 | 25.25 | 398 | 90 | 0.06 | 0.03 | 0.44 | 0.32 | 0.16 | 1.87 |
| 28 | Great Fish River | 110 | 33.58 | 25.36 | 576 | 576 | 0.10 | 0.04 | 0.42 | 0.33 | 0.11 | 1.92 |
| 29 | Mutaroshange | 112 | 17.1 | 30.42 | 873 | 1500 | 0.08 | 0.03 | 0.39 | 0.33 | 0.17 | 1.94 |
| 30 | Thornhill | 98 | 33.54 | 25.08 | 570 | 320 | 0.08 | 0.03 | 0.42 | 0.41 | 0.06 | 1.74 |
| 31 | Coega | 92 | 33.44 | 25.35 | 500 | 50 | 0.16 | 0.05 | 0.35 | 0.32 | 0.13 | 2.08 |
| 32 | Warmbaths | 56 | 28.29 | 18.41 | 105 | 750 | 0.19 | 0.07 | 0.34 | 0.35 | 0.05 | 2.00 |
| 33 | Doornspruit | 42 | 23.2 | 28.4 | 476 | 1000 | 0.17 | 0.00 | 0.28 | 0.41 | 0.13 | 1.86 |
| 34 | Bloemfontein | 99 | 29.07 | 26.14 | 547 | 1422 | 0.09 | 0.15 | 0.38 | 0.26 | 0.13 | 2.14 |
| 35 | Boknes | 112 | 33.48 | 26.3 | 575 | 0 | 0.11 | 0.04 | 0.53 | 0.33 | 0.00 | 1.55 |
| 36 | Hazyview | 42 | 25 | 31 | 950 | 900 | 0.00 | 0.10 | 0.18 | 0.45 | 0.27 | 1.81 |
| 37 | Tzaneen | 65 | 23.5 | 30.09 | 850 | 1300 | 0.00 | 0.01 | 0.19 | 0.44 | 0.35 | 1.57 |
| 38 | Insiza | 99 | 19.48 | 29.1 | 624 | 1450 | 0.01 | 0.04 | 0.37 | 0.25 | 0.34 | 1.81 |
| 39 | Kimberley | 110 | 28.45 | 24.46 | 415 | 1100 | 0.18 | 0.42 | 0.19 | 0.21 | 0.00 | 1.90 |
| 40 | Namib-Naukluft | 95 | 23 | 15.3 | 130 | 1000 | 0.00 | 0.39 | 0.28 | 0.28 | 0.04 | 1.74 |
| 41 | Silkaatsnek | 60 | 25.28 | 28.16 | 735 | 1400 | 0.10 | 0.34 | 0.23 | 0.26 | 0.07 | 2.12 |
| 42 | Maltahöhe | 51 | 24.5 | 17 | 180 | 1490 | 0.00 | 0.28 | 0.66 | 0.06 | 0.00 | 1.15 |
| 43 | Kamieskroon | 53 | 30.12 | 17.56 | 136 | 762 | 0.19 | 0.17 | 0.59 | 0.04 | 0.00 | 1.52 |
| 44 | Richards Bay | 52 | 28.46 | 32.06 | 1050 | 20 | 0.00 | 0.02 | 0.93 | 0.04 | 0.01 | 0.46 |
| 45 | Lake Sibaya | 30 | 27.2 | 32.38 | 1298 | 10 | 0.00 | 0.00 | 0.97 | 0.03 | 0.00 | 0.19 |
| 46 | Lala Nek | 29 | 27.15 | 32.46 | 1298 | 5 | 0.00 | 0.00 | 0.91 | 0.05 | 0.03 | 0.49 |
| 47 | Bazaruto | 45 | 21.32 | 35.29 | 1000 | 1 | 0.00 | 0.00 | 0.99 | 0.00 | 0.01 | 0.08 |
| 48 | Univ. Zululand | 30 | 28.48 | 31.53 | 700 | 64 | 0.00 | 0.02 | 0.72 | 0.18 | 0.08 | 1.19 |
| 49 | Tugela Mouth | 25 | 29.2 | 31.16 | 800 | 58 | 0.00 | 0.02 | 0.60 | 0.24 | 0.14 | 1.45 |
| 50 | Irene | 164 | 25.53 | 28.13 | 741 | 1460 | 0.15 | 0.18 | 0.24 | 0.17 | 0.25 | 2.28 |
| 51 | Leeu-Gamka | 50 | 32.3 | 22.3 | 211 | 553 | 0.16 | 0.14 | 0.25 | 0.17 | 0.28 | 2.27 |
| 52 | Greenbushes | 63 | 33.3 | 27.1 | 559 | 0 | 0.38 | 0.13 | 0.25 | 0.23 | 0.01 | 1.97 |
| 53 | Vanrhynsdorp | 53 | 31.35 | 18.43 | 145 | 122 | 0.37 | 0.14 | 0.30 | 0.15 | 0.04 | 2.05 |
| 54 | Augrabies | 55 | 29 | 19 | 98 | 500 | 0.44 | 0.11 | 0.33 | 0.11 | 0.01 | 1.82 |
| 55 | Prieska | 58 | 29.4 | 22.45 | 232 | 920 | 0.33 | 0.21 | 0.22 | 0.23 | 0.02 | 2.08 |
| 56 | Cookhouse | 65 | 32.45 | 25.49 | 603 | 900 | 0.32 | 0.02 | 0.34 | 0.18 | 0.14 | 2.01 |
| 57 | Bailey | 105 | 31.47 | 26.44 | 560 | 1300 | 0.31 | 0.01 | 0.51 | 0.14 | 0.02 | 1.60 |
| 58 | Potchefstroom | 49 | 26.42 | 27.06 | 608 | 1352 | 0.17 | 0.13 | 0.25 | 0.37 | 0.07 | 2.12 |
| 59 | Roossenekal | 105 | 25.14 | 29.53 | 963 | 1500 | 0.21 | 0.18 | 0.05 | 0.53 | 0.02 | 1.73 |
| 60 | Mowers | 100 | 33.3 | 19.15 | 249 | 280 | 0.24 | 0.24 | 0.08 | 0.28 | 0.17 | 2.23 |
| 61 | Prince Albert | 64 | 32.14 | 22.02 | 183 | 640 | 0.38 | 0.13 | 0.12 | 0.25 | 0.11 | 2.13 |
| 62 | Sardinia Bay | 50 | 33.52 | 25.12 | 570 | 58 | 0.43 | 0.12 | 0.06 | 0.37 | 0.03 | 1.82 |
| 63 | Middelsvlei | 25 | 32.56 | 20.59 | 130 | 900 | 0.63 | 0.01 | 0.10 | 0.25 | 0.00 | 1.32 |

from leaves harvested directly in the field and transported to the laboratory at ambient temperature (BRAIN, 1985), overcoming the potential problems of seed being absent at the time of collection and the practicalities of sample transport for isozyme analysis. This system has been shown previously by BRAIN (1985; 1989) to be both highly reproducible and stable. The present paper reports the results of a study of peroxidase variation in 63 populations from the known natural range of *Acacia karroo*.

## Material and Methods

### *Material*

A total of 4322 individual trees from 63 southern African populations, representing the complete range of *Acacia karroo* were studied to assess peroxidase variation (*Table 1*).

### *Electrophoresis*

Electrophoresis was carried out on single mature pinnae previously collected in the field. Pinnae from individual trees were collected into plastic bags and either sent by post, with a few drops of water added, or brought to Durban (South Africa) for preparation within a week of collection. No change in peroxidase activity was observed under these conditions. Full details of peroxidase extraction and electrophoresis are given in BRAIN (1985); briefly, peroxidases were extracted using POULIK's gel buffer (0.076 M Tris, 0.005 M citric acid, pH 8.65; POULIK, 1957) and the extract stored for up to a year at 4 °C.

Electrophoresis was performed on 8.75% horizontal starch gels, using POULIK's gel and electrode buffers (gel buffer: as above; electrode buffer: 0.05 M NaOH; 0.3 M boric acid). Gels were run at a current of 50 mA for 5 hours or until the buffer front had migrated 6.5 cm at room temperature. Gels were sliced and stained for peroxidase with a mixture of 15 ml water, 0.3 ml glacial acetic acid, 50 mg benzidine and four drops hydrogen peroxide. Peroxidase bands were strongly stained in fifteen minutes and then photographed.

### *Data analysis*

A matrix of phenotypic distances was derived by pairwise comparison between localities and summing the absolute differences in frequencies for each of the 5 bands (BRAIN, 1989). Phenotypic distances were clustered by the unweighted pair-group mean (UPGMA; SNEATH and SOKAL, 1973)) method on the computer package NTSYS (ROHLF, 1988). Single-linkage cluster (SNEATH and SOKAL, 1973) and neighbour-joining (SAITOU and NEI, 1987) analyses were also used to analyse the matrix of phenotypic distances.

Peroxidase phenotype diversity was analysed using SHANNON's information measure, $H = -\Sigma\, p_i \log_2 p_i$, where $p_i$ is the phenotype frequency (KING and SCHAAL, 1989; LEWONTIN, 1972). Apportionment of the peroxidase diversity within and among populations was made using SHANNON's measure of phenotypic diversity (KING and SCHAAL, 1989, SCHAAL *et al.*, 1987; SCHAAL and SMITH, 1980); proportion of peroxidase diver-

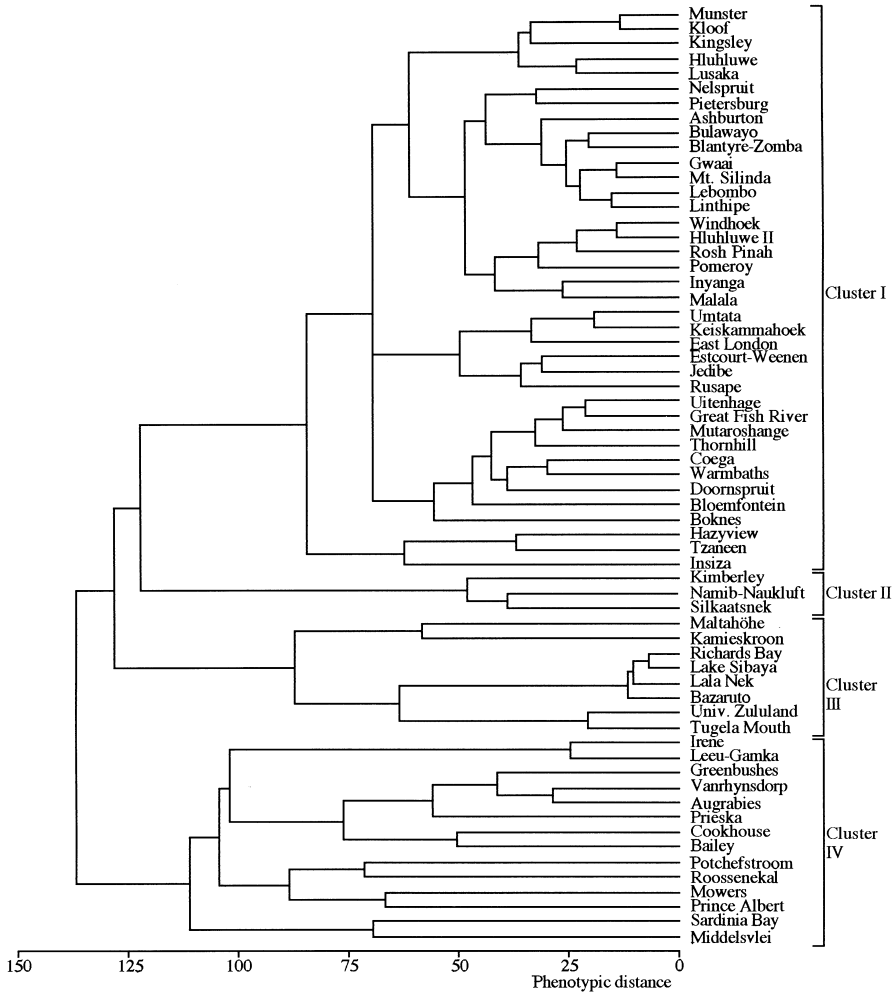


*Figure 1.* – Unweighted pair group mean analysis (UPGMA) clustering of peroxidase phenotypic distance measures between 63 populations of *Acacia karroo* from southern Africa.

sity present within populations ($H_{pop}/H_{sp}$) and among populations [$(H_{sp}–H_{pop})/H_{sp}$], where $H_{pop}$ is the population diversity and $H_{sp}$ is the species diversity.

Environmental data (altitude, mean annual rainfall, mean annual temperature) were recorded from the closest weather station to each locality and these data were used to examine the correlations with band frequency and phenotype diversity. Populations were also classified according to their positions within WHITE's system of African vegetation types or 'phytochoria' (WHITE, 1983).

## Results

Two zones of activity were found, as previously described (BRAIN, 1985, 1989). Of these, only the most anodally migrating zone was scored since the staining intensity of bands in the slow zone was too variable to be relied on, although polymorphism was identified in this region. Five bands, labelled from cathode to anode as K, L, M, N, O were found in the fast or anodal region. The majority of individuals (96.5%) had either 1 or 2 bands, whilst 3.5% had up to 4 bands in this region. Twenty-seven different phenotypes were found in the populations studied, with between 2 (Bazaruto) and 22 (Irene) phenotypes per population (mean 10.7). These differences are reflected in the diversity of the populations, calculated according to SHANNON's information measure ($H_{pop}$), which range from 0.08 (Bazaruto) to 2.28 (Irene) with a mean of 1.67 (*Table 1*). Apportionment of the diversity, within and among populations, showed that, on average, 81% of the peroxidase diversity occurred within populations (*Table 1*).

UPGMA clustering of the phenotypic distances showed 4 broad groups in the populations analysed (*Fig. 1*). Within the dendrogram, one tie was found in Cluster I, which may affect the structure of Cluster I but is unlikely to affect the distribution of populations between clusters (SNEATH and SOKAL, 1973). This may be revealed by the 2 collections from Hluhluwe appearing at 2 separate places in Cluster I, rather than together. Plotting population genetic diversity ($H_{pop}$) with respect to the arrangement of populations shown in *figure 2* revealed that Cluster III (Maltahöhe, Kamieskroon, Richards Bay, Lake Sibaya, Lala Nek, Bazaruto, Univ. Zululand, Tugela Mouth) had a low mean $H_{pop}$ (0.82 ± 0.20; range 0.08 – 1.52) compared to the other 55 populations (1.79 ± 0.03; range 1.18 – 2.28; *Table 1*).

A wide range of variation in the banding patterns within and between populations was found. Across all the populations studied, the frequency of bands M (0.38) and N (0.32) were much greater than those of bands K (0.10), L (0.10) and O (0.10). The distribution of the band frequencies revealed that band K tended to predominate in Cluster IV, whilst Cluster III was characterised by the predominance of band M (*Table 1*). Less marked patterns of band frequency variation were shown by bands L, N and O.

The distribution maps (*Fig. 2*) of the frequencies of bands M and K over the natural of *Acacia karroo* reveal some interesting distribution patterns. Although M is common throughout the range of the species, it is virtually fixed to the exclusion of the other 4 bands in the east coast dune populations between latitudes 20° and 29° South. Band K, on the other hand, is restricted to the Karoo region of the Cape Province of South Africa with lower frequencies in the adjacent highveld region of the central and western Transvaal Province to the North. It is completely absent to the east of the Drakensberg mountains and very rare in the west and north of its range.

Calculation of correlation coefficients between geographical and environmental factors, phenotypic diversity and band frequency revealed 19 significant correlations, from a total of 30 possible correlations (SOKAL and ROHLF, 1981). However, 8 significant correlations were found between the environmental and geographical variables, indicating that these may not be independent. Furthermore, associations between assumed independent variables may increase the number of apparently significant correlations. The 63 populations in this study were classified into 6 of WHITE's phytochoria for southern Africa (WHITE, 1983), however there were no apparent associations between vegetation type and population distribution in the dendrogram. Those groups of populations with a high frequency of band M and a high frequency of band K have some environmental factors in common, for example the high frequency M populations are all on unconsolidated sand, whilst the high frequency K populations of the Karoo-Highveld probably experience the lowest winter temperatures.

## Discussion

Data from the 4322 individual trees from 63 southern African populations representing the complete natural range of *Acacia karroo* were scored and analysed as phenotypes, rather than genotypes, for 2 reasons. Firstly, BRAIN (1989) indicated that the nature of the polymorphic substance detected by the acidified benzidine-peroxidase reagent was 'not altogether clear' and went on to state that if peroxidase activity was being detected, then it was 'remarkably robust and undemanding' since activity remained detectable after many years storage of the extract at 4 °C. The acidified benzidine-peroxidase reagent has been used as a standard method for the detection of heme proteins, particularly cytochromes (BURSTONE, 1962). Comparisons between peroxidase activities detected using the acidified benzidine reagent and the more usual 3-amino-9-ethyl-carbazol reagent (WENDEL and WEEDEN, 1990) on leaf samples of *Faidherbia albida* (DEL.) A. CHEV. revealed identical regions of biochemical activity, but different phenotypes (BRAIN and HARRIS, unpubl.), indicating that different biochemical groups may be responsible for the observed activity using these 2 different reagents.

Secondly, *Acacia karroo* is a tetraploid species with 2n = 4x = 52 chromosomes (OBALLA and OLNG'OTIE, 1994), and the interpretation of isozyme phenotypes in terms of genotypes is complex and unreliable without associated segregation studies. Previous studies by BRAIN (1985; 1989) suggested that the single and double-banded phenotypes represented codominant alleles at a single locus, although no explanation was offered for the genetic basis of those phenotypes with 3 or more bands. 153 (3.5%) of the 4322 individuals sampled possessed three or more banded-phenotypes, with an average of 2.4 (range 0 to 15) individuals per population in this category. Bands in the double-banded phenotypes showed no evidence of differential staining indicative of unbalanced heterozygotes, as might be expected in a model of tetrasomic inheritance at a single locus. The data were treated in the simplest possible manner, as phenotypes, scoring the presence or absence of bands ('alleles').

The available data would suggest that the phenotypes result from tetrasomic inheritance of the nuclear genome, since apparent heterozygotes are visible as two or more banded types (BRAIN, 1985). Unfortunately the majority of the data was not collected in a manner that would allow the tetrasomic inheritance model to be tested and hence the differentiation of balanced and unbalanced heterozygotes to be determined (WEEDEN and WENDEL, 1990). However, given these limitations of the data set, fascinating patterns are beginning to emerge.

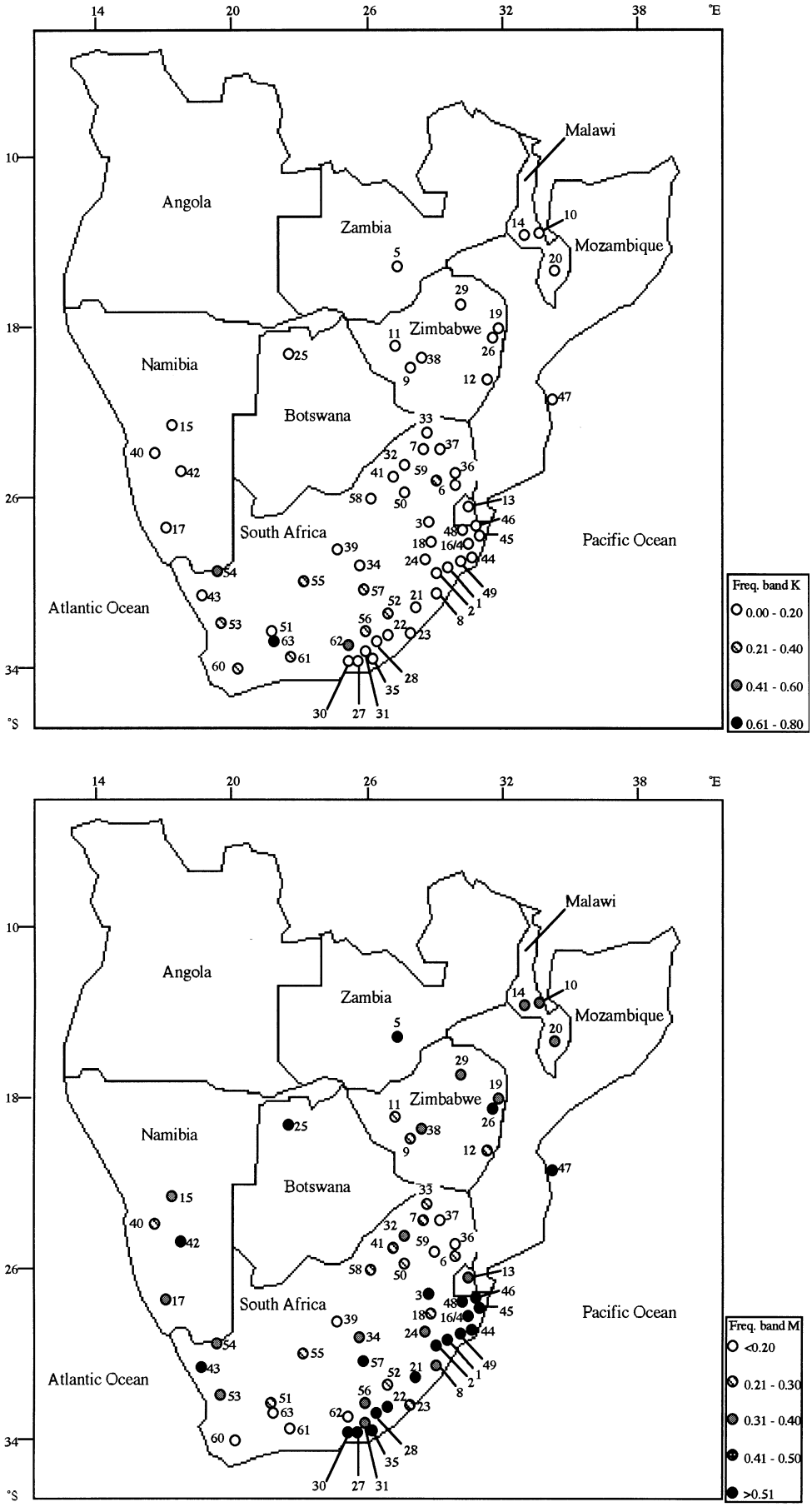


*Figure 2.* – Maps of peroxidase band frequencies in 62 populations of *Acacia karroo:* (a) band K; (b) band M. Populations indicated by numbers according to *table 1.*

The greater part of the phenotypic diversity for the biochemical assay used in this study of *Acacia karroo* was distributed within populations ($H_{pop}/H_{sp}$ = 81%). Similar results were found in a survey of 10 isozyme loci from 26 populations of *A. karroo* ($H_s$ = 84%; OBALLA, unpubl.). Although the apportionment of genetic diversity using SHANNON's information measure and either WRIGHT's F-statistics (WRIGHT, 1978) or NEI's diversity statistics (NEI, 1973; WEIR, 1990) are not directly comparable, this study contrasts with that of the African species *Faidherbia albida* (syn. *Acacia albida*) where approximately 44% of the genetic diversity resided between populations from the complete range of the species (HARRIS *et al.,* submitted). These data are consistent with the observation that there is relatively little differentiation between populations of tropical trees and shrubs (HAMRICK *et al*., 1992; HAMRICK and LOVELESS, 1989; HEYWOOD and FLEMING, 1986; MORAN *et al.,* 1989; PÉREZ-NASSER *et al*., 1993; ROCHA and LOBO, 1996).

Analyses of data on the basis of phenotypic distances and clustering algorithms are known to be susceptible to the assumption of both the method of distance estimation and clustering (SWOFFORD and OLSEN, 1990). Distance measures may be based on the presence of absence of a particular peroxidase band (e.g. JACCARD, 1908) or combined with the frequency of that band (e.g. BRAIN, 1989). Although a simple presence/absence score of phenotypic data makes fewer assumptions, information on frequency is lost. This may become important in those populations where there are many phenotypes that occur at a low frequency (e.g. Irene). Hence BRAIN's (1989) measure, which takes frequency into account, is a better way of determining phenotypic distance.

BRAIN (1989) recognised 3 genetic races within *Acacia karroo*: a Karoo race; an Eastern Cape race; a Natal/Lowveld race. However, when the populations used in BRAIN's (1989) work are plotted on the dendrogram presented here none of the clusters match the three genetic races. Cluster I was composed of a mixture of BRAIN's Natal/Lowveld and Eastern Cape races, Cluster II was composed of BRAIN's Karoo race. Cluster III was composed of those populations in BRAIN's study that were unclassified. Cluster IV was composed of BRAIN's Karoo and Eastern Cape races. The results reported here are based on a range-wide survey of peroxidase variation in *A. karroo*, and suggest that the genetic races identified by BRAIN (1989) are not supported when data from the complete distributional range are analysed, illustrating the importance of including material from the complete range of a species in studies of patterns of genetic variation and not restricting a study to political boundaries.

Two significant features of this study are: the high frequency of the K band in Cluster IV populations and the high frequency of the M band in Cluster III populations. Cluster IV populations are generally associated with the Karoo environment, although populations associated with the Karoo environment also occur in Cluster II. Other populations (Uitenhage, Great Fish River, Mutaroshange, Thornhill, Coega, Warmbaths, Doornspruit, Bloemfontein, Boknes) that display a high frequency of K are found in Cluster I. The 6 coastal populations of Cluster III are the only group that remain together independent of the clustering method used in the analysis. Abrupt, or discontinuous, genetic differences among populations have been reported in other tree species, for example localised genetic differentiation is found in *Pinus muricata*, where ecotypes meet at abrupt ecotones only 0.5 km wide (MILLAR, 1989). In the case of *Acacia karroo* those populations with a high frequency of the M band are associated with deep sandy, coastal soils and are distinct from those populations that are only a short distance (15 km to 30 km) inland.

Analysis of correlations between band frequency and climatic factors showed that though correlations did occur (19 from 30 possible correlations), some of the climatic factors themselves were highly correlated, indicating that they may not be independent. This may be the result of the very generalised nature of the meteorological data which, for almost all sites, was interpolated, often from distant stations. Furthermore, the distribution of *Acacia karroo* is determined to a large extent by soil type although this can change across the range; for example in the Cape province of South Africa it is limited to clay sopils whereas on the Zululand coast it grows on unconsolidated sands. The conclusion must be that a very much more detailed description of the environment would be needed to assess whether band frequency is correlated with any site factor. It is tempting to speculate that there is a selective advantage to either having fixed M or low N in the coastal dune environment or presence of K conferring adaptability to the dry, cold Karoo environment. However, given the limited within site sampling, the doubts over the identity of the substance being assayed and the lack of understanding of the genetics of the bands identified, any speculation of this nature would be premature. Much more intensive local sampling of individuals and environmental data would be necessary (GILLESPIE, 1991). In addition it would be desirable to have information on populations of *A. karroo* that occur on the east coast of Mozambique.

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# Variation in Stem Properties in a IUFRO 1964/1968 *Picea abies* Provenance Experiment in Southern Sweden

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**Abstract**

79 provenances from 3 blocks in a 17-year-old IUFRO 1964/1968 *Picea abies* (L.) KARST. provenance trial in southern Sweden were chosen for special measurements. The provenances originated from 11 zones in eastern Europe and southern Scandinavia and belonged all to the most fast-growing half of provenances. Carpathian provenances had the highest stem volume and dry weight and harvest index. Provenances from the Baltic States and Belarus combine high growth, high basic density and low incidence of spike knots. Provenances from southern Scandinavia had rather thin branches and high wood density but very low stem volume. The zonal variation in root anchorage could be attributed to variation in tree size. Partial correlations eliminating effects of zones showed that stems with high volume had poor form, thick branches and low basic density but high harvest index.

*Key words:* basic density, dry-matter contents, harvest index, *Picea abies,* provenance variation, root anchorage, stem form, wood quality.

*FDC:* 232.12; 165.5; 811; 174.7 *Picea abies;* (485).

## 1. Introduction

Studies on genetic variation have much concentrated on survival and growth capacity. However, there may be many other factors important for the value of the wood produced, including factors influencing wood properties and the manifestation of growth capacity in stands. Some potentially important factors are focused in the present study.

Wood density is the major wood property of concern for both pulp wood and lumber, and could be expected to continue as such in future (ZOBEL and VAN BUIJTENEN, 1989). Basic density serves as a wood-quality index as it is highly correlated with pulp yield and wood strength. Low basic density results in high transport costs for a given quantity of biomass, as wood has low dry-matter contents but high moisture contents per volume. Low basic density is also a prerequisite for stem cracks to develop (PERSSON, 1994).

In provenance and progeny testing, growth differences are usually expressed in terms of volumes rather than dry biomass. For *Picea abies* (L.) KARST., basic density is negatively correlated with annual ring width (ELLIOT, 1970; OLESEN, 1976). Negative genetic correlations with basic density to diameter and height have also been found for *Picea abies* ($r_g$ = – 0.56: BIROT and NEPVEU, 1979). For other species, but far from all, the same pattern is found (see review by ZOBEL and VAN BUIJTENEN, 1989). Consequently, genetic improvement of exclusively for volume growth, or selection of fast-grown provenances, may have negative influence on wood density for *Picea abies*. This also means that a certain gain in volume may not reflect a corresponding gain in dry biomass.

The proportion of the total growth of a tree accounted for by stem growth (harvest index) is an important determinant for acreage productivity (DICKMANN, 1985). If competitive entries are favoured to entries that are able to use limited resources efficiently, yield may be much lower than expected (CANNELL, 1982). This is an obvious risk when using single-tree plots on provenance and progeny testing. Fast-growing provenances of *Picea abies* such as the eastern-continental ones are prone to develop thick branches. As stem growth also is rapid, branch to