CHEMOTAXONOMY OF THE GENUS EUCALYPTUS

by

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DECLARATION

Except as stated herein, this thesis contains no material which has been accepted for the award of any other degree or diploma in any University, and to the best of my knowledge and belief, it contains no copy or paraphrase of material previously published or written by another person, except when due reference is made in the text.

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SUMMARY

Chemical characters in the majority of the Tasmanian species of Eucalypts have been investigated to determine their value to the taxonomy of the genus. The investigation has included three classes of chemicals.

1. The leaf and twig bark anthocyanins of each of twenty-two species were isolated and identified; it was found that anthocyanin distribution generally paralleled the split of the Tasmanian Eucalypts into two sections, namely, Sections Macrantherae and Renantherae. Cyanidin compounds containing glucose are the most widespread in the genus, other anthocyanidins found being delphinidin, malvidin and peonidin. The anthocyanin pattern of the Macrantherae is generally simple, and is based on the 3-glucoside and 3,5-diglucoside of cyanidin. The Section Renantherae, is further subdivided into two groups on the basis of the distribution both of the galactosides of cyanidin and delphinidin and of the methylated anthocyanins - this division parallels that based on morphological characters. The anthocyanin patterns of some renantherous species, (E. coccifera, E. risdoni and E. tasmanica) reflects the simplicity of the patterns found in macrantherous species.

2. Other flavonoids in leaf and twig bark were classified according to their R_f values in different solvent systems. Certain aglycones and alcohol-soluble polyphenols were restricted to either section or to groups within these sections. Again, these divisions agreed with morphological classification excepting for E. coccifera.

3. Protein and isoenzyme patterns of seedlings of the twenty-two species were obtained. Peroxidase and esterase patterns were useful in showing taxa relationships. All macrantherous species, except E. aggregata, showed similar peroxidase patterns, while E. aggregata showed a typically renantherous pattern. Quantitative differences in peroxidase bands reflect intra-section variation. Qualitative and quantitative variations in esterase patterns also provide useful taxonomic markers in the two sections.

Chemical characters were also used in an investigation of the E. viminalis/E. dalrympleana complex in Tasmania. Macromolecular characters of seedlings did not aid in distinguishing the two species, but differences in the phenolic compounds of the seedling leaves enabled positive identification of a "viminalis-type" or a "dalrympleana-type" tree.

Unless where stated otherwise, the nomenclature and classification used in this thesis is according to Blakely (1955. Second Edition).

1. REVIEW OF EUCALYPT TAXONOMY

1-1 Introduction:

The genus Eucalyptus, which belongs to the family Myrtaceae, is one of the largest and most complex genera of woody plants in the world. It has reached its highest development in Australia, only a few species being found on certain land masses to the north of the continent. In recent years the genus has found favour overseas as a timber source (especially in re-afforestation schemes), besides being extensively planted in certain areas for the harvesting of its essential oils.

Blakely (1955) listed 675 species and varieties of Eucalypts, although 225 of these have since been disputed on the grounds that they are either hybrids or clinal or other forms not of specific rank (Johnston and Marryatt, 1965).

The delimiting of species within the genus Eucalyptus is wrought with difficulties, many of which are inherent in the genus itself (Gardner, 1945 gives interesting examples of this). Also, the original description and sampling of certain species has created havoc in some areas. Incomplete, non-scientific description from inadequate samples is not uncommon, and often the 'type specimen' obtained has been hybrid in nature, or in some way abnormal. This is often due to a simple human frailty - selecting an unusual tree, and not a characteristic one. Workers at different times and in different locations have studied similar material.

They have given different names to what by later study has been shown to be the same species so that there is quite extensive synonomy.

In the eucalypts the fixity of the species is not 'real', as original descriptions in most cases fail to cover the full range of variation in the species. Man's intrusion into natural communities has often precipitated a breakdown in breeding barriers between species which then hybridise, e.g. E. pauciflora and E. linearis*. Furthermore, this upset balance has allowed the reduction of selection pressures allowing the resultant hybrids, and many other hybrids lost previously in the struggle for survival, to grow to maturity. In natural communities hybrids are found along the ecological boundaries of two species which could hybridise if brought into contact, but now hybrids are found in most artificial clearings, and often are selected for in gardens, etc.

Another type of variability is indicated by populations which are evolving from mixed origin, but have yet to reach stable species level (e.g. E. vitrea in South Australia - Hamilton, 1961; E. tasmanica in Tasmania - Hamilton, D., unpublished).

Again, there are the clinal variations seen even within well-defined species (Barber and Jackson, 1957; Barber, 1955, 1956). Such forms stress that eucalypts should be studied as organisms living in a particular range of field conditions, and not merely through the morphological

* nomenclature after Blakely (1955)

characters of dried herbarium material.

Eucalypts have been planted as exotics overseas, and this has further complicated the area of classification, for in other places, species which may never mix in Australia, even with the interference of man into natural habitats, are brought together. New varieties may result from the contact with new environments - better silviculture methods in plantations will alter the phenotype of the tree, often leading to increased growth, larger, thinner leaves and drooping branches (Hamilton, 1961). Also, the absence of burning, a very important aspect of eucalypt forests in Australia, will further modify eucalypt growth. Thus, some eucalypts have developed into local 'strains' under cultivation, e.g. in Africa, Zanzibar, Mysore, Italy and Israel (Larsen and Cromer, 1970). All this indicates the difficulty of presenting a valid and unambiguous classification of the genus Eucalyptus.

1-2 Origin of the Eucalypts:

The family Myrtaceae was widely distributed in the Australasian region by the end of the Cretaceous period (Blake, 1953), and a fossil discovery by E.W. Gill near Hobart (quoted by Pryor, 1959) has definitely established that the genus Eucalyptus existed in Tasmania in the early Tertiary, when it should be noted, there were no serious physiographic or climatic barriers to interfere with the distribution of existing species. Blake supports the theory that the genus arose in the northern part of the continent with a relatively warm climate, and present distribution patterns reflect migration, evolution and re-migration of different species. As new soils with a fertility level higher than

that of the old land surfaces became available to the eucalypts during the Tertiary, there was a climatic change which resulted in more arid conditions. Eucalypts produced new species to occupy the new niches in conditions which were rather more fertile than those to which the ancestral types had become adapted (Pryor, 1962).

In the Pliocene, arid phases continued, and desert conditions and certain earth uplifts seem to have split the west and the east of the continent, with a consequent divergence in evolution of the vegetation of both areas (Pyror, 1959). Such divergence is reflected in the observation that of the seventy species in the less harsh parts of the south-west of the Australian continent, not a single one is found in the east, and vice versa.

Rises in sea level also isolated Tasmania and New Guinea from the Australian mainland, but Pryor does not believe that the isolation of Tasmania by Bass Strait had any appreciable effect on divergent development due to isolation, but refers to similar variation on the Australian Mainland in areas that extend over the same latitude range as that between the Australian continent and Tasmania.

Blake (1953) maintains that the genus acquired considerable diversity in southern Australia and Tasmania in Pliocene times, and in Tasmania leaves like those of the Corymbosae* - a group no longer found in the island - have been discovered. But even after the genetic isolation of the various subgenera, each subgenus still possessed hereditary material similar in that it was capable of the same kind of

* as used by Blakely (1955)

variation which has led to the independent emergence in each subgenus of species which are similar in certain aspects, such as bark type (Pryor, 1959).

Some differentiation within the genus must have occurred while Australia had a relatively homogeneous topography and climate. Six species from four distinct Series* occur in Australia and New Guinea, and the group to which they belong must have differentiated before the separation which occurred in the last Pleistocene (Blake, 1953). No endemic species exists in South West Papua so presumably the genus did not occur there prior to the appearance of these six species. Some climatic sorting of species later occurred in the north of the continent and migration followed. Earth movements and erosion further influenced species in the north.

Carr (1972) disputes the idea that eucalypts migrated to Indonesia and the Phillippines across relatively short-lived land bridges, believing the assumption is unjustified due to the non-existence of the necessary land connections in the geological times in which the species are thought to have been in existence. Rather, she theorises a 'cataclysmic wind dispersal', and notes that if such is the case, then it will cause migration into, as well as out of, Australia. Such an interchange may still be occurring, but at a different rate. Carr also allows for speciation occurring outside Australia in the islands to the north (compare Blake, 1953).

1-3 Evolution of the Eucalypts:

Blake has 'synthesised' the ancestral eucalypt type by bestowing it with opposite, feather-veined, dorsi-ventral

* terminology after Blakely (1955)

leaves, axillary, three-flowered inflorescences, four calyx lobes, four petals, versatile anthers with parallel longitudinal slits, a four-celled ovary, fleshy fruits and winged seeds. Darnley-Gibbs (1958) believes it would have much pinene in its essential oils (the more advanced species contain cineole and pinene, while the even more advanced ones have oils with phellandrene and piperitone or geranyl acetate).

Evolution has proceded with either a reduction in flower number to one (e.g. E. globulus) or an increase to seven or more (renantherous species), and towards a fusion of flower bracts in varying degrees, with the shedding of the bracts at a relatively early stage in the maturation of flowers (Carr and Carr, 1959). Evolution has also been from a bi- to a mono-operculate condition. Pryor and Knox (1971) consider that all operculum pathways are modifications of the bi-operculate pathway pattern. During evolution four pathways of operculum development have been successful, the sequence of development being strictly and genetically controlled. Furthermore, the adaptive advantage which the presence of an operculum gives the eucalypts has been achieved in three ways - the development of both perianth whorls into opercula (Corymbia, Symphyomyrtus), the development of calycine opercula and the suppression of the corolla (due to mutation?), as in the Monocalyptus*, and the development of the petals into an operculum with the calyx remaining insignificant, as in Eudesmia.

The slow maturation of flowers seems an advanced character, although certain primitive eucalypts show such a condition. Advance is also reflected in the decrease in size of individual flower buds and increase in the height of

* terminology as used by Pryor and Johnson, 1971

trees. This progression can be considered to be brought about by selection against relative growth rates of some parts in favour of those of others. Anthers are derived from the typical macrantherous type (Carr & Carr, 1959; 1962a).

Carr and Carr, in reflecting on their proposed split of the genus contend that the 'genus Eucalyptus' must have had its beginnings at a time when east and west were not geographically isolated from one another, and the dichotomy between 'genus Eucalyptus' and genus Symphyomyrtus' must have already taken place at or by this time (Carr and Carr, 1962a). It is suggested that the beginning of evolution of 'genus Eucalyptus' took place under unfavourable conditions and that subsequent improvement of the climate made available sites of better quality for which intense competition led to selection for increased growth rate. Thus one expects the most advanced species in what are climatically the most favoured areas. Thus the Renantherae and Renantheroideae have evolved from a eudesmioid complex, the few present day representatives of which display a degree of variation to be expected in a remnant group (Carr and Carr, 1962b).

In its present distribution 'genus Eucalyptus' is sympatric with 'genus Symphyomyrtus' - that is, it has evolved alongside the other 'genus' and has developed biotypes able to occupy sites in common with the other 'genus' (Carr and Carr, 1962a). In Tasmania, we thus have mixed forest stands containing both a renantherous, and a macrantherous species, e.g. E. viminalis and E. obliqua; E. simmondsii and E. ovata.

While limited support may be given to these various ideas of the origin and evolution of the eucalypts, complete

justification for any remains impossible, one of the main reasons for this stalemate being the inadequacy of the fossil record.

1-4 Early Attempts at Classification:

Willdenow (1799) in his Species Plantarum (cited Penfold and Willis, 1961) attempted the first classification of what has proved to be an unwieldy genus - he classified the twelve known species on the shape of the operculum. This was followed by numerous systematic approaches utilising morphological characters which often separated closely related species. Von Mueller (1879) used the character of the bark, a character which is of considerable value in the field, although it does not indicate relationships within the groups.

Bentham (1866, vol. 3) introduced anther shape into eucalypt taxonomy, and this character has been the pre-occupation of eucalypt taxonomists for a considerable time. However clumsy such a character may be however, Bentham must be credited with bringing a certain order into what was even then recognised as a difficult genus. Also, he appreciated the ambiguities arising with anther shapes and warned that his five groups graded into one another through a series of transitional forms.

After Bentham, taxonomists elaborated on the scheme, adding or reducing the number of groups recognised until Blakely's elaborate classification of eight sections, and eighteen sub-sections, all utilising anther characters, was published (1934). Although criticised for its inconsistency, confusion and reliance on what are often intangible distinctions, the system remained, until just recently, the best general synopsis of the genus available.

1-5 Anthers and Classification:

The anther has advantages not possessed by other organs, and the affinities of some species are often best indicated by the anther, but there is the disadvantage that anther shape is often shared by a large number of species of diverse affinities. In the two major anther groups of Blakely's scheme (both of which are represented in Tasmania) there are 295 species and varieties in the single section Macrantherae, and 145 species and varieties in the Renantherae, all individuals within these very large groups sharing similar anther shape within each section. Furthermore, anthers of hybrids are intermediate in varying degrees between those of the parents, as shown by Pryor (1953) when studying a hybrid swarm from E. sideroxylon (anther group Terminales) and E. albens (anther group Porantheroidae). He found that anther shape was intermediate, as well as other floral characters, inflorescence and fruit characters being blended in the hybrids. These are therefore interbreeding groups in which anther shape is morphologically distinct. However, the Renantherae and Macrantherae are two sections with different anther shapes and are genetically isolated groups of species, interbreeding between species only occurring within each group. Thus the anther groups within the genus do not always correspond with genetically-isolated groups, although at some points they may be in agreement.

Anthers are often rather small, and this presents problems in field identification. Unfortunately, the value of anther shape and mode of dehiscence has been over-emphasised;

but now the emphasis has moved to other areas of morphology. Also, any classification based on a single character is necessarily an artificial one and may cut across true phylogenetic units, so there is value in using further characters to distinguish between closely related species and to allow delimitations of 'natural groups' where possible. Among some of the secondary characters now in use are bark types, the nature of the inflorescence, number of pairs of opposite juvenile leaves, the presence of lignotubers and seed morphology, to mention a few. It is acknowledged that many of these characters blend in hybrid populations, but the use of many characters is a better method of obtaining firm identification.

1-6 The Perianth:

Carr and Carr (1959, 1962a) made a study of floral morphology and showed that most of the species in the Renantherae and all those in Renantheroideae show a suppression of sepal primordia, so that a single perianth whorl is present. They suggest the removal of the few species with a double operculum that Blakely had placed in the Renantherae, to the Macrantherae, and advocated that such mono-operculate species form the sub-genus Monocalyptus. Similarly, they would remove certain mono-operculate species (e.g. E.preissiana and E. megacarpa) from the Macrantherae.

1-7 Division or Grouping of Individual Eucalypts:

Carr and Carr (1962b) later contended that the present genus Eucalyptus be divided into two genera on the basis of perianth characters, a division which would be supported by the presence or absence of stellate hairs on the juvenile leaves, and by differences in the inflorescences. Such a

split would be upheld by the characters of the seeds which were revealed by Gauba and Pryor (1958, 1959 and 1961), namely, that certain seeds have double seed coats while others have a testa formed from the outer integument only. That Renantherous species exhibited such a seed coat difference had been suspected earlier by Boden (1957), who also mentioned the seed dormancy possessed by certain Renantherous, but no Macrantherous, species. Also, there is no recorded evidence of hybridisation between species possessing different perianth and seed coat structures.

Thus Carr and Carr proposed that mono-operculate species with a double seed coat belong to one genus retaining the name Eucalyptus, while the rest of the Eucalypts were to be separated into a genus Symphyomyrtus. They contend that these are two separate but convergent genera which, it must be noted, cut right across Bentham's original classification based on anthers. However, under such a system anther shapes would now acquire a new meaning, for within the mono-operculate species, there is a smooth transition from the typically macrantherous condition (e.g. in the Eudesmieae) through the stages of developing confluence of the lines of dehiscence, decrease in size and divergence of the anther lobes, eventually culminating in the typical renantherous anther. If such a scheme is applied to Tasmanian Eucalypts, then the macrantherous species would be in the genus Symphyomyrtus, and the rest in the genus Eucalyptus.

Further support for the establishment of two genera was found in a study of ovules and placentas (Carr and Carr, 1962b). The number of rows of ovules and ovulodes (con-

gentially-sterile, ovule-like structures) and their placental arrangement, is taxonomically important for in the 'genus Eucalyptus' there is a progressive reduction in the number of longitudinal rows of ovules from six (Series Eudesmieae*, some of which have less than six rows) to two in the Renantherae, a transition which parallels anther changes. In the genus Symphymomyrtus in contrast, the number of longitudinal rows is never less than four.

In the most recent, and very exhaustive, Eucalypt classification, Pryor and Johnson (1971) have tended to group, rather than split, individuals, populations and taxa at a level on the basis of features held in common, and have thus weighed characters which show stability and least likelihood of being affected by local conditions or recent adaptations. The characters they have used include inflorescence and floral characters (ovary, anthers, staminophores, stamen groupings, filament oil glands, staminoidal condition of outer stamens in certain groups, different flexure conditions of the filament), operculum, ovule and seed coat structures, and they have recognised also the value of chemical characters, pollen morphology, wood and bark anatomy, distribution of oil glands, lignotubers, trichomes and cotyledon details (Pryor and Johnson, 1971; Johnson 1972).

Pryor and Johnson consider that the large amount of evidence available indicates several major groups within the Eucalypts, which they advocate should be considered as subgenera, at least until more complete and precise information is available. They recognise seven groups within the

* nomenclature after Johnston and Marryat (1965)

existing genus - Blakella, Corymbia, Eudesmia, Gaubaea, Idiogenes, Monocalyptus and Symphyomyrtus, of which only the last two are found in Tasmania. They contend that subgenera Blakella and Corymbia are related to each other and to the genus Angophora, (which genus the authors would like to include in the genus Eucalyptus as an eighth sub-genus), but that the three are genetically isolated. In fact, all eight groups are genetically isolated, i.e. are fertility groups between which no hybrids have been recorded. Eudesmia is clearly separated from the other groups, while Gaubaea and Idiogenes are set up to accommodate certain species which fail to fit into any of the other groups. Monocalyptus includes the majority of the Renantherae and Renantheroideae of Blakely, and is an isolated group still vigorous in evolutionary radiation. Symphyomyrtus is a very large and diverse sub-genus (containing the section Macrantherae) but still coherent, although within the sub-genus the placement of certain species is not finalised.

At this stage, the above classification seems to have much to recommend it, and practically, the authors have set out individual species and their placement in a manner which simplifies reference difficulties. I feel there is more value in retaining species in the traditional genus, rather than splitting the genus as other authors advocate, but perhaps most important of all it should be realised that eucalypt taxonomy is fluid rather than fixed in its terms of reference. The extent of morphological and other information relevant to the genus continues to expand.

1-8 Other Promising Taxonomic Markers:

1. Operculum formation

Extensive histological surveys of the developmental pathways leading to the formation of the operculum enabled Pryor and Knox (1971) to detect four principal pathways of development which correlated with their seven proposed subgenera. The corolline whorl may be entirely suppressed and absent, so that a single calycine operculum is present, as found in the subgenus Monocalyptus (compare with Carr and Carr, 1968, who interpret the components of the operculum as petals). If the corolline whorl is present it may be initiated soon after the sepals as in some species of the subgenus Eudesmia, in which cases the operculum can show considerable variation among the species, e.g. sepals may be free, thus the operculum is corolline, or sepals may be partially or more fully suppressed. This is demonstrated in single species of the subgenera Gaubaea and Idiogenes studied. Again, the corolline whorl may be initiated after a short interval, as in subgenera Blakella, Corymbia and some Symphyomyrtus, or after some delay (in other Symphyomyrtus). In either case the operculum is formed from both whorls (and so is bi-operculate), but with varying contributions of calycine and corolline whorls.

2. Seedling morphology

Another morphological character just beginning to be examined and one which may aid in classification of certain taxa, is seedling morphology. To date, Brooker (1970) has studied this in the Section Bisectae, with some promising taxonomic implications resulting; e.g. in three of the four series examined (Fruticosae, Aridae and Eremophilae) there was no unusual phyllotaxis (species exhibiting decussate phyllo-

taxis). However, in series Subulatae, individuals of five of the eleven species exhibited spiral phyllotaxis. In the species E. formanii, E. longicornis, E. oleosa var. obtusa and E. brockwayi 100% of the individuals showed this spiral phyllotaxis, while in E. oleosa, the character occurred in 99% of individuals.

3. Oil Glands

The essential oils of Eucalyptus were early to figure in some form of chemical taxonomy. Recently a taxonomic and ontogenic study involving oil glands and their ducts has been completed (Carr and Carr, 1969, 1970; Carr, Carr and Milkavits, 1970). In this, oil glands in the bark and pith were reported in only nine species which are in the eudesmioid complex. The bark and pith characters of the species of this complex range from no glands, through glands in the pith only, to both pith and bark glands, and this allows correlation with any natural breakdown of the Eudesmieae. The heterogeneity of the complex may be regarded as being characteristic of a fragmented and ancient group of species. For all other groups which have species in which glands are recorded, glands in the pith and in the bark appear to be mutually exclusive characters.

The members of the Corymbosae have an elaborate and regular system of glands in the pith of the stem, the peduncle, in the medullary tissue of the leaf, and in the base of the flower, but within the series there are distinctly different patterns of arrangements in different parts of the plant and when species are grouped on the basis of these

different arrangements, the grouping follows that based on other unrelated floral and vegetative characters, such as the persistence or otherwise of the outer operculum, and the presence and development of mesophyll fibres (Carr and Carr, 1969; Carr, Carr and Milkavits, 1970). This series appears to form a natural group, the species of which have a number of morphological features in common which are not found in other Eucalypts, and within the series the consideration of several unbiased characters leads to a splitting into groups.

The only other series showing ducts in the pith in some of its species is the related Clavigerae, and the systems developed fall far short of the complexity reached in the Corymbosae, showing no obvious relationship to leaf traces and leaf gaps as they do in members of the Corymbosae.

The presence or absence of pith glands, taken in conjunction with other characters, e.g. cotyledon shape, has aided subdivision of the very large groups Bisectae and Dumosae proposed by Pryor (1962).

The occurrence of glands in the bark is an important taxonomic character, and it appears that glands in the root bark are only expected in the series in which there are glands in the stem bark. Species having bark glands and no pith glands form the natural group Macrantherae Normales, and the character provides further evidence for the removal of certain species from the section, preserving a relatively uniform group.

This morphological character is thus of value when it is present in species, but it should be noted that 379 of

the eucalypts studied lack oil glands in both pith and bark.

4. Structure of Waxes

Another non-reproductive plant region studied has been leaf wax structure (Hallam and Chambers, 1970). Three main wax types, as exposed by electron microscopy, occur within the genus - tubes, plates and a mixture of both. There is a marked correlation between the presence of β-diketones in waxes and the formation of tubes, and a tendency towards the absence of β-diketones in waxes which form only plates. The subtypes of each of these basic wax forms can be related to existing taxonomic groupings within the genus, e.g. within the series Globulares, compoundly-branching tubes are characteristic. From this series, the species E. preissiana, E. megacarpa and E. coronata, which have plate waxes, should be removed to a renantherous group, on the basis of this, and of other characters (ovules and ovulode pattern, perianth characters, seed coat anatomy and the presence of renantherin - Hillis, 1966b, 1967a).

With reference to the proposed split into two genera (Carr and Carr, 1962a and b), the genus Eucalyptus contains an extremely heterogeneous range of leaf waxes, the majority being simple and unornamented. If a split is supported, we can assume that the same wax types have evolved several times or only become apparent with certain genetic combinations. Wax morphology may indicate an evolutionary trend from the complex waxes of the "genus" Symphyomyrtus to the relatively simple waxes of the "genus" Eucalyptus.

5. "Phytoglyphs"

Carr, Milkovits and Carr (1971) and Carr (1972)

have prepared "phytoglyphs" which depict various micro-anatomical features of the leaves of eucalypts, and although the work has been restricted to a small group of species, it provides interest as an important taxonomic item in a plastic scheme of numerical taxonomy. Leaves have often been considered as too plastic and subject to environment to have much use in taxonomic and evolutionary schemes, but the characters of the cuticle as are exposed by electron microscopy and light microscopy appear to be genetically determined and little subject to variation.

1-9 Chemicals and Taxonomy:

The characters discussed to date have been primarily morphological ones, but the essential oils of the Eucalypts were investigated early, and Baker and Smith (1920) had stated that the amount of oils in any species was reasonably stable. However variations have since been reported and the value of oil types is now thought to be very limited (Penfold and Morrison, 1928, 1930, cited by Penfold and Willis, 1961).

In 1962 Hergert predicted that taxonomy might be enriched by a study of flavonoid chemistry. He referred primarily to the phenolics of wood and bark, and Hillis and other workers have done considerable investigations into this, but with few results of taxonomic import (Hillis and Carle, 1960; Hillis and Hasegawa, 1962; Hillis, 1964; Hillis and Isoi, 1965).

Hillis later completed a survey of leaf polyphenols in 80% of known eucalypts and although his results are of

limited use because of certain factors already mentioned (Sharma, 1970), his chemotaxonomic survey supported many of the natural groupings of eucalypts, also proposing a few minor adjustments to classification. For example, he supported the removal of E. preissiana, E. megacarpa and E. coronata from the Globulares to the Renantherae, not only on their possession of renantherin, but also on their very large amounts of myricetin, low amounts of quercetin and the absence of chlorogeneic and p-coumarylquinic acids (Hillis, 1966a and b; 1967a, b, c, d).

Besides the presence of renantherin and macrantherin Hillis found myricetin, ellagic acid, quercetin, leucoanthocyanins, chlorogenic, p-coumarylquinic and gallic acids and certain unknowns to show qualitative and quantitative variations which will surely be of use in numerical taxonomy.

Earlier work on phenolic distributions in leaves and twig bark of some Tasmanian Eucalypts (see Appendix, pp. 1 - 14) has presented certain correlations with classical taxonomy (Sharma, 1970). Generally myricetin is absent only in the closely related macrantherous species, E. viminalis, E. dalrympleana, E. globulus and E. gunnii, but is also found in only low amounts in the anomalous renantherous species, E. coccifera. Other unidentified aglycones appear to be species-specific, and such compounds are found in E. delegatensis, E. obliqua and E. coccifera. Still others are more common to either section Macrantherae or section Renantherae.

Similarly, with alcohol-soluble substances, putative species-specific compounds were found and others

showed variation between the two major sections. Often, groups within a section are characterised by a certain amount and/or distribution of polyphenols, and morphologically-strange eucalypts, like E. coccifera, differ from related species in their flavonoid chemistry. Generally, the chemistry of the Renantherae seems to be the more complex. Other interesting points to arise from this earlier work involve the environmental influence on compounds, and show that certain compounds seem to develop with ontogeny. A pursuit of ontogenic biochemical changes or "dynamic chemo-taxonomy" (a term coined by Hegnauer, 1965) could aid in the elucidation of biosynthetic pathways which may solve taxonomic problems resulting from parallelism and diversifications of chemical characters; that is, become acquainted with evolutionary tendencies, namely progressions and regressions in metabolic pathways and their resulting plant constituents.

Looking to the future, the value of all such characters will be in the application of numerical taxonomic techniques to the range of data available.

2. ANTHOCYANINS

2-1 Introduction: Literature:

Anthocyanins occur frequently in Angiosperm species, in fact are more characteristic of these higher plants than of the lower phyla. They have been recorded in flowers, fruit, leaves, roots, twig bark and tubers (Harborne, 1965) and are responsible for, or contribute to, most flower colour.

The anthocyanins are all based on the flavylium skeleton (fig. 1, Appendix), and usually occur as glycosides with sugars attached at position 3 or positions 3 and 5. Two naturally occurring 3,7-glycosides are known, but substitution on a B-ring hydroxyl has not been recorded. The 5 or 7 sugar appears always to be glucose - sugars recorded at the 3 position are glucose, galactose, arabinose, rhamnose, xylose and fructose. Monosaccharides, disaccharides, trisaccharides and d-monosides have been reported (Harborne, 1967), the linkages in most of the common biosides and triosides being fairly well established. Gascoigne and co-workers (1948) in their survey on the anthocyanins of the Australian flora, recorded a much lower proportion of diglycosides in the Australian plants than had occurred in flora previously surveyed (e.g. by Beale et al., 1941). It was assumed that previous surveys had included a greater number of cultivated plants than had the Australian survey, and so the difference was tentatively ascribed to mutation and artificial selection increasing diglycosides at the expense of monosides in the cultivated forms.

All naturally occurring anthocyanins have the 4', 3, 5 and 7 positions occupied by a hydroxyl or substituted group, and in addition, hydroxylation can occur at the 3' and 5' positions. 6-hydroxylation is unknown, except for the rare compound carajurin, which occurs in the Bignoniaceae. Also rare are those anthocyanins which lack the usual 3-hydroxyl group - apigeninidin, luteolinidin and tricetinidin - their presence being detected so far only in four families (Bignoniaceae, Gesneriaceae, Steruliaceae and Theaceae) in the moss, Bryum sp., and ferns (Harborne, 1963, 1966). Within the family Gesneriaceae (order Tubiflorae),the occurrence of certain 3-deoxyanthocyanins is of systematic interest as they occur in only one of the two sub-families (Harborne, 1967).

Many anthocyanins occur which are acylated - it appears that the only acyl groups attached are hydroxycinnamic acids, which are attached to the anthocyanins through sugar in the 3-position, and not to one of the free hydroxy groups (Harborne,1967). The most common acylating agent appears to be p-coumaric acid (Harborne,1963, 1967), others reported being ferulic, sinapic and caffeic acids. Pomilio and Sproviero, (1972a and b), and Anderson et al., (1970) have reported acetic acid also as a major acylating agent of anthocyanin pigments in grapes. The majority of the acylated pigments known at present have only one (rarely two) acylating groups. Their occurrence is of taxonomic interest - for example, the only five sympetalous families with acylated pigments are in Engler's order Tubiflorae (Harborne,1967). These families are the Convolvulaceae,

Polemonaceae, Labiatae, Solanaceae and the Orobanchaceae. Acylated anthocyanins are undetected in the other five sympetalous orders investigated. In the Archichlamydae, acylated pigments occur in a single family of each of four orders studied (family Cruciferae, order Papaverales; family Vitaceae, order Rhamnales; family Violaceae, order Violales; and family Melastomaceae, order Myrtiflorae). Of these, the family Cruciferae is the most distinctive re the extent to which the anthocyanins are acylated. In the Monocotyledons only sporadic occurrence of acylated anthocyanins has been reported, and this is in the families Liliaceae and Iridaceae (order Liliiflorae) and Commelinaceae (order Commelinales).

The aglycone of the anthocyanin, which can be obtained by acid hydrolysis, is called the anthocyanidin. Addition or removal of hydroxy groups, methylation and glycosylation result in a wide range of anthocyanin types all based on the anthocyanidin, cyanidin. Pelargonidin, cyanidin and delphinidin are the three dominant anthocyanidins cyanidin being the most common. The three differ in structure only by the number of their hydroxyl groups (Fig. 2, Appendix).

In nature, nearly all blue flowers contain anthocyanins based on delphinidin; the majority of red flowers contain a cyanidin type and the occurrence of pelargonidin is almost entirely restricted to red flowers (Gascoigne et al., 1948; Harborne, 1965). As plants are cultivated, mutations delphinidin ⟶ cyanidin ⟶ pelargonidin occur as the brilliant scarlet and orange-red colours are selected for (Harborne, 1965). In a survey on the anthocyanins

of 228 species of Australian wild flowers, delphinidin was found in more than 60% of the species, cyanidin in 41% and pelargonidin in 1.7% (Gascoigne et al., 1948). The authors indicate that the occurrence of pelargonidin may be even less as they had difficulty separating pelargonidin and peonidin (this latter pigment is recorded in the eucalypts). The more limited occurrence of pelargonidin and the reversal of cyanidin and delphinidin as the dominant anthocyanin distinguishes the Australian flora from floras of other countries (Beale et al., 1941).

Methylation in the anthocyanin series is normally restricted to the 3', 5' and 7 hydroxy group, although a rare pattern of methylation in the A ring occurs in three sympetalous families (Primulaceae, Apocynaceae, and Plumbaginaceae) but in no members of the Archichlamydae. The methylated anthocyanins concerned are based on the anthocyanidins hirsutidin, rosinidin and capensinidin. There are only three methyl ethers of the anthocyanidins which are at all common - peonidin (cf. cyanidin), malvidin and petunidin (cf. delphinidin). (Fig. 2,Appendix).

Malvidin, the more fully methylated of the three appears to be the most abundant of the trio in floral organs (Harborne, 1963), while peonidin is uncommon in wild flowers. It appears that the systems for methylation and adding the 3-hydroxyl group to the B ring evolved together, thus malvidin is the dominant of the methylated pigments.

In the survey of Australian plants mentioned previously, there had appeared a higher degree of methylation in wild species than in cultivated ones, the assumption being

that cultivation preserves mutations from methylated to unmethylated types.

In the present work, a survey of the structure and distribution of anthocyanins in certain Tasmanian species of Eucalypts is reported. A preliminary survey (Sharma, 1970) had indicated that the majority of anthocyanins were cyanidin and delphinidin glycosides, but no positive identification of the different types was made. This has now been achieved, and the systematic importance of different anthocyanins has been investigated.

2-2 Experimental Methods:

a) Origin of Plant Material

The organs of the eucalypt species studied were young juvenile leaves (young adult leaves in E. perriniana, and in one sample of E. viminalis) and twig bark. The location of each species sampled is shown in Table 1, together with the name of the collector.

b) Method of Extraction

The material was crushed and then extracted with cold methanol containing 1% concentrated Hydrochloric acid. Problems arose when chlorophyll and brown pigments caused streaking and interference with the anthocyanin bands during the paper runs, and two different methods were tried to overcome this difficulty. The second one was permanently employed as it was the more successful of the two.

(i) The extractant was shaken a number of times with fresh volumes of petroleum ether - a good percentage of the chlorophyll was removed this way. Then, after the solution was concentrated in vacuo, it

was redissolved in a small volume of methanol/HCl and streaked onto Whatman's No. 3MM chromatography papers. These papers were run in a 90:10 mixture of ethyl acetate/1% conc. hydrochloric acid in methanol for about three hours. Most of the brown compounds were soluble in this solvent and migrated away from the anthocyanins. Acetone was another tank solvent tried for this purpose. After the papers had dried, the region containing anthocyanin was cut out and eluted overnight in MAW (see Appendix). The elutant was concentrated in vacuo, and streaked onto more No. 3MM papers for anthocyanin separation. With this method subsequent runs were often still incompletely separated due to further interference by brown and other compounds.

(ii) The extractant was evaporated in vacuo, and then re-dissolved in water and a minimum of methanol. This solution was run slowly onto a Bio-Rex $70H^{+}$ (Bio Rad Lab.)column (Jarman and Crowden, 1973). After all the pigment had become adsorbed onto the column, it was eluted with MAW. The elutant was concentrated in vacuo, and then streaked onto No. 3MM papers.

After this first partial purification, the extract already streaked on paper was run overnight in BAW (see Appendix). The distinct bands were cut out and eluted overnight in MAW. The elutant was concentrated and streaked onto papers which were then run for about 4 to 5 hours in 5% Acetic acid. The different bands after this run were cut out

and eluted. Sometimes further runs in these two solvents were necessary for better separation or the separation of additional bands. The final elutant was concentrated in vacuo, then redissolved in a little methanol and allowed to evaporate to dryness on a watch glass in a fast air stream.

c) Identification (All were isolated and purified before identification was attempted).

Many of the anthocyanins were identified by co-chromatography with reference compounds. When necessary, partial hydrolysis was carried out. The compounds from earlier isolations were often contaminated by brown compounds, and before these were hydrolysed they were further purified. This was done by redissolving them in water, and allowing them to run through a polyamide + celite column (Jarman and Crowden, 1973), when the brown substances were adsorbed onto the polyamide. The elutant from this first column was adsorbed onto a Zeo-Karb 226H^+ (Permutit Co.) column, from which it was eluted with MAW (Jarman and Crowden, 1973). The elutant was concentrated to dryness and then partially hydrolysed in the following way.

Solid anthocyanin was dissolved with methanol and placed in a test tube. A sample of this solution was spotted onto two No. 1 Whatman's Chromatography papers, and the spot was labelled 'time 0'. 1 ml. of 2N hydrochloric acid was added to the test tube, which was placed in a boiling water bath for 30 minutes. A sample was withdrawn from the tube and spotted onto the two papers at times 6 and 12 minutes. One of the papers was run overnight in BAW, the other for six hours in 3% hydrochloric acid (V/v). The

results of this partial hydrolysis show the number of intermediates in the breakdown of the anthocyanin, and this number indicates whether the sugars present are attached to one or more hydroxyl groups. Thus, 3-monosides yield no intermediates, 3-diglycosides yield one intermediate and 3,5 diglycosides give two intermediates (Harborne,1967).

After the thirty minutes boiling, the test tubes were removed from heat and distilled water was added to cool them, and to increase the volume of solution. To extract the aglycone n-amyl alcohol was added, the mixture was shaken, and on settling the upper layer (in which the aglycone had dissolved) was withdrawn. This was evaporated to dryness on a watch glass. Later, the aglycone was spotted onto No. 1 papers with appropriate reference compounds, and a paper was run overnight in each of two solvents, forestal and formic acid (see Appendix). The formic acid run was necessary to identify the anthocyanidin, peonidin.

The lower layer contains the sugar molecules attached to the original anthocyanin. To extract these amounts of N, N di-n-octylmethyl amine/chloroform (Appendix) were shaken with the solution several times (up to seven times). This neutralises mineral acid. When the less dense layer was neutral, the last chloroform layer was withdrawn, and the sugar solution was washed twice or thrice with chloroform. The sugar layer was then withdrawn, and evaporated to dryness on a watch glass in a vacuum dessicator for at least 24 hours.

The sugar was later redissolved in a minimum of water, spotted onto No. 1 paper with reference sugars, and run overnight in pyridine solvent (Appendix). The reference sugars were 1% concentrations of xylose, arabinose, glucose,

galactose and rhamnose. After the paper had dried in a fume cupboard, it was sprayed with aniline hydrogen phthalate, and placed in an oven set at 100°C for 5 minutes. After this time spots representing sugars became evident in visible light (Lederer and Lederer, 1957).

2-3 Results:

Table 2 shows the anthocyanins isolated and their occurrence in the twenty-four species examined, and in table 3, the R_f value of each compound recorded in the current work is given. The compounds for which the R_f's were measured were not under standardised conditions, and contamination with other chemicals produced lagging. However, the reference compounds used in co-chromatography gave similar R_f's.

In a number of species an unusual and inconsistent compound was found. The species are E. ovata, E. dalrympleana, E. gunnii, E. urnigera, E. perriniana, E. cordata, E. globulus, E. johnstonii, E. sieberi, E. pauciflora, E. amygdalina, E. coccifera, E. risdoni and E. tasmanica. Subsequent test runs showed this compound to be an artifact of cyanidin 3-glucoside, produced as a result of the interaction of methanolic-hydrochloric acid extraction and the BAW solvent. Runs in BAW solvent resulted in two bands from a single original band of cyanidin 3-glucoside; one of these bands corresponded to cyanidin 3-glucoside and the other was a faster moving region of an acylated derivative of cyanidin 3-glucoside (Timberlake et al., 1971).

It was difficult to separate the glucoside and galactoside of each of cyanidin, delphinidin and malvidin,

their R_f's being very similar. The presence of the two together was indicated during hydrolysis when the mixture hydrolysed as a monoside, but both glucose and galactose sugars were present. This was not detected in the early work, but back-checking enabled me to discover in which species the galactoside had occurred. Such a check was not made on E. linearis, so it is possible that the absence of the cyanidin 3-galactoside shown for this species is incorrect. It was usually difficult to estimate which of the two was in greater concentration, but in most cases it appeared to be the glucoside.

Similarly, in the few instances in which it was found, cyanidin 3-rhamnosylgalactoside was difficult to separate from the rutinoside (rhamnosylglucoside).

It will be noted that several of the minor compounds (see part b of table 2), are but tentatively identified, if identified at all. These compounds were not available in sufficient quantity to allow isolation and subsequent identification, and are the ones called multi-sugar, cyanidin 3-sambubioside (this is only thought to be a cyanidin compound as it occurred in macrantherous species; the aglycone was not positively identified), cyanidin 3-rhamnoside, cyanidin 3-arabinoside, 'pauciflora' compounds 14 and 15, and "special sugar" compounds 1 and 2. The sambubioside is queried in the case of E. gunnii as the R_f's were out (again, the compound was not hydrolysed) - the compound in E. gunnii has a BAW R_f of 0.19, its R_f in 3% hydrochloric acid is 0.50 and in WAH (see Appendix) is 0.60. The two unusual compounds found in E. pauciflora appeared influenced

by environmental factors for they were only detected in highland samples, and then not universally.

The 3,5-diglycosides containing both glucose and galactose present some problem. Two of these ran very close together in the solvents used, and mixed also with cyanidin 3,5-glucoside. n-pentanol was another solvent used in attempting to separate them, but with no success. Eventually, it became possible to remove them from the diglucoside, but a mixed band of peonidin and malvidin glycosides remained, a band in which one or other anthocyanin predominated, but never to the complete exclusion of the other. This mixed band was hydrolysed and produced the typical breakdown products of the two diglycosides (each distinguished by the colour), and the aglycones were shown to be peonidin and malvidin, the sugars glucose and galactose. However, as neither anthocyanin could be obtained in completely pure form, the position of the glucose and galactose sugars could not be determined unequivocally. I have called the compounds peonidin 3-galactose, 5-glucoside and malvidin 3-galactose, 5-glucoside respectively as galactose has never been reported in the literature substituted at the 5 position. The band containing the breakdown intermediate (i.e. the monosides of peonidin and malvidin) was cut out and eluted. The small quantity of material obtained was hydrolysed, in the hope that a sugar analysis would show galactose in greater quantity than glucose; in other words, supporting the hypothesis that the intermediate product is the galactoside. However, due to an unsatisfactory chromatogram, the situation was no further clarified.

Similarly, cyanidin 3-galactose, 5-glucoside was shown by hydrolysis to be a diglycoside of cyanidin with galactose and glucose sugars present. Again, because of the small quantity of substance remaining after hydrolysis, positive identification of the position of particular sugar attachments was not possible. The considerable number of anthocyanins in those species where the more highly methylated anthocyanins abound resulted in a great deal of mixing of the cyanidin 3-galactose, 5-glucoside, so that even though a very large quantity of raw extract was used, sometimes more than once, the amount of purified anthocyanin at the end of the isolation procedure, was very meagre.

2-4 Interpretations from the Data:

It is not valid to include any of the minor compounds as diagnostic chemotaxonomic markers, these compounds often only occurring as small amounts in single species. Thus, the eleven minor compounds (cyanidin 3-sambubioside, cyanidin 3-rhamnoside, cyanidin 3-arabinoside, malvidin 3-rhamnosyl-glucoside, malvidin + xylose, multi-sugar, special sugar 1 and special sugar 2, bioside with glucose, and 'pauci' 14 and 15) will generally be ignored in the following interpretative analysis.

a) Anthocyanins and their Distribution:

It is obvious that the most widespread anthocyanins in the genus are those based on cyanidin - such anthocyanins have occurred in every organ of every species examined in the current review and in past work (Sharma, 1970). It is significant also that quercetin, the flavonol analogue of

cyanidin, was the most dominant flavonol, and one found in every organ in the earlier work.

Cyanidin 3-glucoside is the most common anthocyanin, being absent from no sample studied. In certain renantherous species it is difficult to separate from the cyanidin 3-galactoside, but in every case hydrolysis has shown the glucoside to be present. It is of interest that the glucoside is generally in higher concentration in bark tissue than in leaf tissue. Cyanidin 3-galactoside cannot be detected in any macrantherous species, and seems absent from the Stringybarks and E. coccifera, E. risdoni and E. tasmanica, in which species it was specifically looked for. The diglucoside of cyanidin is common to both twig bark and leaves in all the macrantherous species, but the ratio of the amounts of cyanidin 3-glucoside and cyanidin 3,5 diglucoside in leaf and twig bark varies. There is a tendency for cyanidin 3,5 diglucoside, to replace cyanidin 3-glucoside as the more dominant anthocyanin in young juvenile leaves.

In renantherous species, cyanidin 3,5 diglucoside is undetected in the bark of all species except E. delegatensis, and in this species it was found in trace amounts. It is interesting that in the leaves of all the renantherous species except E. coccifera, the diglucoside is a major compound, usually in greater concentration than the cyanidin 3-glucoside, which is the dominant cyanidin compound in bark. Apparently bark of renantherous species has a more reduced ability (if any) to synthesise cyanidin 3,5 diglucoside than has the bark of macrantherous species.

Cyanidin 3-rhamnosylglucoside (rutinoside) has been

found in all renantherous species, but is absent in a few of the macrantherous species. Of interest is the fact that a bioside of cyanidin having as the sugars rhamnose and galactose (robinobioside?), and having similar chromatographic properties as cyanidin 3-rutinoside, is present in the Ashes and in E. pauciflora twig bark. This occurs as a minor compound only, but it can be noted that the occurrence of galactose as a substitution sugar is in this specific case restricted to the renantherous section, being not detected at all in macrantherous species. Lowry (1971) suggests that the production of galactoside/arabinosides is a taxonomic character alternative to the production of glucosides at species level. This is not so in eucalypts, for where galactosides occur, they do so together with glucosides.

The only other major cyanidin compound found was cyanidin 3-galactose 5-glucoside, and this was restricted to the young juvenile leaves of E. sieberi.

Some delphinidin glycosides occur, but not as frequently as their cyanidin counterparts (myricetin, the flavonol analogue of delphinidin, was earlier found to be the second-most widely distributed flavonol). The delphinidin compounds are more frequent in renantherous species, but two pigments, delphinidin 3-glucoside and delphinidin 3,5 diglucoside occur as major compounds in both leaves and bark of E. ovata, a macrantherous species. The 3-glucoside of delphinidin is also a major pigment in E. urnigera, E. perriniana, E. cordata and E. globulus, but only in the bark of these macrantherous species. The amount of delphinidin 3,5 diglucoside in other macrantherous species besides E. ovata, varies from a major amount in E. perriniana bark to nil in the majority. It is noted that no delphinidin compound (including leuco-

delphinidin) has been found in the Lowland White Gums (E. viminalis, E. dalrympleana and E. rubida).

In the renantherous species delphinidin 3-glucoside is of universal occurrence, except that it has not been detected in the young juvenile leaves of E. delegatensis. The 3,5-diglucoside is a major compound in the young juvenile leaves of all renantherous species except E. coccifera (in which species it is absent), while the 3-glucoside of delphinidin tends to replace it as the more dominant of the two delphinidin anthocyanins in the bark of the same species.

The galactoside of delphinidin, which is similar chromatographically to delphinidin 3-glucoside, is found in many renantherous species, but again, as was cyanidin 3-galactoside, it is absent from the Ashes and from E. coccifera, E. risdoni and E. tasmanica. It does not occur in the Section Macrantherae.

The methylated anthocyanidins, peonidin and malvidin, form part of the major pigments in the young juvenile leaves of the Ashes, Stringy Barks and the three Peppermints, E. amygdalina, E. linearis and E. simmondsii. The major pigments are peonidin 3-galactose, 5-glucoside and malvidin 3-galactose, 5-glucoside (the Ashes produce other peonidin and malvidin glycosides, but only as minor components). In the Ashes and Stringy Barks these pigments are synthesised in the bark as well as in the young juvenile leaves, but to a lesser extent. This synthetic ability does not appear to have been retained by the bark of the Peppermints.

Outside the Section Renantherae, the methylated

anthocyanins have only been detected in small amounts, and this has been in the high altitude species, E. gunnii, E. urnigera, E. johnstonii and E. perriniana. The confinement of these methylated anthocyanins (as major pigments) to particular renantherous groups provides a very useful taxonomic marker. It is also of interest that galactose is only found outside the section Renantherae when it forms part of the methylated anthocyanins which even then only occur as minor components.

I would suggest that these methylated anthocyanins could in fact be the "pelargonin-like" compound (pelargonidin 3,5-diglucoside) reported by Banks and Hillis (1967) in E. camaldulensis. In current and in previous work there has been no detection of pelargonidin in any of the species studied. Harborne (1967) gives similar R_f values for pelargonidin 3,5-diglucoside and peonidin 3-galactose, 5-glucoside, so on R_f values alone the two could be confused.

b) Chemical characters and the Taxonomy of the Eucalypts:

One cannot distinguish a species by its chemical profile alone, but within each of the two sections in Tasmania some division into groups can be made.

i) Ovata: delphinidin 3-glucoside and delphinidin 3,5-diglucoside as major pigments, along with their cyanidin analogues. Such a grouping needs to be supported by the presence of the delphinidin compounds in E. aggregata, in which species they have not to date been detected. E. ovata and E. aggregata are the only species of their group represented in Tasmania, but they cannot be regarded as closely-related species within this group. The Tasmanian

form of E. aggregata (E. rodwayi, Pryor and Johnson, 1971) differs from mainland members of the species.

ii) Lowland White Gums: Absence of any delphinidin compounds.

iii) Stringy-barks: (a) Absence of cyanidin 3,5-diglucoside and delphinidin 3,5-diglucoside in bark, but their presence, and as major pigments, in young juvenile leaves; (b) Absence of the galactosides of cyanidin and delphinidin; (c) Presence of peonidin 3-galactose, 5-glucoside and malvidin 3-galactose, 5-glucoside as major compounds in the young juvenile leaves.

iv) Ashes: (a) Absence (or trace amounts only) of cyanidin 3,5-diglucoside and delphinidin 3,5-diglucoside in bark but their presence as major pigments in young juvenile leaves; (b) presence of delphinidin 3-galactoside and cyanidin 3-galactoside as major pigments in bark and young juvenile leaves; (c) the presence of the methylated anthocyanins as major pigments in the young juvenile leaves.

v) E. pauciflora: (a) absence of cyanidin 3,5-diglucoside and delphinidin 3,5-diglucoside in the bark, but their moderate occurrence in young juvenile leaves; (b) Presence of cyanidin 3-galactoside and delphinidin 3-galactoside in major amounts in the bark; (c) The absence of methylated anthocyanins in both organs.

vi) Peppermints (E. amygdalina, E. linearis and E. simmondsii): (a) Possible occurrence of the galactosides of cyanidin and delphinidin in some organs; (b) Lack of cyanidin 3,5-diglucoside and delphinidin 3,5-diglucoside in the bark, but their occurrence as major pigments in the young juvenile leaves; (c) Presence of methylated

pigments, but not as major compounds, in the young juvenile leaves, but never in the bark.

vii) E. coccifera: (a) Absence of galactosides; (b) Absence of cyanidin 3,5-diglucoside from the bark and its occurrence as only a minor pigments in the leaves; (c) complete absence of delphinidin 3,5-diglucoside in both organs; (d) complete absence of methylated anthocyanins from both organs.

viii) E. risdoni and E. tasmanica: (a) Absence of galactosides; (b) Complete absence of methylated anthocyanins.

Chemical trends observed in the earlier survey of polyphenols support the above groupings (see Appendix - pages 1 - 14), and it is relevant to mention these at this point.

1. Aglycones

i) Myricetin: This flavonol analogue of delphinidin is more common in renantherous species, and tends to replace quercetin in the Peppermints (except E. coccifera). It is only found occasionally, and then in trace amounts, in the Lowland White Gums.

ii) E. coccifera: Certain yellow aglycones (numbers 22 and 23) are specific to this species, as is the blue-fluorescing aglycone number 21. E. coccifera has a higher level of ellagic acid than other Peppermints. Aglycone number 14 is present in a large quantity in it (and in certain macrantherous species), while it seems a relict chemical in the other Peppermints. E. coccifera is also characterised by having lower amounts of delphinidin in the bark, and myricetin in

the bark and in young and older juvenile leaves, than other Peppermints. It shares 'polyphenol' aglycone number 16 with E. perriniana.

iii) E. delegatensis and E. obliqua: E. delegatensis contains the species-specific aglycone number 12, and E. obliqua contains species-specific 'polyphenol' aglycone 19.

iv) Purple-blue fluorescing aglycones: E. dalrympleana has a compound (number 9) shared with E. risdoni, but one not present in any other macrantherous species. A group of purple compounds (numbers 13,14 and 15) is common to macrantherous species, and to E. coccifera, but is never found in E. dalrympleana.

v) Macrantherous aglycones: Compounds 17 and 20 are restricted to species in the section Macrantherae.

2. Alcohol-soluble Substances

i) Species-specific compounds: These occur in E. obliqua (compound 72), E. coccifera (compound 74), E.tasmanica (compound 75), E. perriniana (compound 76), E. amygdalina (compound 77), E. pauciflora (compunds 80 and 81) and E. archeri (compounds 82 and 83).

ii) Compounds of restricted occurrence: These are three in number; compounds 73, which occurs in E. archeri, E. risdoni, E. coccifera and E. perriniana; compound 78 occurs in E. linearis and E. amygdalina; and compound 79 occurs in E. amygdalina and E.pauciflora.

iii) Compounds 70 and 71: These compounds are restricted to renantherous species.

iv) Compound 39: This compound is restricted to the section Macrantherae.

v) Complexes A and B: These consist of several yellow-fluorescing substances which showed interesting quantitative variation. The Blue Gums had low quantities of both A and B, while other macrantherous species possessed medium to low amounts of these. Both Ashes and Stringy-barks showed high amounts of each complex, but with slightly more of A in the Stringy-barks, and slightly more of B in the Ashes. E. coccifera, E. tasmanica and E. pauciflora possessed only low to medium amounts of both complexes, while the other Peppermints had high A concentration and low B concentration.

vi) Complex C: This complex comprises three compounds with intense blue fluorescence in ultra-violet light. These occur in high concentrations in every macrantherous species examined, and in E. coccifera. They appear as minor pigments in E. risdoni, E. pauciflora and E. tasmanica, and are completely absent from the remaining renantherous species.

vii) Complex D: Two blue-fluorescing pigments which turn duck-egg green on addition of ammonia solution make up complex D. Again, these occur as important pigments in organs of all the macrantherous species examined, and in only E. pauciflora, E. tasmanica, E. simmondsii and E. coccifera of the section Renantherae. Again, of these renantherous species, the compounds are found in greatest quantity in E. coccifera.

vi) Complex F: This consists of five purple-fluorescing compounds and is restricted to macrantherous species except in one instance, when two of the five pigments

occur as minor compounds in the young juvenile leaves of E. delegatensis. Within the Macrantherae, they are absent from the ovata group only, and are always in the twig bark of the other species. Within the Lowland White Gums the complex occurs in high quantity in the young juvenile leaves as well as the twig bark of E. dalrympleana and E. rubida, but only in the bark of E. viminalis.

c) Evolutionary Aspects:

The difficulty which exists when deciding if one morphological character is advanced over any other morphological character (and this difficulty is due to scarcity of information regarding the development of different morphological characters from one another), is not evident when dealing with chemical characters which are biosynthetically related; e.g. different anthocyanins.

Leaf pigments tend to be simpler, both in anthocyanidin and in the pattern of glycosidation, than flower or fruit pigments - similarly do bark pigments. Of the pigments found in eucalypts, cyanidin is considered the most primitive because a) it exists in the greatest amounts in the least specialised of organs of all sorts of species studied; and b) it is present in greater amount in the Archichlamydae than in the Sympetalae, the cyanidin ⟶ delphinidin sequence being considered a gain mutation associated with evolution towards a blue colour (Harborne, 1963, 1966). Thus delphinidin is the more advanced of the two pigments.

As flower colour has further evolved, there has been a tendency for plants to produce more complex pigments, hence the sequence, cyanidin 3-glucoside ⟶ complex acylated co-pigmented malvidin triglycoside, represents considerable

evolutionary advance. That malvidin predominates among the methylated anthocyanins is explained by the hypothesis that the systems for methylation and for adding the third hydroxyl group to the anthocyanidin B ring evolved together (Harborne, 1963). The advance has resulted in greater pigment stability, as a complex pigment without o-dihydroxy groups, and having several sugars attached is much more stable to light and to enzymatic attack than is a simple cyanidin derivative (Harborne, 1963).

Furthermore, Harborne considers the evolutionary sequence monoside ⟶ diglycoside to have occurred. In the eucalypts the diglucoside does tend to replace the monoside in bark as the dominant anthocyanin in young juvenile leaves, the leaves being the more specialised of the two organs. Diglycosylation appears to be associated with a higher degree of methylation of the aglycone - this explains why the monosides of peonidin and malvidin are only minor pigments in those groups which possess large quantities of the methylated anthocyanins. Glycosidic character may be more useful as a marker than the anthocyanidin identity, because the former is much less variable genetically.

Of the two eucalypt sections investigated, the section Macrantherae is considered the less advanced of the two, and one in which the final placement of certain species is not final, while the section Renantherae is the more advanced, and one still vigorous in evolution radiation. The chemical trends support this - the 'advanced' delphinidin, malvidin and peonidin glycosides are more common to renantherous species (although within this section, certain species, e.g. E.coccifera, E. pauciflora, E. risdoni and E. tasmanica, may be less

advanced). Such 'advanced' compounds are only minor components in the macrantherous species in which they occur. Furthermore, the methylated anthocyanins occurring frequently in renantherous species are in the 'advanced' diglycoside form, rather than in monoside form.

One final point that is of interest concerns those eucalypts which fill strange or rigorous ecological niches, e.g. E. coccifera, E. dalrympleana, E. urnigera, E.perriniana. They may vary greatly in their chemistry from closely-allied species. As an example, E. coccifera is more aligned with macrantherous than renantherous species. This perhaps shows a retention of primitive compounds once common to all species in an "odd" member of a certain group, simply due to its extreme environment. The synthesis of the advanced methylated anthocyanins in E. urnigera, E. perriniana, E. johnstonii and E. gunnii, all high altitude species, but ones less advanced than the renantherous species, indicates the parallel but separate evolution of the ability to synthesise these pigments. It would be interesting to learn if there is a common adaptation significance for these compounds in the species of the two sections in which they occur.

TABLE 1

Species	Location(s)	Collector
E. ovata Labill.	Mt. Nelson	Self
E. aggregata Deane & Maiden	Central Plateau	Dr.R.K.Crowden
E. viminalis Labill.	Ti-tree area; Uni.	Self
E. rubida Deane & Maiden	Upper Plenty Road	Self
E. dalrympleana Maiden	Central Plateau	Dr.R.K.Crowden
E. gunnii Hook.f.	Central Plateau	Dr.R.K.Crowden; self
E. urnigera Hook. f.	Mt. Wellington	Self
E. perriniana F.Muell. Rodw.	Sandy Bay	Dr.R.K.Crowden
E. cordata Labill.	Ridgeway	Self
E. globulus Labill.	Tasman Peninsula; Southern Outlet Rd.	Dr.R.K.Crowden; self
E. johnstonii Maiden	Mt. Wellington	Self
E. obliqua L'Herit.	Huon Rd. near Pillinger's Drive	Self
E. regnans F.Muell.	Hartz Mountains	Dr.R.K.Crowden
E. sieberi L. Johnson	East Coast	Dr.R.K.Crowden
E.delegatensis R.T.Baker	Tasman Peninsula	Dr.R.K.Crowden
E.pauciflora Sieb.	Central Plateau; Ti-tree	Dr.R.K.Crowden self
E. linearis Dehn.	Mt. Nelson	Self
E. amygdalina Labill.	Howell	Self
E. simmondsii Maiden	West Coast	Dr.R.K.Crowden
E.coccifera Hook.f.	Mt. Wellington	Self
E. risdoni Hook.f.	Ridson	D.Hamilton; self
E. tasmanica Blakely	Huon Road	D. Hamilton; self

Table 2a: Distribution of Anthocyanins

	cy.3-G.	cy.3-Gal.	cy.3-RG.	cy. 3-RGal.	cy.3G,5G.	cy.3Gal. 5-G.	dp.3-G.	dp.3-Gal.	dp.3-G,5-G.	pn.3Gal. 5G.	mv.3-Gal. 5G.	2nd pn. cpd.	mv.3-G.	mv.3-Gal.
E. ovata B	3+	-	-	-	5+	-	2+	-	3+	-	-	-	-	-
E. ovata LY	4+	-	-	-	5+	-	2+	-	3+	-	-	-	-	-
E. aggregata B	5+	-	2+	-	5+	-	-	-	-	-	-	-	-	-
E. aggregata LY	4+	-	3+	-	5+	-	-	-	-	-	-	-	-	-
E. viminalis B	5+	-	-	-	4+	-	-	-	-	-	-	-	-	-
E. viminalis LY	2+	-	t	-	5+	-	-	-	-	-	-	-	-	-
E. viminalis AY	t	-	-	-	3+	-	-	-	-	-	-	-	-	-
E. rubida B	5+	-	t	-	+	-	-	-	-	-	-	-	-	-
E. rubida LY	5+	-	t	-	3+	-	-	-	-	-	-	-	-	-
E. dalrympleana B	5+	-	t	-	3+	-	-	-	-	-	-	-	-	-
E. dalrympleana LY	4+	-	+	-	5+	-	-	-	-	-	-	-	-	-
E. gunnii B	4+	-	-	-	5+	-	-	-	-	-	-	-	-	-
E. gunnii LY	4+	-	t	-	5+	-	-	-	-	t	t	-	-	-
E.urnigera B	5+	-	t	-	4+	-	2+	-	+	+	-	-	-	-
E. urnigera LY	5+	-	t	-	4+	-	-	-	-	t	-	-	-	-
E. perriniana B	5+	-	3+	-	4+	-	2+	-	2+	+	?	-	-	-
E. perriniana AY	4+	-	3+	-	5+	-	-	-	-	-	-	-	-	-

Table 2a cont.

E. cordata B	5+	-	+	-	3+	-	2+	-	t	-	-	-	-	-
E. cordata LY	3+	-	3+	-	5+	-	-	-	t	-	-	-	-	-
E. globulus B*	2+	-	t	-	5+	-	-	-	t	-	-	-	-	-
E. globulus LY*	2+	-	t	-	5+	-	-	-	t	-	-	-	-	-
E. globulus B+	3+	-	2+	-	5+	-	2+	-	+	-	-	-	-	-
E. globulus LY+	3+	-	t	-	5+	-	-	-	+	-	-	-	-	-
E. johnstonii B	5+	-	-	-	5+	-	-	-	-	-	-	-	-	-
E. johnstonii LY	2+	-	-	-	5+	-	-	-	-	t	?	-	-	-
E. subcrenulata	Very little anthocyanin but Cy3G present in both B and LY													
E. obliqua B	5+	-	+	-	-	-	2+	-	-	+	?	t	-	-
E. obliqua LY	3+	-	2+	-	5+	-	2+	-	4+	2+	2+	t	-	-
E. regnans B	5+	-	+	-	-	-	2+	-	-	t	?	-	-	-
E. regnans LY	3+	-	?	-	5+	-	+	-	4+	2+	2+	-	-	-
E. delegatensis B	3+	2+	2+	+	t	-	2+	+	-	+	+	-	+	+
E. delegatensis LY	+	+	+	+	5+	-	-	-	2+	+	+	?	-	-
E. sieberi B	3+	2+	t?	t?	-	-	2+	2+	-	+	?	-	-	-
E. sieberi LY	2+	+	-	-	4+	2+	2+	2+	2+	2+	3+	+	t	t
E. pauciflora B	3+	2+	t	t	-	-	2+	+	-	-	-	-	-	-
E. pauciflora LY	5+	-	+	-	3+	-	2+	-	2+	-	-	-	-	-

Table 2a Cont.

E. linearis B	5+	-	-	-	-	-	4+	-	-	-	-	-	-	-
E. linearis LY	3+	-	t	-	5+	-	3+	-	4+	?	?	-	-	-
E. amygdalina B	3+	2+	-	-	-	-	2+	2+	-	-	-	-	-	-
E. amygdalina LY	3+	2+	2+	-	5+	-	4+	-	4+	+	+	-	-	-
E. simmondsii B	5+	?	-	-	-	-	4+	-	-	-	-	-	-	-
E. simmondsii LY	3+	2+	+	-	4+	-	3+	-	2+	?	?	-	-	-
E. coccifera B	5+	-	+	-	-	-	4+	-	-	-	-	-	-	-
E. coccifera LY	5+	-	+	-	+	-	+	-	-	-	-	-	-	-
E. risdoni B	5+	-	t	-	-	-	4+	-	-	-	-	-	-	-
E. risdoni LY	5+	-	+	-	5+	-	3+	-	4+	-	-	-	-	-
E. tasmanica B	5+	-	-	-	-	-	4+	-	-	-	-	-	-	-
E. tasmanica LY	4+	-	+	-	4+	-	3+	-	5+	-	-	-	-	-

Key Table 2a - t trace amount; * Tasman Peninsula species; + Southern Outlet Road species;
B Bark; LY Young juvenile leaves; AY Young adult leaves.

Cy. cyanidin
dp. delphinidin
pn. peonidin
mv. malvidin

G. glucose
Gal. galactose
R. rhamnose

Table 2b: Distribution of Anthocyanins

	cy.3-samb.	cy.3-arab.	cy.3-R.	mv.3-R.	mv.+X	M.S.	S.S. 1	S.S. 2	G.Bioside	P14	P15	BAW acylation
E. ovata B	-	-	-	-	-	t	-	-	-	-	-	-
E. ovata LY	-	-	-	-	-	-	-	-	-	-	-	✓
E. aggregata B	-	-	-	-	-	-	-	-	-	-	-	-
E. aggregata LY	-	-	-	-	-	-	-	-	-	-	-	-
E. viminalis B	-	-	-	-	-	-	-	-	-	-	-	-
E. viminalis LY	-	-	-	-	-	-	-	-	-	-	-	-
E. viminalis AY	-	-	-	-	-	-	-	-	-	-	-	-
E. rubida B	-	-	-	-	-	-	-	-	-	-	-	-
E. rubida LY	-	-	-	-	-	t	-	-	-	-	-	-
E. dalrympleana B	-	-	-	-	-	-	-	-	-	-	-	✓
E. dalrympleana LY	-	-	-	-	-	-	-	-	-	-	-	-
E. gunnii B	+?	-	-	-	-	-	-	-	-	-	-	-
E. gunnii LY	-	-	-	-	-	t	-	-	-	-	-	✓
E. urnigera B	-	-	-	-	-	+	-	-	-	-	-	✓
E. urnigera LY	-	-	-	-	-	-	-	-	-	-	-	✓
E. perriniana B	+	-	-	-	-	+	-	-	-	-	-	✓
E. perriniana AY	-	-	-	-	-	-	-	-	-	-	-	✓

Table 2b cont.

E. cordata B	+?	-	-	-	-	t	-	-	-	-	-	✓
E. cordata LY	-	-	-	-	-	-	-	-	-	-	-	-
E. globulus B*	-	-	-	-	-	-	-	-	-	-	-	-
E. globulus LY*	-	-	-	-	-	-	-	-	-	-	-	-
E. globulus B+	-	-	-	-	-	-	-	-	-	-	-	-
E. globulus LY+	-	-	-	-	-	-	-	-	-	-	-	✓
E. johnstonii B	-	-	-	-	-	-	-	-	-	-	-	-
E. johnstonii LY	-	-	-	-	-	-	-	-	-	-	-	✓
E. obliqua B	-	-	-	-	-	-	-	-	-	-	-	-
E. obliqua LY	-	-	-	-	-	-	-	-	-	-	-	-
E. regnans B	-	-	-	-	-	-	-	-	-	-	-	-
E. regnans LY	-	-	-	-	-	-	-	-	-	-	-	-
E. delegatensis B	-	+	-	+	-	-	+	-	-	-	-	-
E. delegatensis LY	-	-	-	-	-	-	-	-	-	-	-	-
E. sieberi B	-	?	-	-	-	-	-	-	-	-	-	✓
E. sieberi LY	-	-	-	-	t	-	-	-	2+	-	-	-
E. pauciflora B	-	-	-	-	-	+	-	+	-	+	+	✓
E. pauciflora LY	-	-	-	-	-	+	-	-	-	-	-	✓
E. linearis B	-	-	-	-	-	-	-	-	-	-	-	-
E. linearis LY	-	-	-	-	-	-	-	-	-	-	-	-

Table 2b cont.

E. amygdalina B	-	t	-	-	-	-	-	-	-	-	-	-
E. amygdalina LY	-	-	+	-	-	-	-	-	-	-	-	✓
E. simmondsii B	-	-	-	-	-	-	-	-	-	-	-	-
E. simmondsii LY	-	-	-	-	-	-	-	-	-	-	-	✓
E. coccifera B	-	-	-	-	-	-	-	-	-	-	-	-
E. coccifera LY	-	-	-	-	-	-	-	-	-	-	-	✓
E. risdoni B	-	-	-	-	-	-	-	-	-	-	-	✓
E. risdoni LY	-	-	-	-	-	-	-	-	-	-	-	✓
E. tasmanica B	-	-	-	-	-	-	-	-	-	-	-	✓
E. tasmanica LY	-	-	-	-	-	-	-	-	-	-	-	-

"pauciflora" compounds 14 and 15 are restricted to some highland samples. Special Sugar compound 2 was found in highland and lowland samples of E. pauciflora.

Key Table 2b - t trace amount; * Tasman Peninsula species; + Southern Outlet Road species; B Bark; LY Young juvenile leaves; AY Young adult leaves.

cy.	cyanidin	arab.	arabinoside	S.S.	"special sugar"
mv.	malvidin	R.	rhamnose	M.S.	multi-sugar
samb.	sambubioside	X	xylose	G.	glucose
				P	pauciflora

Table 3

R_f Values

Pigment	R_f BAW	R_f 3%HCl	R_f WAH	Other information
Cyanidin 3,5 diglucoside	0.09	0.21	0.33	Identified by hydrolysis
cyanidin 3-glucoside	0.23	0.073	0.18	"
cyanidin 3-galactoside	0.21	0.073	0.18	"
delphinidin 3-glucoside	0.12	0.04	0.12	"
delphinidin 3-galactoside	0.11	0.04	0.12	"
delphinidin 3,5-diglucoside	0.05	0.10	0.20	"
malvidin 3-glucoside malvidin 3-galactoside	0.33	0.14	-	"
malvidin 3-rhamnose, 3-glucoside	0.36	0.25-0.30	0.50	"
"pauciflora" 14	0.12	0.41	0.52	Aglycone lost; arabinose, trace of glucose
"pauciflora" 15	0.14	0.64	0.66	Aglycone lost; arabinose, galactose
malvidin + xylose	0.19	-	-	Although hydrolysed, too weak a spot to detect breakdown products
bioside with glucose	0.23	0.22	-	Aglycone lost
second peonidin cpd.	0.23	0.37	0.48	Total hydrolysis showed presence of peonidin and glucose
cyanidin 3-sambubioside	0.29?	0.33	0.53	No breakdown products, but cyanidin, glucose found, and xylose queried.

Table 3 cont.

cyanidin 3-rhamnoside	0.31	0.08	-	Its position relative to cy 3-G suggests this identity; rhamnose was found after hydrolysis
cyanidin 3-arabinoside	0.28	0.08	0.16	Identified by hydrolysis
multi-sugar compound	0.13	0.33	0.50	cyanidin and glucose detected; could be the sophoroside?
special sugar 1	0.10	0.53	0.62	Aglycone lost - suspected to be cyanidin: complete hydrolysis performed - glucose detected plus an unusual darkish-blue spot in the position for arabinose; fits cyanidin 3-sophoroside, 5-arabinoside, but no fluorescence could be detected in the very small quantity available.
special sugar 2	0.10	0.34	-	Aglycone lost; heavy galactose, arabinose; also dark spot in the position of xylose; appears to be a monoside, but insufficient quantity for firm test.
cyanidin 3-rhamnose, 3-glucoside	0.21	0.17	0.35	Identified by hydrolysis
cyanidin 3-rhamnose, 3-galactoside	0.18	0.17	-	"
cyanidin 3-galactose, 5-glucoside	0.08	0.12	0.25	"
peonidin 3-galactose, 5-glucoside	0.21	0.23	$\simeq$ 0.31	"
(3-glucoside - hydrolysis information)	0.31	0.12	-	
malvidin 3-galactose, 5-glucoside	0.19	0.17	$\simeq$ 0.29	"
(3-glucoside - hydrolysis information)	0.27	0.06	-	

There is the possibility of a compound similar to peonidin 3-glucose, 5-glucoside occuring in young juvenile leaves of E. sieberi, but only in trace amounts.

KEY TO ANTHOCYANINS IN FIGURE 2-1

1. Cyanidin 3-glucoside and cyanidin 3-galactoside.
2. Cyanidin 3,5-diglucoside.
3. Delphinidin 3-glucoside and delphinidin 3-galactoside.
4. Delphindidin 3,5-diglucoside.
5. Cyanidin 3-rhamnose, glucoside (=cyanidin 3-rutinoside).
6. Cyanidin 3-rhamnose, galactoside.
7. Peonidin 3-galactose, 5-glucoside.
8. Malvidin 3-galactose, 5-glucoside.
9. Cyanidin 3-galactose, 5-glucoside.
10. Malvidin 3-glucoside and malvidin 3-galactoside.
11. Malvidin 3-rhamnose, 5-glucoside (malvidin rutinoside).
12. Second peonidin compound.
13. Cyanidin 3-sambubioside.
14. Multi-sugar compound.
15. Cyanidin 3-rhamnoside.
16. Cyanidin 3-arabinoside.
17. 'Pauciflora' compound 14.
18. 'Pauciflora' compound 15.
19. Special sugar compound 1.
20. Special sugar compound 2.
21. Bioside with glucose.
22. Position of BAW acylated compound (i.e. acylated cyanidin 3-glucoside).

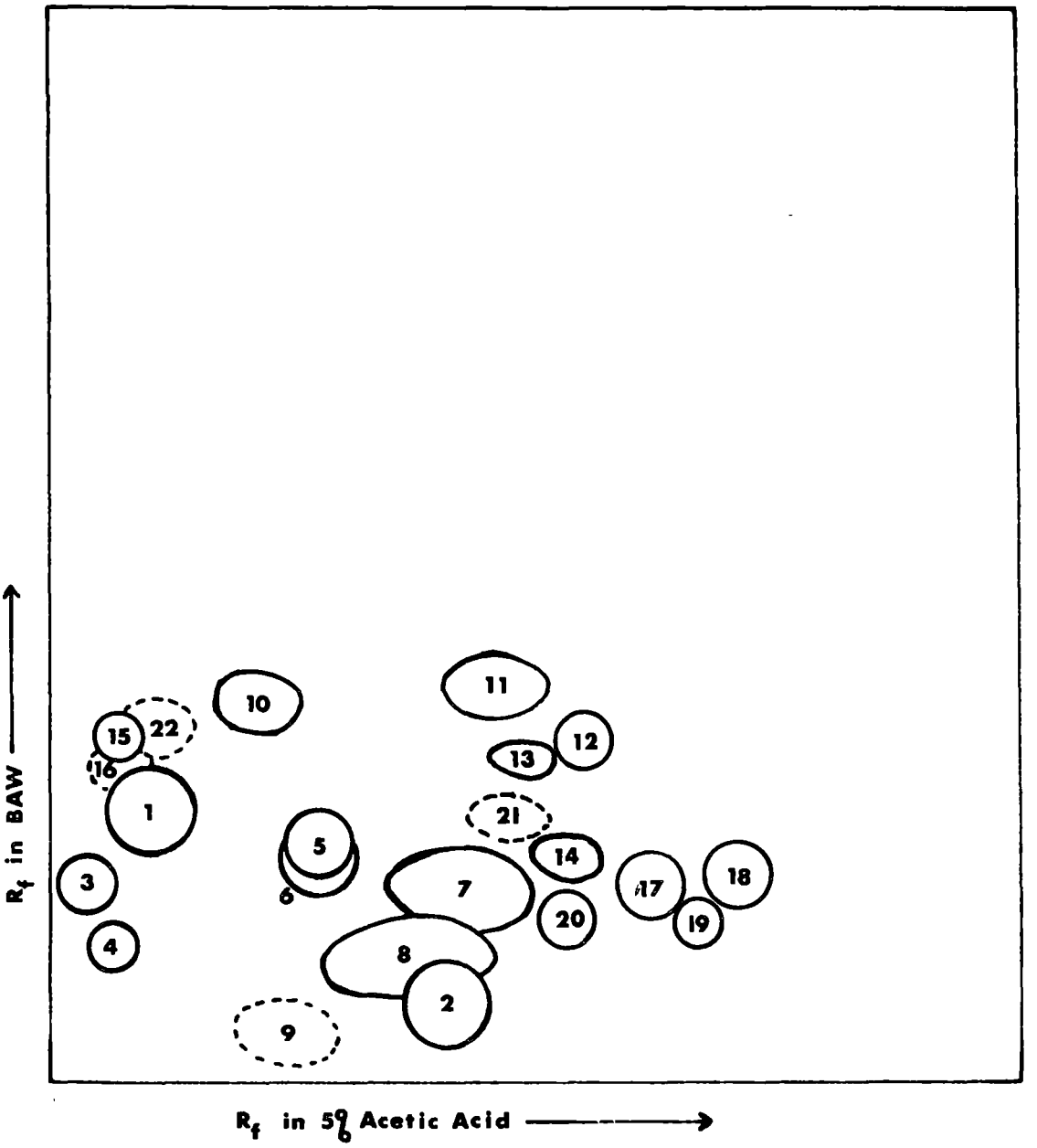


Figure 2-1: Anthocyanins of Eucalypts.

3. MACROMOLECULAR STUDIES IN EUCALYPTUS

3-1 Introduction

Exact knowledge of the base sequences in the DNA of a species would provide the most direct approach to biochemical taxonomy. Evolution of a species is the result of changes in nucleotide base sequence which are able to survive natural selection and be perpetuated. Specific proteins can give some indirect evidence of these sequences; hence diverging species would be expected to differ with respect to protein molecules originally homologous. As substitutions, additions or deletions occur during the course of evolution, progressively greater differences in the shape and charge on the protein molecule will occur. Thus the migration velocity of proteins under an electric current can give the degree of homology between protein bands within and between related taxa.

Proteins of species can be compared by electrophoresis in gels of low porosity where the electrophoretic mobility or migration velocity of proteins in the gel is determined by the charge on the protein and by the size and shape of the protein molecule. The pore size of the gel acts as a molecular sieve, for as the average pore size approaches the range of dimensions of the proteins, the various protein components will be differentially slowed in velocity proportional to their dimensions (Davis, 1964). The degree of homology of protein bands of different species as resolved by electrophoresis can give an estimate of their genetic similarity, from which evolutionary relationships may be inferred.

At the centre of origin of a species, one would expect

a simple, but heterogeneous banding pattern - as populations become isolated, progressive genetic divergence would lead to increased differences in the genetic make-up, until populations diverge to the extent that they are unable to hybridise. Thus a greater increase in specific banding patterns is observed in the 'derived' species. This has been shown in the seed proteins of Gossypium sp. (Cherry et al., 1970 and 1972; Johnson and Thein, 1970). Cultivated varieties show a varied and complex banding pattern. This has probably been induced by man selecting for greater modification of those characters which are important agronomically (Cherry et al., 1970).

Differences in banding patterns may be due to small genetic changes in the genome which had been selected for in different environments. These differences would result in slight changes in amino acid composition in the proteins, which could lead to variations in mobility.

It has been shown that remakable uniformity of protein patterns of like genotypes exist at comparable stages of development (Dessauer and Fox, 1962) and considerable use has been made of variations in total protein patterns in various taxa for delimiting species and genera, and determining the ancestors of hybrids and polyploids. Fox et al. (1964) and Boulter et al. (1967) found seed proteins of taxonomic use in the Leguminosae, as did Crowden et al. (1969) in the Umbelliferae; Vaughan et al. (1966) employed proteins in the study of Brassica spp. Protein banding has also been employed in fungal taxonomy - in Neurospora (Chang et al., 1962), Phytophora (Clare and Zentmeyer, 1966) and Pythium (Clare et al., 1968). Considerable use has been made of the method in investigating the ancestry of hybrids and polyploids - Johnson and Hall, 1965, 1966; Johnson, 1967 a and b; Johnson et al., 1967; Vaughan and Waite, 1967; Desborough

and Peloquin, 1966, 1969; Johnson and Thein, 1970; Cherry et al., 1970; Levin and Schaal, 1970; and Waines, 1969. Generally, the results paralleled those previously obtained from morphological, cytogenetical and chromatographic studies.

In addition to those stains available to react with total protein, there have been developed specific stains for particular enzymes - this allows classification of the proteins present. When specific stains are employed, many bands usually appear, indicating the presence of different protein molecules having the same enzymic specificity. Jaaska and Jaaska (1968) propose that all the structurally distinct forms of enzymes catalysing the same types of chemical reactions be called isoenzymes. With such a definition a further classification to distinguish homologous and non-homologous isoenzymes, is desirable. Homologous isoenzymes are those whose structure is genetically determined by the same locus or loci and which are therefore structurally related.

Comparison of isoenzyme patterns or "zymograms" have been employed in taxonomy of Hordeum (Mitra et al., 1970); Fabaceae (Thurman et al., 1967); Maize (Scandalios, 1964); Triticinae and Aegilops (Barber et al., 1968, Bhatia, 1968, Jaaska, 1970, 1971a and b); Umbelliferae (Crowden et al., 1969); Nicotiana (Reddy and Garber, 1971); Gossypium (Cherry et al., 1972) and peanut cultivars (Cherry and Ory, 1973). The majority of these support affinities based on other studies. In addition, "zymograms" from mycelial extracts of fifteen species of Aspergillus provided a useful taxonomic tool (Nealson and Garber, 1967), and esterase typing has helped in the identification of some lactic acid bacteria (Morrichi et al., 1968).

Many workers have reported enzyme patterns to be tissue-specific (Steward et al., 1965; Upadhya and Yee, 1968; Jaaska

and Jaaska, 1969; Parish, 1969, Mitra et al., 1970 and Reddy and Garber, 1971), and to change in an orderly fashion during differentiation (Mitra et al., 1970; Chen et al., 1970 and Hall et al., 1972). As all cells in different tissues of a plant carry the same genetic information, this indicates that the phenotype as expressed by enzyme patterns is determined by controlling genes which turn the synthesis of particular molecules on or off at certain times, and in specific tissues. The mechanism by which the genes are thus differentially activated is not known. The practical implication of this is that it is only valid in any comparison between taxa to use tissue of the same type and at a comparable stage in development from each organism. Also, it may be valuable to survey a range of organs to determine which source has the greatest number of invariant sites of enzyme activity to be of use in comparative studies.

Batteries of proteins which are enzymatic show a high degree of polymorphism; and this causes a problem when documenting banding patterns of species - thus one needs an initial survey of many organisms within a population to determine those enzyme sites which are invariant i.e. those for which high selective pressures to the exclusion of alternatives, are operating. As a conservative estimate, Lewontin and Hubby, (1966) and Hubby and Lewontin (1966), found an average polymorphism percentage of 30 for all loci in Drosophila pseudo-obscura when esterase, malic dehydrogenase, leucine aminopeptidase and three alkaline phosphatases all showed allelic variation. These polymorphic proteins are controlled by a pair or series of co-dominant alleles at each locus in the fruit fly (Wright, 1963). One must assume some evolutionary significance for this polymorphism, in view of the fact that heterozygotes are maintained in the population at a level of 12% - again a conserv-

ative and under-estimate (Lewontin and Hubby, 1966).

Similarly, within species polymorphism has been illustrated in plants, for example Maize (Scandalios, 1964; Macdonald and Brewbaker, 1972), Avena (Clegg and Allard, 1973), Gossypium (Cherry et al., 1972). The genetics of electrophoretic variants in Avena fatua were intensively investigated to show 5 loci each with 2 alleles governing the esterase banding pattern and one locus with 2 alleles for the peroxidase activity.

If a locus controls the formation of a particular polypeptide, then the production of hybrid enzymes in allopolyploids such as the bread-wheats (Barber et al., 1968) is explained, where the genes must still be able to be expressed in a background of 'foreign' genetic material. Thus the enhanced biochemical versatility and heterosis in hybrids could be due to the presence of more isoenzymes.

Cherry et al., (1970) maintain that storage proteins from seed, a dormant tissue, represent a more stable reflection of the genomic state in a given species than does that from developing seedlings. Such proteins may be under selective pressures: vernalisation and photoperiodic responses have evolutionary implications, so mutations affecting these and other early development processes could affect endosperm and embryo proteins, and result in variation between taxa (Johnson, 1968). Also, it is expected that mutations in reserve proteins will not impair the survival ability of the plant as long as the enzymes necessary for the establishment of the embryo can be hydrolysed from the changed protein. Jaaska and Jaaska (1969), and Juo and Stotsky (1970) demonstrated that the degradation of reserve proteins during seed germination was accompanied by the induction of the biosynthesis of new enzymes such as peroxidases. On the other hand, the plant cannot tolerate

indiscriminate mutations in the enzymes controlling vital cell processes. Thus, Mitra et al., (1970) observed inter-specific variations in Hordeum spp. for esterase and peroxidase (the precise role of these enzymes in plant metabolism is unknown), but not for glutamic acid dehydrogenase, (key enzyme providing link between nitrogen metabolism and the TCA cycle) and malate dehydrogenase (vital enzyme in the TCA cycle).

However, preliminary work with Eucalypt seed (Sharma, 1970) has indicated that the germinated seed yields some electrophoretic information about the enzymes which was not obtained using ungerminated seed. In the present study germinated seed was used.

3-2 Experimental Methods

(a) Seed origin

Seed was generally collected from a single tree, but more than one tree per species was sampled. Mr. Tom Walduck and the Forestry Department kindly provided seed from those species I was unable to sample personally.

(b) Methods of Germination

All seed was stratified at $6^{o}C$ for a minimum of eight days (maximum 12 days). Two methods of germination were tried, the second being permanently employed as it successfully eliminated mould growth which occurred in seedlings germinated following the first method.

1. Whatman's Seed Test Thick Paper was placed in a petri dish, moistened with distilled water and sprinkled with Thiram 80, a fungicide. Seed and chaff were spread out on the moistened paper. The petri dish and contents were stratified and later left at approximately $25^{o}C$ until germination occurred. High humidity built up in the petri dish and fungal contamination

occurred - so this method was replaced by the following one.

2. Equal quantities of vermiculite and perlite were mixed, placed in an apple juice can (diameter 155 cm., height 165 cm.) and moistened. The seeds were spread on this and a thin layer of the perlite and vermiculite mixture was placed on top and pressed down. After stratification these cans were left in the glasshouse and the seedlings were sampled some 5 to 10 days after germination.

The seedlings were raised under as controlled conditions as possible to cut down variation due to environmental factors. Lee and Ronalds (1967) and Reddy and Garber, (1971) pointed out the effect of environmental influences on some electrophoretic components; total proteins and different isoenzymes are influenced by substrate nutrient levels (van Lear and Smith, 1970); De Jong et al., (1968) reported that different peroxidases were secreted at different temperatures.

(c) Sampling

Reference has already been made to the differences existing between isoenzyme patterns in different organs and at different ontogenic stages. In the present study, total seedlings of a few species (e.g. E. aggregata, E. ovata, E. viminalis and E. sieberi) were sampled at intervals of two to four days between the unfolding of the cotyledon leaves from the seed coat, until the first sign of the first pair of leaves. The only differences which could be detected during this time were slight quantitative ones (this parallels changes found by Graham and Morton, 1963, in wheat endosperm developing over 28 days).

Many samples were also taken to determine the degree of variation between individual seedlings from a single tree, or from different trees. No qualitative variation was shown with total protein, esterase and acid phosphatase bands, but some quantitative variation occurred. Then batches of 6

seedlings were sampled. Between these the qualitative variation was very low (shown by an asterisk in the diagrams), and very little quantitative variation occurred. Peroxidase activity had been virtually undetected in single seedlings, but was very marked when samples of six were used, the number and intensity of bands per species being remarkably consistent between samples.

Subsequent runs to determine the enzyme activity profiles for the species were made using six seedling samples, each of these seedlings being 5 - 6 days germinated (this age did vary, but seedlings showing any development of the first true leaves were never used).

(d) Method of Extraction

Seed coats which may have adhered to seedlings were removed, and six seedlings selected at random were ground in a small agate mortar and pestle with a little acid-washed sand, and 12 - 18 drops of extracting solution (after the method of Bowling; private communication - see appendix).

Samples were pipetted from the mortar in 3 ml centrifuge tubes and were centrifuged at 11,000 - 14,000g for 15 - 20 minutes. On removal from the centrifuge, the samples were either used immediately in electrophoresis, or stored for up to 2 hours at 5°C before use.

(e) Electrophoretic Methods

Horizontal electrophoresis was carried out in 8% acrylamide gels in a Shandon apparatus. The gel mixture (see appendix) polymerized in a template as described by Mills and Crowden (1968). The tank buffer was a borate buffer giving a pH of 8.8 at 20 - 25°C (see appendix). Contact between the gel and the tank buffer was made with muslin wicks. Electrophoresis was carried out at 5°C, and after pre-electro-

phoresis, and at a constant current of 35 mA, the front, marked by bromothymol blue dye, migrated about 4.5 cm in 4 hours.

After electrophoresis, the gels were sliced horizontally into three using a Shandon 'slicer' and gels were then stained for protein or esterase or acid phosphatase or peroxidase.

A few trial runs were done using starch gels in an effort to detect transaminase activity, but these gave unsatisfactory 'short-lived' results, so were discontinued.

(f) Detection of Protein and Enzymes (for basis of staining reactions, see appendix):

i) Protein: Gels were placed in a saturated solution of amido black (1.4 gm amido black and 200 ml of 7% acetic acid) for upwards of an hour, and then destained by rinsing in fresh solutions of 7% acetic acid (method after that of Stewart et al., 1965).

ii) Peroxidase: 0.12 gm o-dianisidine was added to a 10 ml aliquot of 50% ethanol and heated gently until all crystals dissolved. 100 ml. acetate buffer at pH 5.5 (see appendix) was then added. Then 0.1% of hydrogen peroxide (30% w/v) was added. Dark brown bands indicating peroxidase were usually visible after half an hour and they sometimes began to fade in 24 hours (method of Mills and Crowden, 1968).

iii) Esterase Activity: Gels were incubated for 20 minutes in 100 ml phosphate buffer of pH 6.5 (see appendix). 20 mg of α-naphthyl acetate was dissolved in 1 ml. acetone; 1 ml of water was added, then all of this solution was put into 100 ml of phosphate buffer, pH 6.5. 20 - 50 mg Fast Blue RR (Gurr Chemicals)dissolved in a few millilitres of water was added to the above. Green-brown bands indicating esterase

activity began to appear after an hour, but did not fully develop for about 4 hours. (Method of Mills and Crowden, 1968). The bands were fixed in a fixative solution (see appendix).

iv) Acid Phosphatase: Gel was incubated for 20 minutes in 100 ml acetate buffer, pH 5.0 (see appendix). The staining reagent was made by combining in a further 100 ml acetate buffer (pH 5.0) 250 mg soluble polyvinyl pyrrilidone, 1 gm sodium chloride, 10 drops 1.0N magnesium chloride solution, 100 mg α- naphthyl acid phosphate and a few mls water containing 100 mg Fast Blue RR (Gurr Chemicals). Brown and purple bands appeared after several hours - these could be fixed as for esterase (Bowling, private communication).

3-3 Results and Discussion

The location and intensity of the bands developed with each specific staining reaction are shown in diagrams 3-1 through 3-4. Photographs of some gels are included to show protein and peroxidase banding patterns in a few cases. Also recorded is the average electrophoretic mobility value for each band, the electrophoretic mobility value (e_m) being defined as

$$= \frac{\text{distance from the origin to the leading edge of the band}}{\text{distance from the origin to the borate ion boundary}}$$

For anodic-migrating bands e_m is positive, but for the back-running or cathodic-migrating bands its value is negative.

The general protein survey revealed nothing of value, only two bands being present in the whole of the 22 species examined. Band I was always present, as was the weaker band II in the majority of species. The absence of this second band in a handful of species could be due to its concentration

in those instances being too low to be detected (Fig. 3-1).

Eight bands were detected using the acid phosphatase stain. Bands I and II were shared by all species. Of the others no pattern in distribution was obvious and the fact that the bands did not occur in every sample from each species may be caused by their concentration being below the threshold of detection (Fig. 3-2).

The remaining enzyme band patterns, peroxidase and esterase, reflected species groupings. There are 12 peroxidase bands, and it is obvious that in every renantherous species examined on every occasion there is an absence of backrunning bands I, II and III, but bands IV and V consistently occur in medium to high amounts. Also, bands X and XI are present in medium to high amounts in renantherous species other than E. coccifera, but even in this species both bands are always present. Within the section Macrantherae bands X and XI occur sporadically, if at all, and then in low quantities - however, E. aggregata consistently has these bands present in medium concentration. Bands I - III are present in high amount in all macrantherous species except E. aggregata where they are completely absent, but in which species bands IV and V are major bands. These two bands do occur in other macrantherous species, but only as minor bands. The seed profiles then of all macrantherous species except E. aggregata are distinguished from those of renantherous species and E. aggregata by the occurrence, as major components, of bands I, II and III.

Of the remaining bands, VII occurs consistently in every species, although in varying amount, and bands VI and XII are variant bands of both sections. Band VII is restricted to three closely related renantherous species, E. obliqua, E. regnans and E. sieberi. Band IX seems invariant within section

Macrantherae, but is lacking in six renantherous species, E. linearis, E. simmondsii, E. coccifera, E. pauciflora, E. tasmanica and E. risdoni (Fig. 3-3).

Of the nineteen esterase isoenzymes detected, band V is consistently present in every species, while bands I - III, VI and VIII are variant sites of activity and can be ignored in taxonomic considerations. Band IV seems restricted to macrantherous species but its inconsistency prevents it being a useful marker. Band VII is an invariant major band in E. aggregata and renantherous species except the three peppermints, E. linearis, E. amygdalina, and E. simmondsii, while it only occurs as a minor component of some macrantherous species.

E. aggregata and all renantherous species (other than E. coccifera in which the amount is low) have high quantities of combinations of IX, X and XI; again these are minor components of macrantherous species. Band XII only occurs in E. aggregata and E. pauciflora, while either one or other or both of bands XIII and XIV occur in macrantherous species except E. aggregata; these are only minor and variant components of the three renantherous species E. obliqua, E. delegatensis and E. sieberi.

E. coccifera, E. pauciflora, E. risdoni, E. tasmanica and E. aggregata all show band XV. Bands XVI and XVII are both major bands in the Ashes and Stringybarks; one or other is major in the peppermints and E. aggregata, while XVII is major, but XVI is absent in E. coccifera and E. pauciflora. In E. tasmanica and E. risdoni band XVII is minor, and band XVI is absent. These two bands show no consistent distribution in macrantherous species.

Band XVIII is consistent to certain macrantherous species only, while band XIX occurs only in E. dalrympleana. Generally this species differs from the other two Lowland White Gums in having bands VI and VIII, but not VII; and bands XVI, XVII and XIX, but not XVIII (Fig. 3-4).

The peroxidase and esterase results were statistically analysed in an effort to give a measure of the genetic similarity or genomic homology among the species. Two statistical procedures were used, the first the Similarity Index Method developed by Sokal and Sneath (quoted by Sheen, 1972). The formula for measuring s is given as follows:

$$s = \frac{\text{similarities}}{\text{similarities + dissimilarities}} \times 100$$

Table 3 - 2 gives values of s for peroxidase bands, esterase bands and the combined value of s for these two bands. In tables 3 - 1 a and b are shown the figures used in determining s values. Table 3-3 is based on the combined value of s.

Using peroxidase values, s within sections is high (greater than 50%), but the between sections value is low (less than 50%). E. aggregata is an exception as the similarity index of this species with all other macrantherous species except E. globulus and E. cordata is low, but it is high with renantherous species.

With esterase results the division is not as clear cut but generally within section similarities are high, between section similarities low, again with E. aggregata proving the exception. Looking at the combined similarity indices, the species which have unexpected s values (except E. aggregata) are those in which the total number of bands is proportionately larger - i.e. in E. globulus, E. cordata, E. dalrympleana, E.

amygdalina. This would indicate that more consistent results are obtained when the number of bands per species is relatively constant. Again, the discrepancy of certain esterase results has been lessened in the combined results; thus the similarity index method of analysis is best employed when a number of different characters is used, not simply two as in the present instant.

A statistical method based on a hypergeometric distribution model from Smith et al., (1970) was also used to assess the degree of phylogenetic association between the Eucalyptus species in terms of matching species bands. The method gives an estimate of the probability under random matching of obtaining the observed number of matching bands between the electrophoretic patterns of any two species. A low probability is taken to indicate that the shared pattern is not due to random matching and that the high frequency of matched bands reflects genetic similarities. The equation for the hypergeometric series is:

$$P_{(i)} = \frac{\binom{k_2}{i}\binom{n-k_2}{k_1-i}}{\binom{n}{k_1}}$$

where $P_{(i)}$ is the probability under random matching of obtaining i matches;

k_1 is the number of bands in one species;

$k_2 \geqslant k_1$ is the number of bands in the other species;

n is the total number of bands possible (12 for peroxidase, 19 for esterase). (Smith et al., 1970).

The equation was programmed for the computor and print-

out information gave the probabilities between and within sections as listed in table 3 - 4.

Within the section Macrantherae, between E. aggregata and each of the other 10 species there is high probability of the bands occurring by random matching (this probability ranges from 0.11 to 0.38), except for the low probability of the esterase bands being randomly matched between E. aggregata and E. gunnii (i.e. 0.05). This indicates E. aggregata is not as close phylogenetically as other macrantherous species. The between section comparison shows a closer relationship of this species to renantherous rather than macrantherous species - this parallels the information obtained from similarity indices.

With the majority of the remaining macrantherous species there is a very low probability of either the peroxidase or esterase band matchings occurring by chance. Exceptions include comparisons between peroxidase bands of each of E. globulus and E. cordata with E. ovata, E. viminalis, E. rubida, E. dalrympleana, E. urnigera and E. gunnii. This is not paralleled by a high probability for these same species with the esterase bands, except in the case of E. dalrympleana. This result may be influenced by the larger number of peroxidase bands scored for E. cordata and E. globulus compared to the number scored for other species (i.e. 9 against 5 or 6). This is supported by the between section comparisons which show high probability between these two macrantherous species and all renantherous species (this probability was usually greater than 0.30). The hypergeometric expression uses a value of $k_2 \gg k_1$, and where k_1 and k_2 are unbalanced to a degree which greatly favours k_1 then the probability calculated may be biased.

However, when the comparison is made using the k_1 value for the species with the lesser number of bands, and k_2 for the species with the greater number, e.g. E. globulus, exactly the same probability is obtained.

A high probability occurs between E. dalrympleana and a few macrantherous species (E. ovata, E. viminalis, E. perriniana), mainly with esterase bands; but as E. dalrympleana also shows high probability of sharing bands with renantherous species by random matching rather than genetic homology, these results may be influenced by the slightly higher than normal number of esterase bands scored within this species. A similar situation occurs within the section Renantherae when E. delegatensis and E. amygdalina are compared with certain species - both these had a high band score compared to other species; however, the between sections comparisons are high for these and all macrantherous species except E. aggregata, showing they share less genetic homology with this section, than with the remaining renantherous species. Other between sections comparisons also show high probabilities (except with E. aggregata).

Analysing phylogenetic relationships between the species on the basis of isozymic affinities assumes that the isozymes represent the products of different genetic units and the presence of any isozyme in two or more species implies a genetic similarity. There remains the possibility of the occurrence of different isozymes with the same substrate specificity and e_m value which would exaggerate the degree of genetic similarity between species. On the other hand, the less satisfactory

results obtained with esterase bands are probably due to a certain amount of polymorphism displayed at various loci, and which has not been taken into account. However, one can still conclude that the classical division of Tasmanian Eucalypts into two sections is supported, with but one exception, by this estimate of the phylogenetic relationship of the various species.

Table 3-1(a) Distribution of Peroxidase Bands

Species	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII
E. ovata	1.0	1.0	1.0	0.5	0.5	0.0	1.0	0.0	1.0	1.0	0.0	0.0
E. aggregata	0.0	0.0	0.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	0.0
E. viminalis	1.0	1.0	1.0	0.0	0.0	0.1	1.0	0.0	1.0	0.0	0.0	0.1
E. rubida	1.0	1.0	1.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0
E. dalrympleana	1.0	1.0	1.0	0.1	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.1
E. globulus	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	0.0	1.0	0.1
E. cordata	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	1.0	0.0	1.0	0.1
E. perriniana	1.0	1.0	1.0	0.0	0.1	0.0	1.0	0.0	0.9	0.2	0.1	0.0
E. urnigera	1.0	1.0	1.0	0.2	0.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0
E. gunnii	1.0	1.0	1.0	0.0	0.0	0.1	1.0	0.0	1.0	0.2	0.0	0.0
E. johnstonii	1.0	1.0	1.0	0.1	0.1	0.0	1.0	0.0	1.0	0.0	1.0	0.0
E. obliqua	0.0	0.0	0.0	1.0	1.0	0.1	1.0	1.0	0.4	1.0	1.0	0.0
E. regnans	0.0	0.0	0.0	1.0	1.0	0.1	1.0	1.0	0.4	1.0	1.0	0.0
E. delegatensis	0.0	0.0	0.0	1.0	1.0	0.1	1.0	0.0	1.0	1.0	1.0	1.0
E. sieberi	0.0	0.0	0.0	1.0	1.0	0.1	1.0	1.0	1.0	1.0	1.0	0.0
E. amygdalina	0.0	0.0	0.0	1.0	1.0	1.0	1.0	0.0	1.0	1.0	1.0	0.0
E. linearis	0.0	0.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0
E. simmondsii	0.0	0.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0
E. coccifera	0.0	0.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	1.0	1.0	0.0
E. pauciflora	0.0	0.0	0.0	1.0	1.0	0.1	1.0	0.0	0.0	1.0	1.0	0.5
E. tasmanica	0.0	0.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	1.0	1.0	0.0
E. risdoni	0.0	0.0	0.0	1.0	1.0	0.0	1.0	0.0	0.0	1.0	1.0	0.0

Key 1 = always present, 0 = never present, fractions indicate frequency of band occuring in several samples

Table 3-1(b) Distribution of Esterase Bands

	I	II	III	IV	V	VI	VII	VIII	IX	X	XI	XII	XIII	XIV	XV	XVI	XVII	XVIII	XIX
E. ovata	0.1	0.0	0.0	0.0	1.0	1.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0
E. aggregata	0.1	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.1	0.0	0.1	1.0	1.0	0.0	0.0
E. viminalis	0.5	0.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	1.0	0.0
E. rubida	0.2	0.0	0.0	0.1	1.0	0.0	0.2	0.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	1.0	0.0
E. dalrympleana	0.2	0.0	0.0	0.0	1.0	1.0	0.0	1.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.5	1.0	0.0	1.0
E. globulus	0.5	0.1	0.1	1.0	1.0	0.0	1.0	0.5	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	1.0	0.0
E. cordata	1.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0
E. perriniana	1.0	0.0	0.0	0.1	1.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	0.0	1.0	0.0
E. urnigera	0.2	0.0	0.0	0.1	1.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0
E. gunnii	0.2	0.0	0.0	0.1	1.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	0.0	0.0
E. johnstonii	0.0	0.2	0.0	0.0	1.0	0.0	1.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0	1.0	0.0	0.0
E. obliqua	0.1	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.1	0.0	0.0	1.0	1.0	0.0	0.0
E. regnans	0.1	0.0	0.0	0.0	1.0	1.0	1.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	0.1	0.0
E. delegatensis	1.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	0.5	1.0	1.0	0.0	0.1	0.0	0.0	1.0	1.0	0.1	0.0
E. sieberi	1.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	0.1	0.0	0.0	1.0	1.0	0.0	0.0
E. amygdalina	1.0	0.0	1.0	0.0	1.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	0.0	1.0	1.0	1.0	0.0
E. linearis	0.0	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0
E. simmondsii	0.0	0.1	0.0	0.0	1.0	0.0	0.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	0.0	1.0	1.0	0.0	0.0
E. coccifera	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0
E. pauciflora	0.2	0.2	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	1.0	1.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0
E. tasmanica	0.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0
E. risdoni	0.5	0.0	0.0	0.0	1.0	0.0	1.0	0.0	1.0	1.0	1.0	0.0	0.0	0.0	1.0	0.0	1.0	0.0	0.0

Key 1 = always present, 0 = never present, fractions indicate frequency of band occurring in several samples

Table 3-2 Similarity Indices

Within Section Macrantherae

Species x Species	Peroxidase	Esterase	Combined
E. ovata x *E. aggregata*	40.0	46.4	43.4
" x *E. viminalis*	69.4	71.8	70.7
" x *E. rubida*	71.4	63.9	67.3
" x *E. dalrympleana*	71.8	47.2	57.0
" x *E. globulus*	59.4	59.8	59.6
" x *E. cordata*	59.4	76.3	66.85
" x *E. perriniana*	73.2	50.5	59.9
" x *E. urnigera*	74.3	54.8	63.2
" x *E. gunnii*	73.2	69.9	71.52
" x *E. johnstonii*	86.7	43.5	60.5
E. aggregata x *E. viminalis*	20.8	33.3	27.8
" x *E. rubida*	20.0	27.4	24.0
" x *E. dalrympleana*	20.8	55.5	39.3
" x *E. globulus*	59.4	34.1	44.8
" x *E. cordata*	59.4	34.7	44.8
" x *E. perriniana*	23.0	31.8	28.0
" x *E. urnigera*	22.0	45.6	34.6
" x *E. gunnii*	23.0	50.0	36.8
" x *E. johnstonii*	32.0	35.96	34.1
E. viminalis x *E. rubida*	96.0	87.7	88.2
" x *E. dalrympleana*	98.0	43.3	59.5
" x *E. globulus*	57.1	81.5	69.4
" x *E. cordata*	57.1	81.3	68.4
" x *E. perriniana*	87.5	71.4	77.6
" x *E. urnigera*	92.6	72.1	80.0
" x *E. gunnii*	94.4	60.5	73.6
" x *E. johnstonii*	78.1	57.5	66.2
E. rubida x *E. dalrympleana*	96.2	46.4	62.4
" x *E. globulus*	55.0	69.6	62.3
" x *E. cordata*	55.0	66.7	60.5
" x *E. perriniana*	90.7	59.3	71.0
" x *E. urnigera*	96.2	65.1	77.0
" x *E. gunnii*	94.3	54.9	70.4
" x *E. johnstonii*	80.7	48.2	61.9

Table 3-2 continued:

E. dalrympleana x E. globulus	57.1	43.2	48.9
" x E. cordata	57.1	31.1	41.6
" x E. perriniana	87.5	41.3	55.5
" x E. urnigera	96.2	57.4	60.3
" x E. gunnii	90.9	48.2	62.6
" x E. johnstonii	76.1	45.9	58.7
E. globulus x E. cordata	100.0	67.0	83.0
" x E. perriniana	54.8	69.6	62.6
" x E. urnigera	57.1	61.8	59.6
" x E. gunnii	54.8	52.0	53.3
" x E. johnstonii	68.1	49.5	58.2
E. cordata x E. perriniana	54.8	65.9	60.3
" x E. urnigera	57.1	57.1	57.1
" x E. gunnii	54.8	64.2	59.2
" x E. johnstonii	68.1	43.5	55.7
E. perriniana x E. urnigera	87.5	52.5	65.0
" x E. gunnii	92.7	42.6	60.3
" x E. johnstonii	79.7	53.8	64.3
E. urnigera x E. gunnii	90.9	86.3	88.3
" x E. johnstonii	81.0	80.0	80.4
E. gunnii x E. johnstonii	75.8	66.7	70.9
Within Section Renantherae			
E. obliqua x E. regnans	100.0	97.6	98.6
" x E. delegatensis	67.9	59.8	63.2
" x E. sieberi	75.3	68.1	73.8
" x E. amygdalina	68.8	55.0	60.7
" x E. linearis	68.9	54.3	60.8
" x E. simmondsii	68.9	53.8	60.5
" x E. coccifera	76.9	54.3	63.7
" x E. pauciflora	72.9	44.3	55.1
" x E. tasmanica	76.9	49.0	59.9
" x E. risdoni	76.9	48.1	59.1

Table 3-2 continued:

E. regnans x *E. delegatensis*	67.9	54.9	60.1
" x *E. sieberi*	80.2	69.0	74.0
" x *E. amygdalina*	68.8	59.3	63.1
" x *E. linearis*	68.9	54.3	60.8
" x *E. simmondsii*	68.9	53.8	60.5
" x *E. coccifera*	76.9	54.3	63.7
" x *E. pauciflora*	72.9	44.3	55.1
" x *E. tasmanica*	76.9	49.0	59.9
" x *E. risdoni*	76.9	48.1	59.1
E. delegatensis x *E. sieberi*	75.3	64.7	69.4
" x *E. amygdalina*	76.3	68.5	71.7
" x *E. linearis*	63.8	53.9	58.2
" x *E. simmondsii*	63.8	53.4	57.9
" x *E. coccifera*	70.4	40.2	51.9
" x *E. pauciflora*	78.9	46.0	57.9
" x *E. tasmanica*	70.4	49.1	57.4
" x *E. risdoni*	70.4	53.6	60.1
E. sieberi x *E. amygdalina*	76.3	59.4	66.9
" x *E. linearis*	63.8	61.7	62.7
" x *E. simmondsii*	63.8	61.0	62.3
" x *E. coccifera*	70.4	61.7	67.8
" x *E. pauciflora*	67.1	48.7	55.9
" x *E. tasmanica*	70.4	54.9	61.7
" x *E. risdoni*	70.4	60.4	64.8
E. amygdalina x *E. linearis*	85.7	50.0	64.7
" x *E. simmondsii*	85.7	49.5	64.3
" x *E. coccifera*	71.4	36.0	49.7
" x *E. pauciflora*	68.0	31.8	44.9
" x *E. tasmanica*	71.4	33.3	47.4
" x *E. risdoni*	71.4	37.5	50.0
E. linearis x *E. simmondsii*	100.0	98.4	99.2
" x *E. coccifera*	83.3	50.0	64.3
" x *E. pauciflora*	78.5	53.2	63.5
" x *E. tasmanica*	83.3	62.5	71.4
" x *E. risdoni*	83.3	58.8	69.0
E. simmondsii x *E. coccifera*	83.3	49.4	63.8
" x *E. pauciflora*	78.5	54.3	64.2
" x *E. tasmanica*	83.3	61.7	70.9
" x *E. risdoni*	83.3	59.3	69.2

Table 3-2 continued:

E. coccifera x *E. pauciflora*	89.3	71.4	82.1
" x *E. tasmanica*	100.0	85.7	91.7
" x *E. risdoni*	100.0	80.0	88.0
E. pauciflora x *E. tasmanica*	89.3	83.3	85.7
" x *E. risdoni*	89.3	82.8	85.3
E. tasmanica x *E. risdoni*	100.0	93.0	96.0
Between Sections			
E. ovata x *E. obliqua*	33.7	51.9	42.6
" x *E. regnans*	33.7	51.9	42.6
" x *E. delegatensis*	39.6	29.2	33.8
" x *E. sieberi*	39.6	47.3	43.6
" x *E. amygdalina*	40.0	34.2	36.8
" x *E. linearis*	30.0	29.7	30.0
" x *E. simmondsii*	30.0	29.4	29.7
" x *E. coccifera*	33.3	48.8	40.9
" x *E. pauciflora*	31.2	36.0	33.8
" x *E. tasmanica*	33.3	39.6	36.6
" x *E. risdoni*	33.3	39.0	36.4
E. aggregata x *E. obliqua*	68.8	88.2	79.2
" x *E. regnans*	68.8	86.2	78.2
" x *E. delegatensis*	76.3	68.1	71.5
" x *E. sieberi*	76.3	60.8	68.9
" x *E. amygdalina*	100.0	50.0	68.2
" x *E. linearis*	85.7	64.5	73.6
" x *E. simmondsii*	85.7	63.8	73.2
" x *E. coccifera*	71.4	50.0	58.7
" x *E. pauciflora*	68.0	50.0	57.5
" x *E. tasmanica*	71.4	59.8	64.5
" x *E. risdoni*	71.4	58.5	63.6
E. viminalis x *E. obliqua*	14.7	36.5	26.3
" x *E. regnans*	14.7	36.2	26.2
" x *E. delegatensis*	21.8	32.3	27.7
" x *E. sieberi*	20.6	46.0	33.2
" x *E. amygdalina*	20.8	37.5	29.9
" x *E. linearis*	10.9	31.3	21.8

Table 3-2 continued:

E. viminalis x *E. simmondsii*	10.9	31.5	21.7
" x *E. coccifera*	10.9	42.1	26.7
" x *E. pauciflora*	12.5	35.9	25.4
" x *E. tasmanica*	10.9	38.1	25.4
" x *E. risdoni*	10.9	42.9	28.7
E. rubida x *E. obliqua*	13.9	30.4	22.5
" x *E. regnans*	13.9	30.4	22.5
" x *E. delegatensis*	19.8	23.3	21.7
" x *E. sieberi*	19.8	34.3	27.0
" x *E. amygdalina*	20.0	37.5	29.3
" x *E. linearis*	10.0	31.9	20.6
" x *E. simmondsii*	11.1	32.3	22.0
" x *E. coccifera*	11.1	36.2	23.9
" x *E. pauciflora*	10.4	29.8	21.0
" x *E. tasmanica*	11.1	31.4	21.9
" x *E. risdoni*	11.1	32.4	22.6
E. dalrympleana x *E. obliqua*	14.7	59.8	38.3
" x *E. regnans*	14.7	59.5	37.7
" x *E. delegatensis*	21.8	37.6	31.0
" x *E. sieberi*	20.6	40.0	31.1
" x *E. amygdalina*	20.8	43.9	33.8
" x *E. linearis*	10.9	40.2	26.3
" x *E. simmondsii*	10.9	39.8	26.2
" x *E. coccifera*	12.1	37.4	25.8
" x *E. pauciflora*	12.5	30.2	23.0
" x *E. tasmanica*	12.1	32.6	24.1
" x *E. risdoni*	12.1	32.3	24.0
E. globulus x *E. obliqua*	40.5	37.0	38.7
" x *E. regnans*	40.5	37.0	38.7
" x *E. delegatensis*	47.3	34.0	39.8
" x *E. sieberi*	46.0	39.3	42.5
" x *E. amygdalina*	59.4	41.9	49.8
" x *E. linearis*	49.5	24.6	35.9
" x *E. simmondsii*	49.5	25.4	36.3
" x *E. coccifera*	39.6	35.7	37.6
" x *E. pauciflora*	40.0	32.3	35.7
" x *E. tasmanica*	39.6	32.8	35.9
" x *E. risdoni*	39.6	36.9	38.1

Table 3-2 continued:

E. cordata x *E. obliqua*	40.5	38.2	39.4
" x *E. regnans*	40.5	38.2	39.4
" x *E. delegatensis*	47.3	39.2	43.0
" x *E. sieberi*	45.9	56.7	50.7
" x *E. amygdalina*	59.4	45.5	52.1
" x *E. linearis*	49.5	30.0	39.8
" x *E. simmondsii*	49.5	29.7	39.6
" x *E. coccifera*	39.6	44.4	41.9
" x *E. pauciflora*	40.0	37.5	38.7
" x *E. tasmanica*	39.6	40.0	39.8
" x *E. risdoni*	39.6	45.0	42.3
E. perriniana x *E. obliqua*	18.0	34.7	27.1
" x *E. regnans*	18.0	34.7	27.1
" x *E. delegatensis*	22.8	35.9	30.2
" x *E. sieberi*	22.8	36.9	30.2
" x *E. amygdalina*	23.0	41.3	33.0
" x *E. linearis*	14.1	16.5	15.5
" x *E. simmondsii*	14.1	16.4	15.4
" x *E. coccifera*	15.7	27.0	22.0
" x *E. pauciflora*	14.9	24.1	20.3
" x *E. tasmanica*	15.7	24.8	21.0
" x *E. risdoni*	15.7	29.2	23.4
E. urnigera x *E. obliqua*	15.8	50.5	33.3
" x *E. regnans*	15.8	49.0	32.7
" x *E. delegatensis*	21.8	39.3	31.4
" x *E. sieberi*	21.8	58.2	39.1
" x *E. amygdalina*	22.0	34.7	29.0
" x *E. linearis*	12.0	43.0	26.9
" x *E. simmondsii*	12.0	42.6	26.8
" x *E. coccifera*	13.3	60.2	35.8
" x *E. pauciflora*	12.5	49.5	31.8
" x *E. tasmanica*	13.3	53.8	33.9
" x *E. risdoni*	13.3	54.2	34.4
E. gunnii x *E. obliqua*	16.8	55.9	35.6
" x *E. regnans*	16.8	44.3	31.5
" x *E. delegatensis*	27.1	39.3	33.6

Table 3-2 continued:

E. gunnii x *E. sieberi*	22.8	65.4	41.8
" x *E. amygdalina*	30.0	37.8	30.8
" x *E. linearis*	13.0	48.2	29.0
" x *E. simmondsii*	13.0	47.6	28.8
" x *E. coccifera*	13.2	68.5	37.5
" x *E. pauciflora*	13.5	61.2	35.9
" x *E. tasmanica*	13.2	60.2	35.6
" x *E. risdoni*	13.2	60.5	36.2
E. johnstonii x *E. obliqua*	25.7	39.8	32.8
" x *E. regnans*	25.7	38.5	32.2
" x *E. delegatensis*	31.7	31.9	31.8
" x *E. sieberi*	31.7	44.6	37.8
" x *E. amygdalina*	32.0	24.6	27.9
" x *E. linearis*	22.0	32.6	27.1
" x *E. simmondsii*	22.0	33.7	27.6
" x *E. coccifera*	24.4	48.8	33.3
" x *E. pauciflora*	22.9	40.4	32.0
" x *E. tasmanica*	24.4	43.5	34.1
" x *E. risdoni*	24.4	41.2	33.2

Table 3-3 Similarity Index Values Among Eucalyptus Species

	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22
1	100																					
2	43	100																				
3	71	28	100																			
4	67	24	88	100																		
5	57	39	60	62	100																	
6	60	45	70	62	49	100																
7	67	45	68	61	42	83	100															
8	60	28	78	71	56	63	60	100														
9	63	35	80	77	60	60	57	65	100													
10	72	37	74	70	63	53	59	60	88	100												
11	61	34	66	62	59	58	56	64	80	71	100											
12	43	79	26	23	38	39	39	27	33	36	33	100										
13	43	78	26	23	38	39	39	27	33	32	32	99	100									
14	34	72	28	22	31	39	43	30	31	34	32	63	60	100								
15	44	69	33	27	31	43	50	30	39	42	38	74	74	69	100							
16	37	68	30	30	34	50	52	33	29	31	28	61	63	72	67	100						
17	30	74	22	20	26	36	40	16	27	29	27	61	61	58	63	65	100					
18	30	73	22	22	26	36	40	15	27	29	28	61	61	58	62	64	99	100				
19	41	59	27	24	26	38	42	22	36	38	33	64	64	52	68	50	64	64	100			
20	34	58	25	21	23	36	39	20	32	36	32	55	55	58	56	44	64	64	82	100		
21	37	65	25	22	24	36	40	21	34	36	34	60	60	57	62	47	71	71	92	86	100	
22	36	64	29	22	24	38	42	23	34	36	33	59	59	60	65	50	69	69	88	85	96	100

Key

1. E. ovata
2. E. aggregata
3. E. viminalis
4. E. rubida
5. E. dalrympleana
6. E. globulus
7. E. cordata
8. E. perriniana
9. E. urnigera
10. E. gunnii
11. E. johnstonii
12. E. obliqua
13. E. regnans
14. E. delegatensis
15. E. sieberi
16. E. amygdalina
17. E. linearis
18. E. simmondsii
19. E. coccifera
20. E. pauciflora
21. E. tasmanica
22. E. risdoni

This table is compiled from the combined value of s for peroxidase and esterase bands.

Table 3-4 Probabilities of Sharing Bands by Random Matching

Species x Species	$P_{(i)}$ peroxidase	$P_{(i)}$ esterase
WITHIN SECTION MACRANTHERAE		
E. ovata x E. aggregata	0.378788	0.112527
" x E. viminalis	0.007576	0.001667
" x E. rubida	0.007576	0.009288
" x E. dalrympleana	0.007576	0.112527
" x E. globulus	0.409091	0.000000
" x E. cordata	0.409091	0.001667
" x E. perriniana	0.007576	0.061126
" x E. urnigera	0.007576	0.027507
" x E. gunnii	0.007576	0.009288
" x E. johnstonii	0.038961	0.085139
E. aggregata x E. viminalis	0.265152	0.300071
" x E. rubida	0.265152	0.371517
" x E. dalrympleana	0.265152	0.109117
" x E. globulus	0.318182	0.350083
" x E. cordata	0.318182	0.300071
" x E. perriniana	0.265152	0.350083
" x E. urnigera	0.265152	0.112527
" x E. gunnii	0.265152	0.046440
" x E. johnstonii	0.378788	0.208978
E. viminalis x E. rubida	0.001263	0.000258
" x E. dalrympleana	0.001263	0.112527
" x E. globulus	0.159091	0.000159
" x E. cordata	0.159091	0.001667
" x E. perriniana	0.001263	0.006113
" x E. urnigera	0.001263	0.001667
" x E. gunnii	0.001263	0.009288
" x E. johnstonii	0.007576	0.009288

E. rubida x *E. dalrympleana*	0.001263	0.046440
" x *E. globulus*	0.159091	0.001032
" x *E. cordata*	0.159091	0.009288
" x *E. perriniana*	0.001263	0.022704
" x *E. urnigera*	0.001263	0.009288
" x *E. gunnii*	0.001263	0.043123
" x *E. johnstonii*	0.007576	0.043123
E. dalrympleana x *E. globulus*	0.200048	0.159091
" x *E. cordata*	0.208978	0.159091
" x *E. perriniana*	0.001263	0.200048
" x *E. urnigera*	0.001263	0.016671
" x *E. gunnii*	0.001263	0.032151
" x *E. johnstonii*	0.007576	0.032151
E. globulus x *E. cordata*	0.004545	0.006113
" x *E. perriniana*	0.159091	0.020375
" x *E. urnigera*	0.159091	0.006113
" x *E. gunnii*	0.159091	0.022704
" x *E. johnstonii*	0.090909	0.022704
E. cordata x *E. perriniana*	0.159091	0.006668
" x *E. urnigera*	0.159091	0.027507
" x *E. gunnii*	0.159091	0.009288
" x *E. johnstonii*	0.090909	0.085139
E. perriniana x *E. urnigera*	0.001263	0.061126
" x *E. gunnii*	0.001263	0.141899
" x *E. johnstonii*	0.007576	0.022704
E. urnigera x *E. gunnii*	0.001263	0.000258
" x *E. johnstonii*	0.007576	0.000258
E. gunnii x *E. johnstonii*	0.007576	0.002875

WITHIN SECTION RENANTHERAE

E. obliqua x *E. regnans*	0.001082	0.000013
" x *E. delegatensis*	0.113636	0.050012
" x *E. sieberi*	0.007576	0.006113
" x *E. amygdalina*	0.113636	0.050012
" x *E. linearis*	0.038961	0.022704
" x *E. simmondsii*	0.038961	0.022704

E. obliqua x E. coccifera	0.007576	0.022704
" x E. pauciflora	0.007576	0.122251
" x E. tasmanica	0.007576	0.061126
" x E. risdoni	0.007576	0.061126
E. regnans x E. delegatensis	0.113636	0.050012
" x E. sieberi	0.007576	0.006113
" x E. amygdalina	0.113636	0.050012
" x E. linearis	0.038961	0.022704
" x E. simmondsii	0.038961	0.022704
" x E. coccifera	0.007576	0.022704
" x E. pauciflora	0.007576	0.122251
" x E. tasmanica	0.007576	0.061126
" x E. risdoni	0.007576	0.061126
E. delegatensis x E. sieberi	0.044192	0.016671
" x E.amygdalina	0.044192	0.017537
" x E. linearis	0.113636	0.046440
" x E.simmondsii	0.113636	0.046440
" x E. coccifera	0.026515	0.208978
" x E.pauciflora	0.026515	0.200048
" x E.tasmanica	0.026515	0.112527
" x E. risdoni	0.026515	0.112527
E. sieberi x E. amygdalina	0.044192	0.016671
" x E. linearis	0.113636	0.009288
" x E. simmondsii	0.113636	0.009288
" x E. coccifera	0.026515	0.009288
" x E. pauciflora	0.026515	0.015281
" x E. tasmanica	0.026515	0.027507
" x E. risdoni	0.026515	0.027507
E. amygdalina x E. linearis	0.007576	0.046440
" x E. simmondsii	0.007576	0.046440
" x E. coccifera	0.026515	0.208978
" x E. pauciflora	0.026515	0.350083
" x.E. tasmanica	0.026515	0.300071
" x E. risdoni	0.026515	0.300071
E. linearis x E. simmondsii	0.001082	0.000037
" x E. coccifera	0.007576	0.043123
" x E. pauciflora	0.007576	0.022704

E. linearis x E. tasmanica	0.007576	0.009288
" x E. risdoni	0.007576	0.009288
E. simmondsii x E. coccifera	0.007576	0.043123
" x E. pauciflora	0.007576	0.022704
" x E. tasmanica	0.007576	0.009288
" x E. risdoni	0.007576	0.009288
E. coccifera x E. pauciflora	0.001263	0.001032
" x E. tasmanica	0.001263	0.000258
" x E. risdoni	0.001263	0.000258
E. pauciflora x E. tasmanica	0.001263	0.000159
" x E. risdoni	0.001263	0.000159
E. tasmanica x E. risdoni	0.001263	0.000020
BETWEEN SECTIONS		
E. ovata x E. obliqua	0.243506	0.061126
" x E. regnans	0.243506	0.061126
" x E. delegatensis	0.378788	0.350083
" x E. sieberi	0.378788	0.152814
" x E. amygdalina	0.378788	0.300071
" x E.linearis	0.243506	0.283798
" x E. simmondsii	0.243506	0.283798
" x E. coccifera	0.378788	0.085139
" x E. pauciflora	0.378788	0.229221
" x E. tasmanica	0.378788	0.152814
" x E. risdoni	0.378788	0.152814
E. aggregata x E. obliqua	0.113636	0.000119
" x E. regnans	0.113636	0.000119
" x E. delegatensis	0.044192	0.109117
" x E. sieberi	0.044192	0.016671
" x E. amygdalina	0.001263	0.109117
" x E. linearis	0.007576	0.003096
" x E. simmondsii	0.007576	0.003096
" x E. coccifera	0.026515	0.046440
" x E. pauciflora	0.026515	0.050012
" x E. tasmanica	0.026515	0.016671
" x E. risdoni	0.026515	0.016671

E. viminalis x *E. obliqua*	0.113636	0.229221
" x *E. regnans*	0.113636	0.229221
" x *E. delegatensis*	0.265152	0.350083
" x *E. sieberi*	0.265152	0.152814
" x *E. amygdalina*	0.265152	0.300071
" x *E. linearis*	0.113636	0.283798
" x *E. simmondsii*	0.113636	0.283798
" x *E. coccifera*	0.220960	0.085139
" x *E. pauciflora*	0.220960	0.229221
" x *E. tasmanica*	0.220960	0.152814
" x *E. risdoni*	0.220960	0.152814
E. rubida x *E. obliqua*	0.113636	0.340557
" x *E. regnans*	0.113636	0.340557
" x *E. delegatensis*	0.265152	0.278638
" x *E. sieberi*	0.265152	0.283798
" x *E. amygdalina*	0.265152	0.208978
" x *E. linearis*	0.113636	0.210820
" x *E. simmondsii*	0.113636	0.210820
" x *E. coccifera*	0.220960	0.210820
" x *E. pauciflora*	0.220960	0.340557
" x *E. tasmanica*	0.220960	0.283798
" x *E. risdoni*	0.220960	0.283798
E. dalrympleana x *E. obliqua*	0.113636	0.050012
" x *E. regnans*	0.113636	0.050012
" x *E.delegatensis*	0.265152	0.343718
" x *E. sieberi*	0.265152	0.300071
" x *E. amygdalina*	0.265152	0.286432
" x *E. linearis*	0.113636	0.208978
" x *E. simmondsii*	0.113636	0.208978
" x *E. coccifera*	0.220960	0.208978
" x *E. pauciflora*	0.220960	0.350083
" x *E. tasmanica*	0.220960	0.300071
" x *E. risdoni*	0.220960	0.300071
E. globulus x *E. obliqua*	0.409091	0.305628
" x *E. regnans*	0.409091	0.305628
" x *E. delegatensis*	0.477273	0.280067
" x *E. sieberi*	0.477273	0.229221
" x *E. amygdalina*	0.318182	0.350083

E. globulus x *E. linearis*	0.409091	0.340557
" x *E. simmondsii*	0.409091	0.340557
" x *E. coccifera*	0.477273	0.141899
" x *E. pauciflora*	0.477273	0.305628
" x *E. tasmanica*	0.477273	0.229221
" x *E. risdoni*	0.477273	0.229221
E. cordata x *E. obliqua*	0.409091	0.229221
" x *E. regnans*	0.409091	0.229221
" x *E. delegatensis*	0.477273	0.350083
" x *E. sieberi*	0.477273	0.152814
" x *E. amygdalina*	0.318182	0.300071
" x *E. linearis*	0.409091	0.283798
" x *E. simmondsii*	0.409091	0.283798
" x *E. coccifera*	0.477273	0.085139
" x *E. pauciflora*	0.477273	0.229221
" x *E. tasmanica*	0.477273	0.152814
" x *E. risdoni*	0.477273	0.152814
E. perriniana x *E. obliqua*	0.113636	0.305628
" x *E. regnans*	0.113636	0.305628
" x *E. delegatensis*	0.265152	0.280067
" x *E. sieberi*	0.265152	0.229221
" x *E. amygdalina*	0.265152	0.350083
" x *E. linearis*	0.113636	0.340557
" x *E. simmondsii*	0.113636	0.340557
" x *E. coccifera*	0.220960	0.340557
" x *E. pauciflora*	0.220960	0.342304
" x *E. tasmanica*	0.220960	0.366754
" x *E. risdoni*	0.220960	0.366754
E. urnigera x *E. obliqua*	0.113636	0.061126
" x *E. regnans*	0.113636	0.061126
" x *E. delegatensis*	0.265152	0.300071
" x *E. sieberi*	0.265152	0.027507
" x *E. amygdalina*	0.265152	0.112527
" x *E. linearis*	0.113636	0.085139
" x *E. simmondsii*	0.113636	0.085139
" x *E. coccifera*	0.220960	0.009288
" x *E. pauciflora*	0.220960	0.061126
" x *E. tasmanica*	0.220960	0.027507
" x *E. risdoni*	0.220960	0.027507

E. gunnii	x *E. obliqua*	0.113636	0.022704
"	x *E. regnans*	0.113636	0.022704
"	x *E. delegatensis*	0.265152	0.208978
"	x *E. sieberi*	0.265152	0.009288
"	x *E. amygdalina*	0.265152	0.208978
"	x *E. linearis*	0.113636	0.043123
"	x *E. simmondsii*	0.113636	0.043123
"	x *E. coccifera*	0.220960	0.002875
"	x *E. pauciflora*	0.220960	0.227040
"	x *E. tasmanica*	0.220960	0.009288
"	x *E. risdoni*	0.220960	0.009288
E. johnstonii	x *E. obliqua*	0.243506	0.141899
"	x *E. regnans*	0.243506	0.141899
"	x *E. delegatensis*	0.378788	0.371517
"	x *E. sieberi*	0.378788	0.085139
"	x *E. amygdalina*	0.378788	0.208978
"	x *E. linearis*	0.243506	0.210821
"	x *E. simmondsii*	0.243506	0.210821
"	x *E. coccifera*	0.378788	0.043123
"	x *E. pauciflora*	0.378788	0.141899
"	x *E. tasmanica*	0.378788	0.085139
"	x *E. risdoni*	0.378788	0.085139

Figures 3-1 and 3-2 are composite diagrams presenting deteeted sites of protein or acid phosphatase activity in seedling extracts on polyacrylamide-gel electrophoretograms.

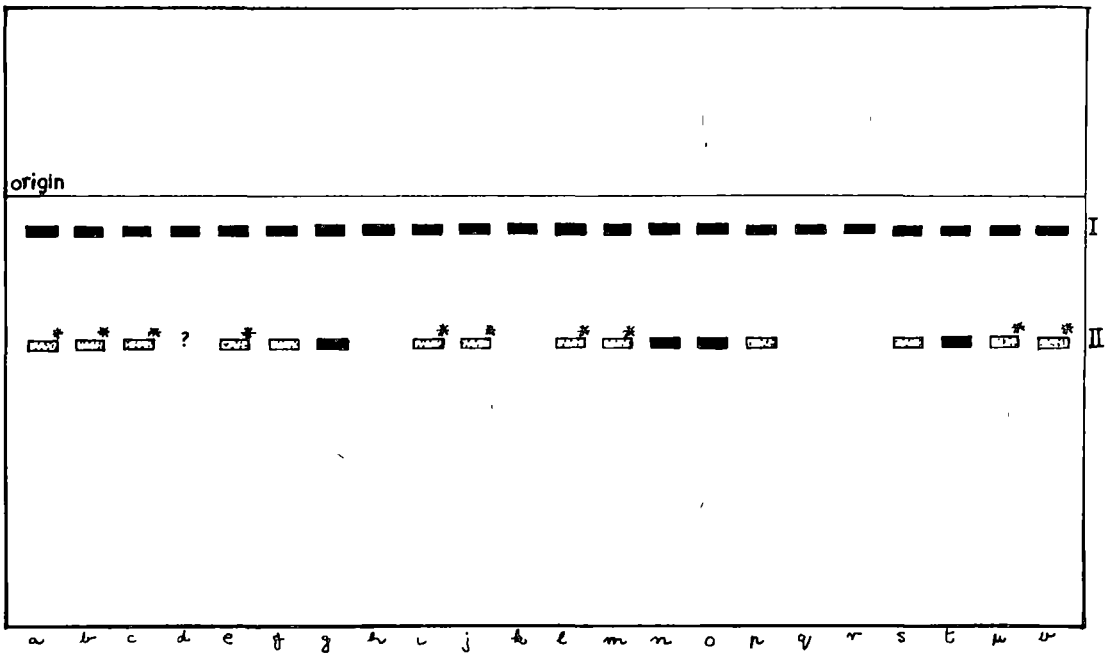


FIGURE 3-1: PROTEIN BANDS

E_m (I) = 4/45 ■ ⩾ 3+

E_m (II) = 16/45 ▭ ⩽ 3+

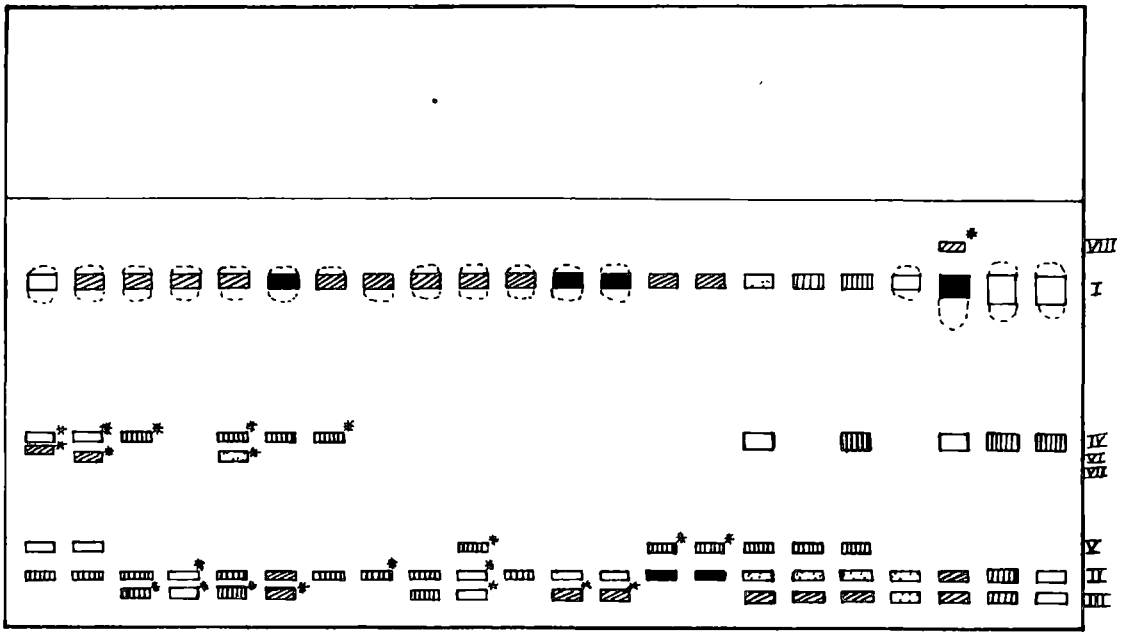


FIG. 3-2: ACID PHOSPHATASE

E_m: I = 8/45
II = 40/45
III = 42/45
IV = 26/45
V = 37/45
VI = 27/45
VII = 29/45
VIII = 4/45

■ 5+
▨ 4+
▭ 3+
▥ 2+
▭ +

* not present in every sample.

Figures 3-3 and 3-4 are composite diagrams presenting detected sites of peroxidase or esterase activity in seedling extracts on polyacrylamide-gel electrophoretograms.

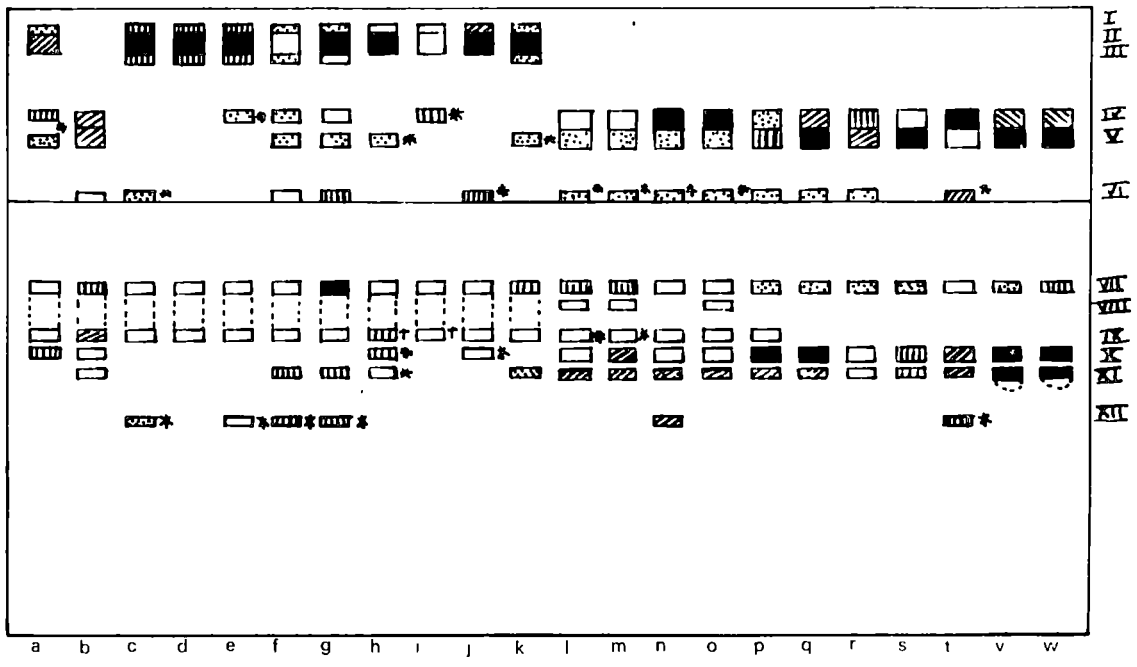


Fig. 3-3 Peroxidase Bands

* indicates band is not present in every sample

\+ indicates band is undetected in one sample

5+
4+
3+
2+
+

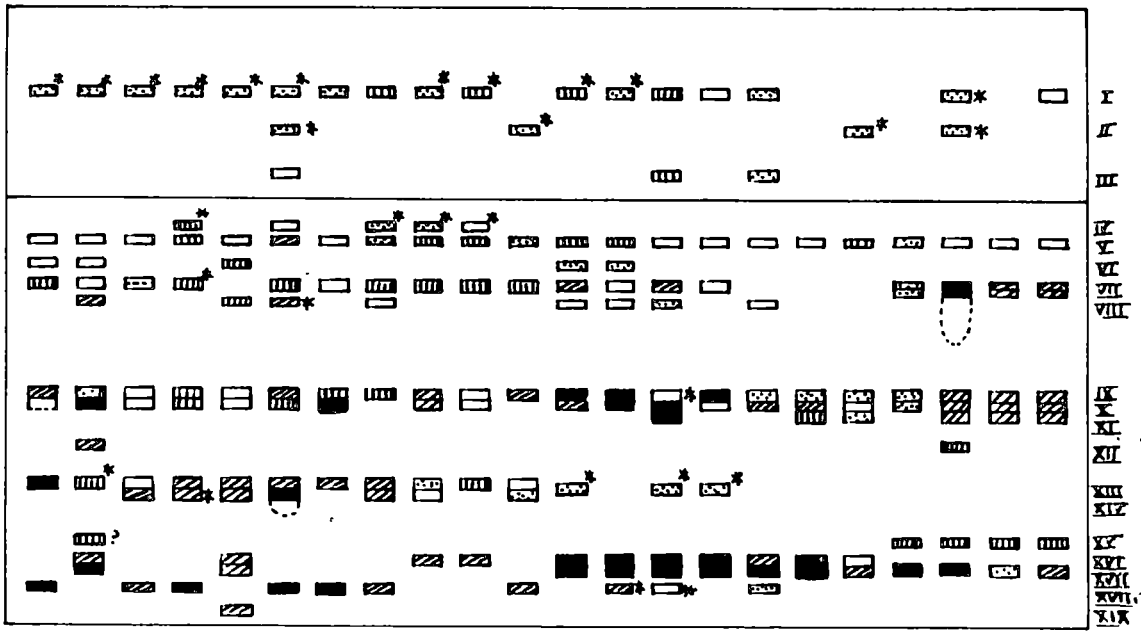


Fig 3-4 Esterase Bands

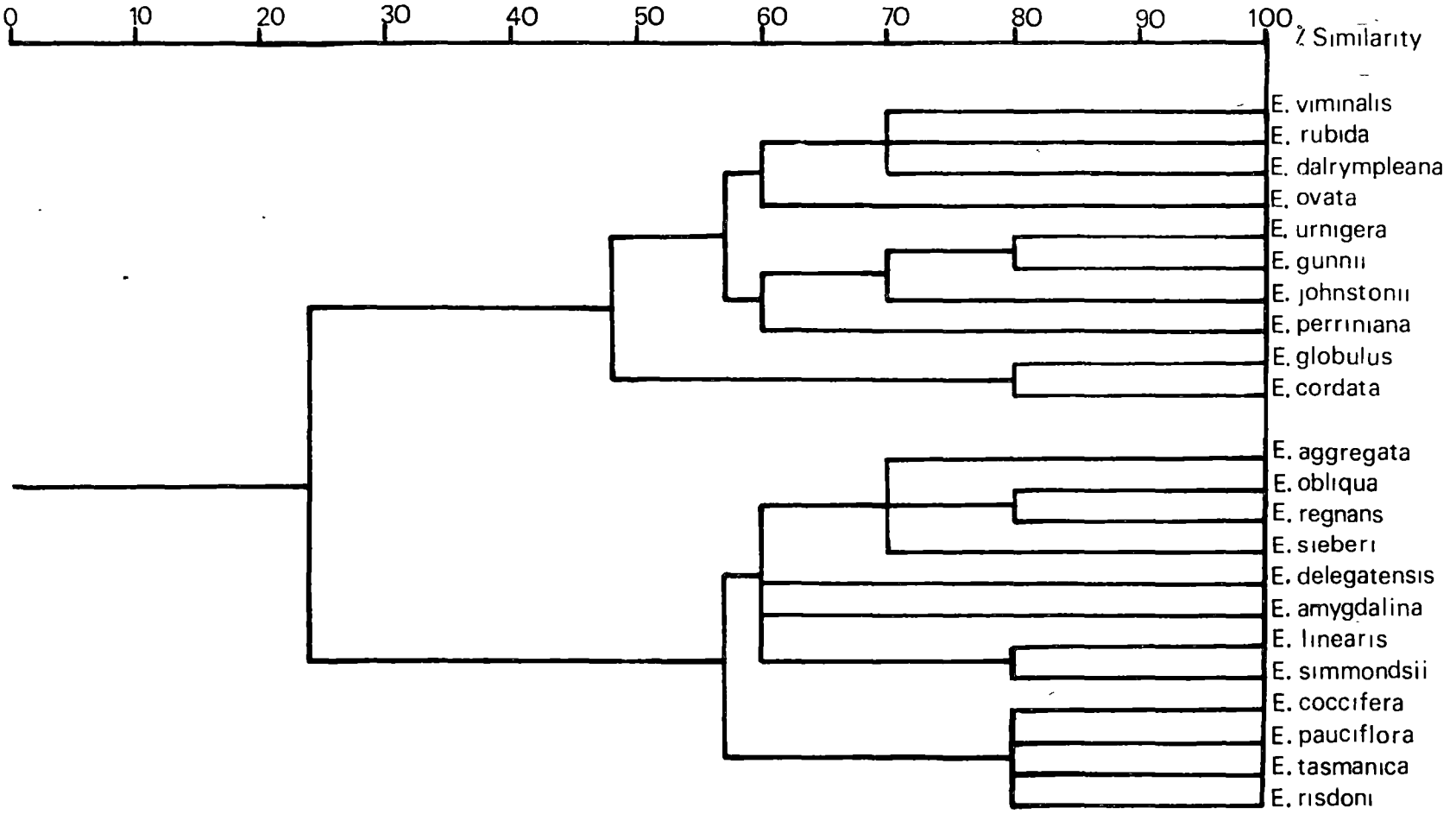


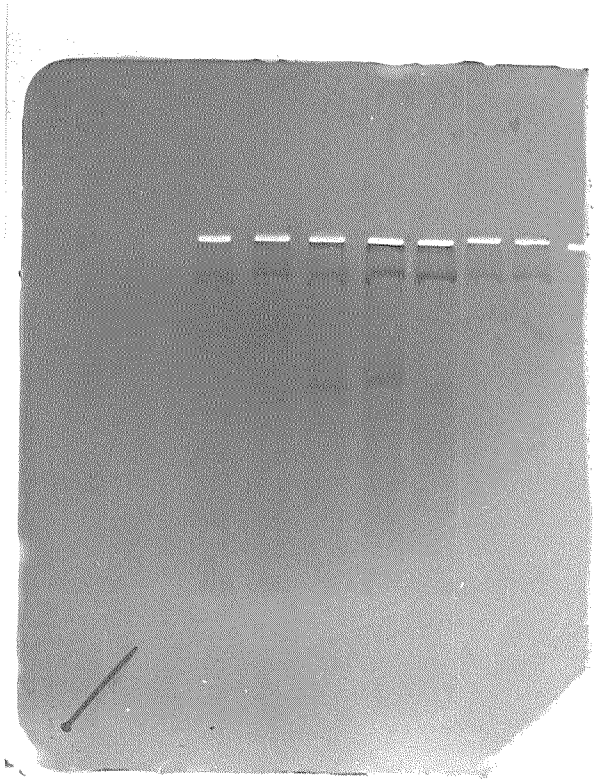
Figure 3-5: Species grouped on Similarity Indices.

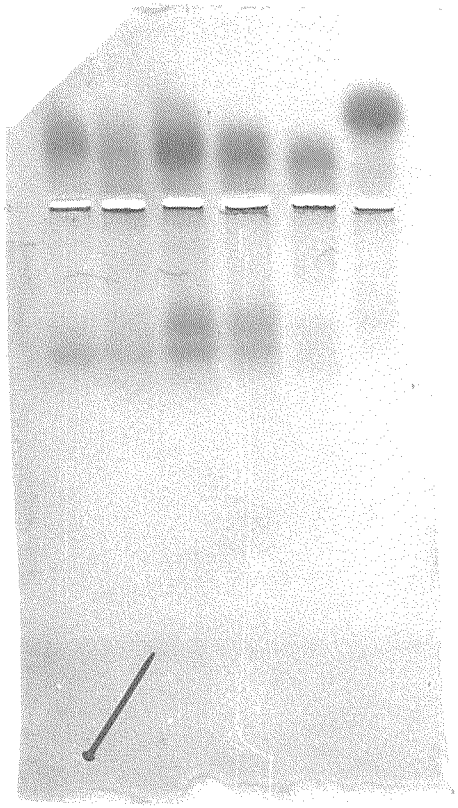
Values from Table 3-3 are used here in grouping species at the level where they show most similarity.

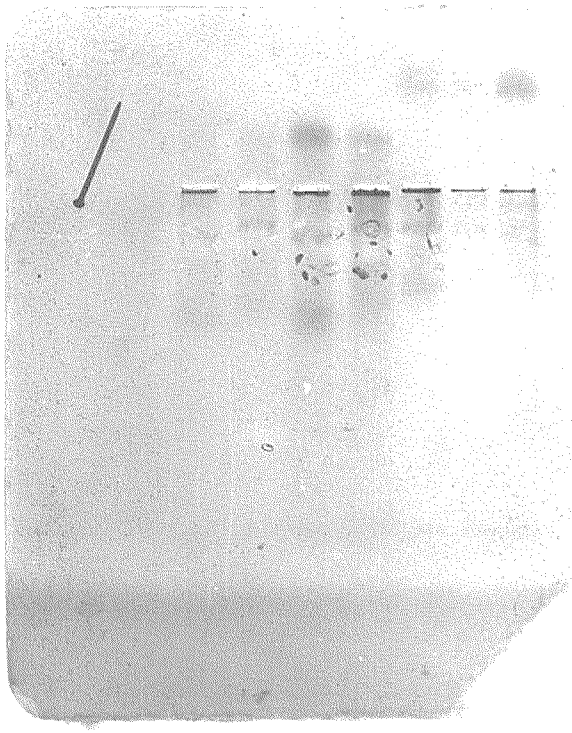
Top Photograph: Polyacrylamide gel stained for peroxidase. Samples 1 - 3 (from top) are extracts from macrantherous species; samples 4 - 7 are from renantherous species.

Centre Photograph: Polyacrylamide gel stained for peroxidase. Sample 1 (from top) is from E. gunnii. Samples 2 - 6 are extracts from renantherous species.

Bottom Photograph: Polyacrylamide gel stained for protein. These samples are from macrantherous species.







4. NUMERICAL CLASSIFICATION FROM CHEMICAL DATA

4-1 Introduction

The chemical data obtained on the twenty-two species of Eucalypts was used in erecting a numerical classification of these Tasmanian trees. Table 4-1, with its key, indicates those chemical characters used in the analysis. An agglomerative system, developed and described by Lance and Williams (1967), was used to obtain the classification. Blake (1973) considers these classificatory procedures of Lance and Williams to have the greatest degree of clustering power and flexibility.

The particular program used was **MULTBET** (Lance and Williams, 1967), which can accept mixed data - in this case qualitative and ordered multi-state attributes. The MULTBET program uses the information statistic. It tends to produce intense clustering during fusion, and a drawback is that individuals having little in common with one another or with other members tend to be fused as non-conformist groups.

Program GOWER was employed to produce ordinations of the similarity matrices produced by **MULTBET**. GOWER performs a principal co-ordinates analysis, the results of which can be represented as points on a set of co-ordinate axes (Gower, 1966; 1967). If the groups produced by the classification appear spatially distant from one another in the ordination, then this may indicate that non-conformist groups have been formed.

Finally, a GROUPER analysis was used to indicate the importance of the different chemical characters in differentiating various groupings (Lance and Williams, 1968).

Blake (1973) oulines features of these numerical methods, and the reader is also referred to the papers of Gower and Lance and Williams.

The programs were used to classify four arrangements of data: 1) peroxidase; 2) peroxidase + esterase; 3) anthocyanin; 4) peroxidase + esterase + anthocyanin.

4-2 Classification based on Peroxidase Attributes

The dendrogram (figure 4-1a) shows that seven macrantherous species (E. gunnii, E. urnigera, E. perriniana, E. dalrympleana, E. rubida, E. viminalis and E. johnstonii) fuse very early; E. ovata joins this group at a higher level, GROUPER analysis indicating the separation is due mainly to differences in bands IV, V and X. Differences in bands IV, V and VI are sufficient to prevent fusion of these species with another two macrantherous species, E. globulus and E. cordata, until quite a high level. The separation of these two species from the rest of Section Macrantherae is best seen in the plot of the results of the principal co-ordinates analysis (figure 4-1b).

MULTBET divides the remaining species into two groups. One contains E. delegatensis, E. amygdalina, E. linearis, E. simmondsii, E. pauciflora, E. coccifera, E. risdoni, E. tasmanica, along with the macrantherous species, E. aggregata. The remaining group contains E. regnans, E. obliqua and E. sieberi. Bands VIII and IX are the main attributes responsible for preventing fusion of the two groups below the 11.4321 information gain level.

GROUPER analysis indicates that many bands, but particularly bands I - V and X, separate the species into two main groups, and are responsible for the clustering of E. aggregata with the renantherous, rather than the macrantherous, species.

4-3 Classification based on Total Protein.

This generally reflects the first classification

although fusion occurs between all of E. aggregata, E. linearis, E. amygdalina, E. simmondsii, E. delegatensis, E. obliqua, E. regnans and E. sieberi at a lower level than does fusion of any of these species with E. tasmanica, E. risdoni, E. coccifera and E. pauciflora (figure 4-2a). The fusion of all twelve species is prevented by differences in esterase bands VIII, XV and XVI, and in peroxidase band IX.

In the macrantherous group, all members but E. cordata and E. globulus fuse early, these two remaining separate due to differences in peroxidase bands IV, V, VI and XI, and esterase band I. However, the co-ordinates plots (4-2b) indicate that the two species are not as spatially distant from the other eight macrantherous species as they appeared when peroxidase data alone was considered.

The GROUPER analysis indicates that peroxidase bands I - V and X (compare with first classification), and esterase band XIII prevent early fusion of all the Eucalypt species.

4-4 Classification based on Anthocyanins

The micromolecular data splits the Eucalypts into two groups, most of the species in one of these groups containing highly-methylated anthocyanins, species of the other group having simpler anthocyanin molecules (figure 4-3a). The presence of galactosides of cyanidin and delphinidin, and the higher amount of delphinidin 3,5-diglucoside in leaves, in the more complex group aided in the separation, as did the presence of cyanidin 3,5-diglucoside in the bark of certain members of the simpler group.

That group containing methylated anthocyanins is composed of eight renantherous species, and within this, E. regnans, E. obliqua, E. simmondsii and E. linearis are separated from the remainder (E. sieberi, E. delegatensis, E. amygdalina and E. pauciflora) due to the quantity of cyanidin 3-glucoside in the

bark and the presence or absence of cyanidin 3-galactoside and delphinidin 3-galactoside in the bark. However, if the co-ordinations plots (figure 4-3b) are examined, it is clear that E. amygdalina is not as distant spatially from the other true peppermints as figure 4-3a indicates. The Ashes, E. delegatensis and E. sieberi, are quite separate in the co-ordinates plots, while the spatial distance of E. pauciflora from the other seven species is quite considerable (this species possesses galactosides but not the methylated anthocyanins).

The co-ordinates plots show the tightness of the cluster formed by the remaining Eucalypts. These include the macrantherous species and those three renantherous species with simple anthocyanins, namely E. tasmanica, E. risdoni and E. coccifera. Although the dendrogram places these three with E. rubida, the ordination plots do indicate their distance from this species, and the other macrantherous species.

4-5 Classification using all the Chemical Data

The dendrogram resulting when all 49 chemical attributes are used is presented in figure 4-4a. The fusion measure, which increases as dissimilarity between individual or groups of samples increases, is given on a logarithmic scale, because of the great difference in level at which groups fuse. Again there are two major groups, one containing the eleven renantherous species and E. aggregata: the other, the remaining macrantherous species. GROUPER analysis indicates that peroxidase bands I - V, IX - XI, esterase bands XIII, XIV and XVII, and the occurrence of cyanidin 3,5-diglucoside in the bark, and delphinidin 3,5-diglucoside in young juvenile leaves, contribute 70% of the information which causes the separation of the two groups. The placement of E. aggregata with renantherous species certainly reflects the main type of information used in the separation, that is, protein characteristics.

The closeness of the macrantherous species, E. viminalis, E. rubida, E. dalrympleana, E. gunnii, E. urnigera and E. johnstonii is supported by the co-ordinates plots (figure 4-4b). E.globulus and E. cordata fuse with one another and with E. ovata and E. perriniana before they group with other macrantherous species. The presence of the two delphinidin compounds (the 3-glucoside and the 3,5-diglucoside) in their bark contributes significantly to the separation of these four from other macrantherous species, differences in peroxidase bands IV, V, VI and in esterase bands I, XVII and XVIII accounting for much of the remaining dissimilarity.

E. aggregata differs from renantherous species in at least 13 attributes, the majority of these being anthocyanin characters. Its fusion with E. obliqua and E. regnans is prevented by differences in delphinidin 3-glucoside, cyanidin 3,5-diglucoside, cyanidin 3-rutinoside, and peonidin 3-galactose, 5-glucoside, all in the bark; and cyanidin 3-glucoside, delphinidin 3,5-diglucoside, peonidin 3-galactose, 5-glucoside and malvidin 3-galactose, 5-glucoside in young juvenile leaves; and in peroxidase band IX. The three species do not fuse at a lower level with E. linearis and E. simmondsii because of differences in esterase bands VI - VIII, in peroxidase band IX, and in the amount of delphinidin 3-glucoside in bark and young juvenile leaves.

GROUPER analysis shows that esterase bands XV, XVI, XVIII, peroxidase IX and the presence of peonidin and malvidin compounds and of cyanidin 3-glucoside in young juvenile leaves, contribute most to the separation of E. aggregata, E. obliqua, E. regnans, E. linearis and E. simmondsii from E. pauciflora,

E. coccifera, E. risdoni and E. tasmanica. Fusion of all these with the remaining three renantherous species, E.amygdalina, E. delegatensis and E. sieberi is prevented by differences in esterase bands I and III; peroxidase band IX; cyanidin 3-galactoside in young juvenile leaves; cyanidin 3-glucoside, cyanidin 3-galactoside and delphinidin 3-galactoside in bark, and by the presence or absence of the malvidin monoside in each species.

The principal co-ordinates analysis generally reflects the groupings presented in the dendrogram; 57% of the trace of the dissimilarity matrix could be explained by the first three principal co-ordinates. The plots show two main groups (one a very tight cluster) within the macrantherous species, and three within the renantherous species plus E. aggregata. The closeness of the members of each of three pairs - E. linearis and E. simmondsii; E. regnans and E. obliqua; E. tasmanica and E. risdoni - is very clearly shown.

4-6 Discussion of Taxonomic Considerations

From the dendrogram of combined data, a classificatory diagram of the Tasmanian Eucalypts has been drawn up (figure 4-5). The cut-off lines which decide subseries and series are somewhat arbitary. However, evidence for the appropriateness of these can be obtained by a study of the first dissimilarity matrix MULTBET prints out - this is presented in table 4-2, each dissimilarity being expressed as a percentage. The matrix illustrates, for example, the low dissimilarity between E. risdoni and E. tasmanica, between E. obliqua and E. regnans, and between E. linearis and E. simmondsii. The members of each of these pairs fuse at a very low level, as they differ in only one or two attributes (see table 4-1).

The members of the Macrantherae are not as chemically diverse as are those of the Renantherae - this supports the view that the Renantherae is a group still vigorous in evolutionary radiation.

The closeness of E. globulus and E. cordata, and also of E. viminalis, E. rubida and E. dalrympleana differs from the Pryor-Johnson classification (1971), but agrees with that of W. D. Jackson (see appendix). Pryor and Johnson separate E. cordata and E. globulus into two subseries, the former species giving its name to the subseries (Cordatinae), which includes E. gunnii, E. urnigera, E. perriniana, E. rubida and E. dalrympleana. These authors place E. viminalis into a third subseries, which placement separates it from E. rubida and E. dalrympleana until the series level. It is surprising that the present classification has E. globulus, E. cordata and E. perriniana so far removed from the other gums, but it must be remembered that the co-ordinates plots (4-4b) very clearly show the coherence of all the macrantherous species, except E. aggregata. Records of known hybridisation bear out the genetic closeness of the different species.

The present work proposes a close similarity between E. obliqua and E. regnans, and also between E. sieberi and E. delegatensis. This is in contrast to the Pryor-Johnson classification which places each of the Ashes and Stringybarks in separate subseries, sometimes unaccompanied by any other species. Ewart (1930) has suggested that E. fastigata is a variety of E. regnans of possible hybrid origin, with its parents being E. obliqua and E. regnans. This indicates a closeness of the species, and Curtis (1956) notes records of hybrids between the two species.

E. amygdalina joins with E. delegatensis and E. sieberi at the series level. There is a report of a hybrid (E. taeniola) between E. amygdalina and E. sieberi (Curtis, 1956), but the former species does form hybrids with several renantherous members - E. risdoni, E. pauciflora, E. linearis, E. simmondsii, and E. coccifera.

E. tasmanica, E. risdoni, E. coccifera and E. pauciflora form another series, the simpler anthocyanins separating this series from the other renantherous species. The co-ordinates plots indicate that E. pauciflora is spatially distant from the other three species. This placement of E. pauciflora differs from that of Pryor and Johnson, which groups it in a series with the Ashes and Stringybarks.

The remaining major grouping contains E. obliqua and E. regnans, which join with E. aggregata, E. linearis and E. simmondsii at the series level. The separation of these from E. amygdalina, E. delegatensis and E. sieberi is surprising (Pryor and Johnson unite E. amygdalina and E. simmondsii as superspecies). A comparison of the dendrograms does illustrate the results of the GROUPER analysis; namely that the anthocyanin data contribute substantially to this separation.

The fusion of E. aggregata with the above renantherous species appears an anomaly, this species being considered close to E. ovata. In comparisons involving only anthocyanins, E. aggregata is close to the macrantherous group; but protein data places it firmly with the Ashes, Stringybarks and Peppermints. As there are more protein than anthocyanin attributes, in the overall survey it comes in as a renantherous type. The protein of the species was checked using seed from an alternate source (the Forestry Department), and similar results were obtained. In

view of these differences between E. ovata and E. aggregata, it would be of interest to investigate the protein characters of the newly-named 'ovata' type, E. barberi (Johnson and Blaxell, 1972) to ascertain its position.

4-7 Evaluation of Numerical Methods

A comparison can be made between the statistical methods employed in the previous section (3-3) and those used to erect this hierarchal classification of the Eucalypts. The Similarity Index Method developed by Sokal and Sneath, and the assessment of the phylogenetic association as taken from Smith et al, were applied to protein characters only, while both anthocyanin and protein characters were used in the MULTBET programs. However, if the phenograms developed using total protein attributes are compared from the two systems (i.e. figures 4-2a and 3-5) few major dissimilarities are evident. These involve the fusion of E. dalrympleana, E. ovata, E. perriniana; Sneath's method fuses E. aggregata with E. obliqua, E. regnans and E. sieberi, while in MULTBET it is most similar to E. linearis and E. simmondsii.

The statistical methods employed after Sokal and Sneath, and Smith et al., are early attempts at achieving some sort of groupings from the data available. The Sokal/Sneath Similarity Matrix has similarities arranged in a haphazard order, and these are subjectively rearranged to compose dendrograms. As a study of the matrix of Table 3-3 and the dendrogram drawn from it (Figure 3-5) will show, the fusion levels are arbitrary, being only the closest possible for groupings involving more than two individuals (e.g. on the dendrogram E. risdoni, E. tasmanica, E. pauciflora and E. coccifera fuse at about the 80% similarity level, whereas the actual similarity between any two of them ranges from 82% to 96%). And as the number of species in any fusion increases, the percentage similarity shared by a group necessarily becomes more approximate, and the dendrogram rather unwieldly.

Neither the Similarity Index of Sokal and Sneath, which employs the Jaccard coefficient, nor Smith's model, take into account negative matches. Obviously, in a group of distantly related organisms, it would not be valid to consider similarity between two species on the mutual absence of a certain character - similarly, it is misleading to assume that certain mutual positive characters provide a basis for similarity in such a large grouping. However, in the present instance a single genus is being considered, so the matching of absences could be very important. MULTBET, which uses the simple matching coefficient, takes into account such joint absences.

The Similarity Index of Sokal and Sneath, is geared to accept qualitative data, although some fractional data was used in this study. MULTBET can accomodate mixed data - qualitative, disordered multistate, ordered multistate - and it also allows for missing values; thus it will accept the mixture of attribute types normally found in the information collected.

Of major importance is the fact that MULTBET and associated techniques allow unprejudiced clusterings - it is quite objective, being free from personal and pre-conceived notions. A drawback of the technique is that certain clusters may be artefacts, due to MULTBET's tendency to form non-conformist groups of individuals which have little in common with each other, or the rest of the population. However, the principal co-ordinates analysis shows such non-conformist groups as spatially distant from each other.

MULTBET can accomodate quite large problems, although there must be a practical limit to the number of attributes which can be used, depending on the computer and the skill of the programmer. This limit can be extended by a repeated analysis of data in which new characters could be added and tested against the known pattern of variability of earlier-used attributes. Then those which add no new information would be rejected.

A drawback with the present research is the limited number of characters employed in the analysis (49 in all). All attributes are chemical ones - it would be desirable to include a range of attributes that would ensure sampling of a maximum proportion of each tree's visible phenotype. Ideally, no assumptions should be made regarding the relative taxonomic importance of any group of attributes, and chemical, anatomical and external morphological features from as wide a range of the parts of the tree as possible could all be utilised, even if this did involve such repeated analysis as mentioned above. Perhaps some selection is needed to ensure the characters chosen are conservative, that is, evolve slowly and have taxonomic significance, rather than being ones which show ecological influence. Argument exists as to the justification of weighting characters - this could introduce bias into an otherwise objective procedure, as the 'importance' of a character will be determined by what feature the taxonomist decides has more evolutionary significance. If weighting is used, then the heavier-weighted characters should be the more conservative ones. Blake (1973) compared dendrograms obtained using weighted and unweighted characters and found that weighting did not upset clusters produced with the unweighted characters.

A considerable number of characters other than chemical ones have been scored for Eucalypts, so theoretically it would be possible to employ these and obtain a classification which reflects more fully the tree's phenotype. Such a project would involve considerable literature research to build up a list of those attributes described and measured in particular species. Of course, there would be a large number of missing values, but MULTBET can accept data containing these. A difficulty is that such a multiplicity of characters leads to greater dimensionality of the chart representing the relationships between the taxa studied, and the type of representation given (normally a dendrogram)

may not reflect this dimensionality. 3-dimensional models have been proposed to overcome this problem, but dendrograms are easier to construct.

The value of chemical attributes to taxonomic problems must surely be in their identification as an important expression of an organism's genotype - certain metabolic pathways reflect very closely genetic action, some being suitable for delimiting smaller groupings than others. An advantage they have over morphological characters is that they can often be very exactly described in terms of definite structural and configurational chemical formulae. Chemical classification is independent of the traditional methods of taxonomy and so can be used to check groupings decided by the latter. Classifications based on chemical data usually support the traditional classificatory schemes, and numerous instances of these have been listed elsewhere (Sharma, 1970). In certain instances, chemical work has clarified problems morphologists have been unable to resolve, and in those instances where a re-grouping is indicated, there has usually existed some doubt about the original placement.

Concerning the Tasmanian Eucalypt taxonomy, the chemical characters have supported the major split in the genus, with just one exception. The close affinities between certain species reflect the conclusions of local taxonomists, it not of those concerned with mainland forms of the island's species. The position of E. aggregata is thrown in doubt if the chemical characters are consulted, although only one aspect of these - protein characters - is responsible for the discrepancy. Without being subjective, it is difficult to decide which is more important, the protein or the anthocyanin attributes, although the latter do support the relationships established by morphological attributes. These flavonoids place the species in the macrantherous section, but just why the protein profile is so obviously

renantherous is difficult to answer. At this stage it is impossible to draw any reasonable conclusion, but one can only present the data as it has been collected. Chemical work involving other compounds could clarify the position, and a comparison of this anomalous species with supposedly-related mainland species, and with E. barberi, would be of value.

Table 4-1

Characters used in classification and ordination studies

(Characters 1-21 Anthocyanins in bark and young leaves; 22-32 Peroxidase bands from whole seedlings; 33-49 Esterase bands from whole seedlings. Note: When a column contains only 0 and 1, 0 = absence, 1 = presence; when a column contains 1,2 and 3, 1 = absence, 2 = presence in moderation, 3 = presence in considerable amount).

	Anthocyanins	Peroxidase	Esterase
E. ovata	300000003302200210000	11122003101	10011111001001010
E. aggregata	310011003301100100000	00033103111	10011311100003100
E. viminalis	210000003301100100000	11111003001	20001111001101010
E. rubida	310000002101100100000	11111003001	10000111001101010
E. dalrympleana	310000003201100100000	11111003001	10010311001102101
E. gunnii	310000003301100100000	11111003001	10001111001001100
E. urnigera	310000003301200110100	11111003001	10001111001101100
E. perriniana	310011003301200110100	11111003001	30001310001101010
E. cordata	210010003201200110000	11133103011	30001111001001010
E. globulus	200000003301200110000	11133103011	20101211001101010
E. johnstonii	210000003301100100000	11111003011	10001110001101100
E. obliqua	210010003102200301110	00033012111	10011311000003100
E. regnans	210000003101200301110	00033012111	10011311000003100
E. delegatensis	101101113101201201111	00033003113	31001301100003100
E. sieberi	201100013112211201111	00033013111	30001111000003100
E. pauciflora	300100012102201200000	00033001112	10001111110011100
E. coccifera	310000001101300100000	00033001111	10001111000011100
E. linearis	210000003102300301010	00033101111	10000111100003100
E. amygdalina	201110003103201301010	00033103111	31000311000003110
E. simmondsii	211000003102300201010	00033101111	10000111100003100
E. risdoni	310000003102300300000	00033001111	20001111100011100
E. tasmanica	310000003102300300000	00033001111	10001111100011100

Table 4-2

Dissimilarity Matrix from (MULTBET)

	E. ovata	E. aggregata	E. viminalis	E. rubida	E. dalrympleana	E. gunnii	E. urnigera	E. perriniana	E. cordata	E. globulus	E. johnstonii	E. obliqua	E. regnans	E. delegatensis	E. sieberi	E. pauciflora	E. coccifera	E. linearis	E. amygdalina	E. simmondsii	E. risdoni	E. tasmanica
E. ovata		41	24	27	33	22	22	29	27	27	31	45	45	63	53	43	39	47	53	47	39	37
E. aggregata			41	43	35	31	39	41	35	41	35	27	27	43	49	39	24	27	37	29	27	24
E. viminalis				10	20	10	14	18	20	18	10	51	47	67	59	55	37	47	55	49	39	41
E. rubida					16	12	16	22	27	29	16	53	49	69	63	49	33	45	55	47	41	39
E. dalrympleana						14	18	29	35	37	18	47	43	67	65	57	39	47	57	49	45	43
E. gunnii							8	22	24	29	8	45	41	61	55	45	27	41	55	43	33	31
E. urnigera								14	24	24	12	45	41	61	55	49	33	47	59	49	39	37
E. perriniana									22	27	22	51	51	63	65	63	47	61	57	63	51	51
E. cordata										14	24	43	43	61	53	51	35	41	41	43	39	39
E. globulus											24	51	47	63	57	53	41	47	47	49	43	45
E. johnstonii												45	41	63	55	49	31	41	55	43	37	35
E. obliqua													4	41	29	41	31	20	29	24	29	27
E. regnans														37	29	41	27	20	31	24	29	27
E. delegatensis															24	39	49	43	31	39	47	47
E. sieberi																35	43	35	39	31	41	41
E. pauciflora																	22	33	43	33	20	18
E. coccifera																		22	43	24	10	8
E. linearis																			27	4	16	14
E. amygdalina																				27	41	41
E. simmondsii																					20	18
E. risdoni																						2

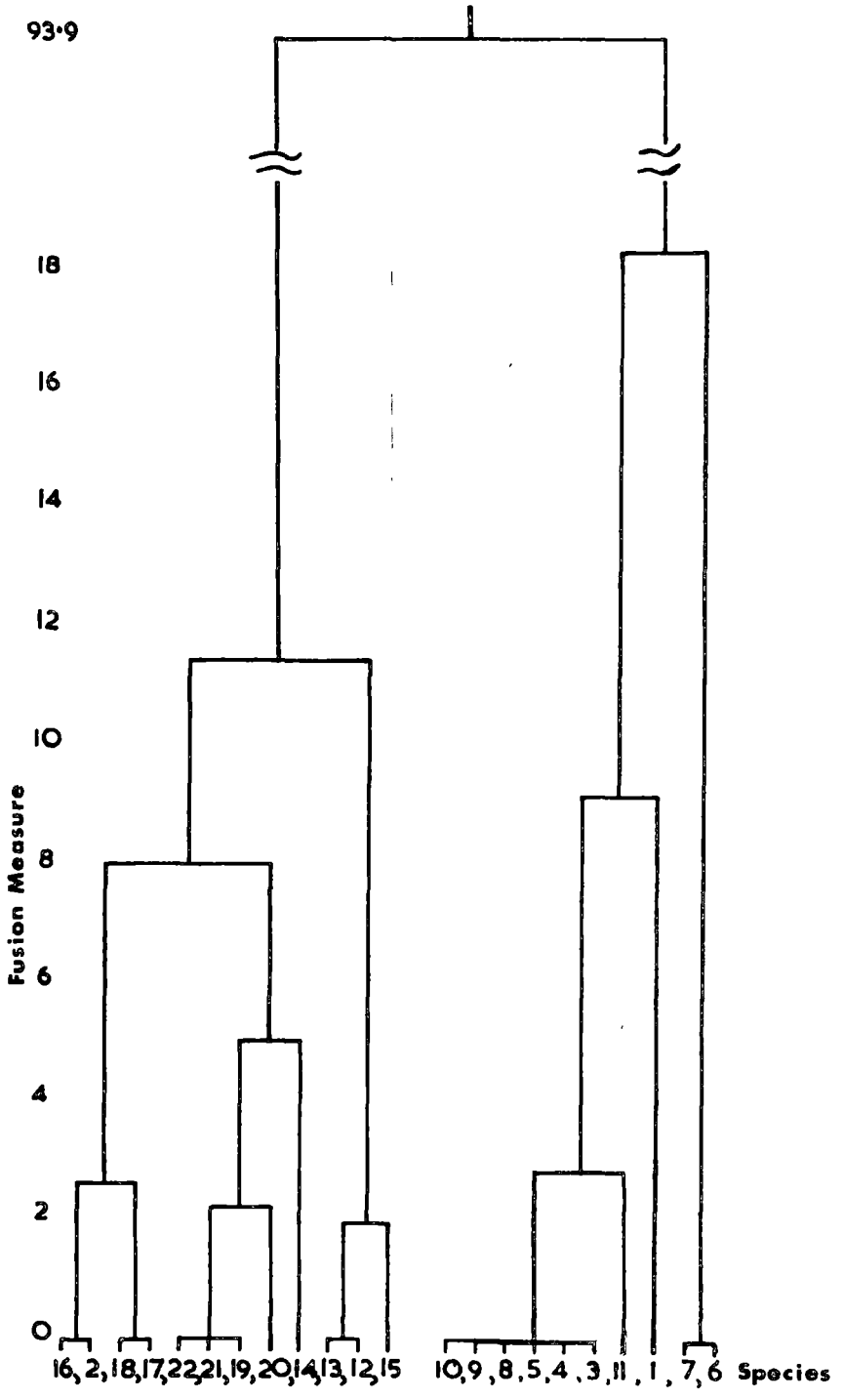


Fig. 4-1a Dendrogram of Classification based on Peroxidase Data.

Key to Species

1. E. ovata
2. E. aggregata
3. E. viminalis
4. E. rubida
5. E. dalrympleana
6. E. globulus
7. E. cordata
8. E. perriniana
9. E. urnigera
10. E. gunnii
11. E. johnstonii
12. E. obliqua
13. E. regnans
14. E. delegatensis
15. E. sieberi
16. E. amygdalina
17. E. linearis
18. E. simmondsii
19. E. coccifera
20. E. pauciflora
21. E. tasmanica
22. E. risdoni

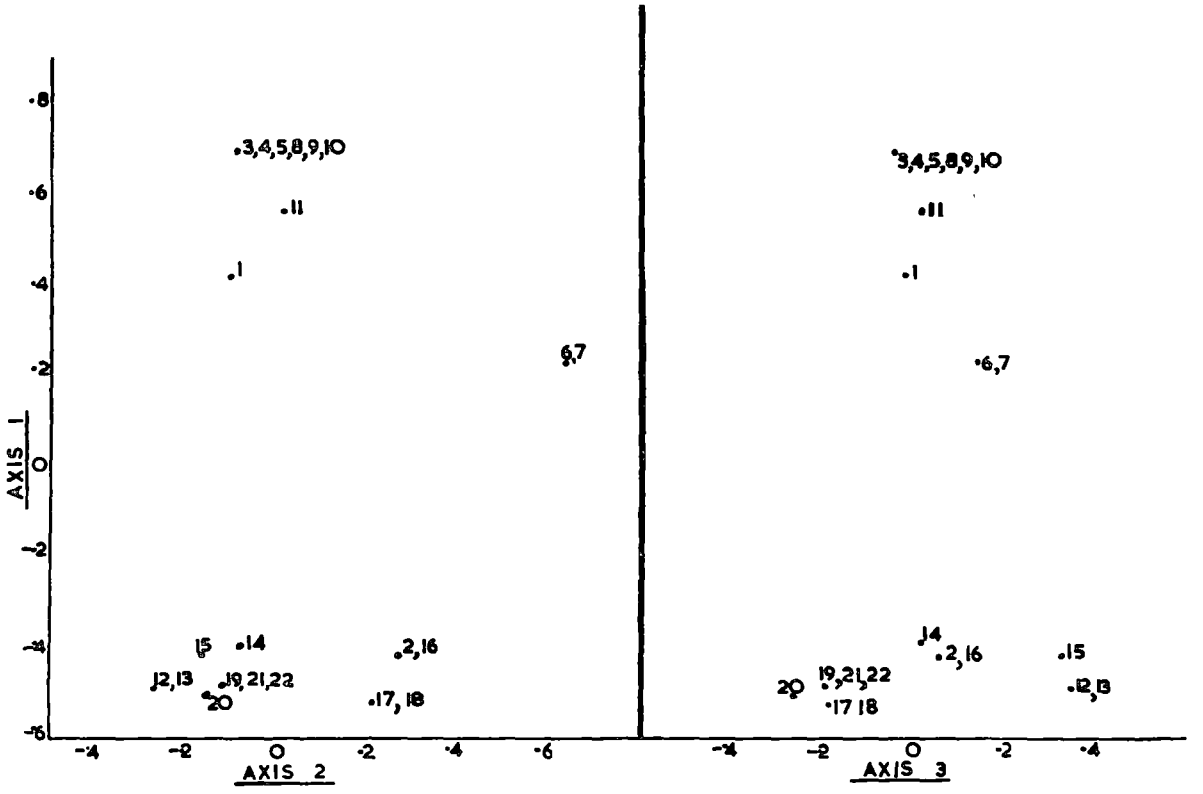


Fig. 4-1b Co-ordination Plots using Peroxidase Data.

Key to Species

1. E. ovata
2. E. aggregata
3. E. viminalis
4. E. rubida
5. E. dalrympleana
6. E. globulus
7. E. cordata
8. E. perriniana
9. E. urnigera
10. E. gunnii
11. E. Johnstonii
12. E. obliqua
13. E. regnans
14. E. delegatensis
15. E. sieberi
16. E. amygdalina
17. E. linearis
18. E. simmondsii
19. E. coccifera
20. E. pauciflora
21. E. tasmanica
22. E. risdoni

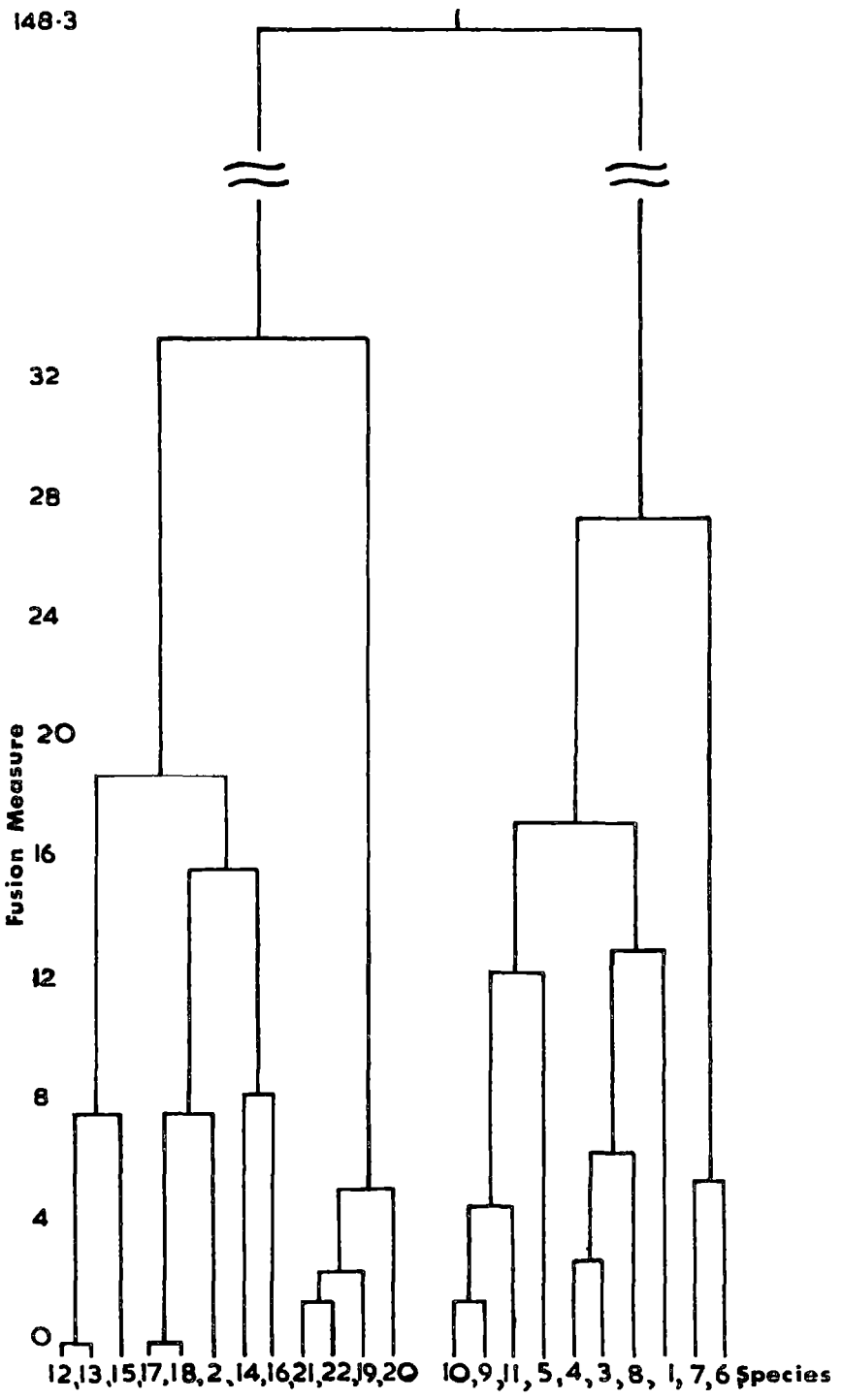


Fig. 4-2a: Dendrogram of Classification based on all Protein Data.

Key to Species

1. E. ovata
2. E. aggregata
3. E. viminalis
4. E. rubida
5. E. dalrympleana
6. E. globulus
7. E. cordata
8. E. perriniana
9. E. urnigera
10. E. gunnii
11. E. johnstonii
12. E. obliqua
13. E. regnans
14. E. delegatensis
15. E. sieberi
16. E. amygdalina
17. E. linearis
18. E. simmondsii
19. E. coccifera
20. E. pauciflora
21. E. tasmanica
22. E. risdoni

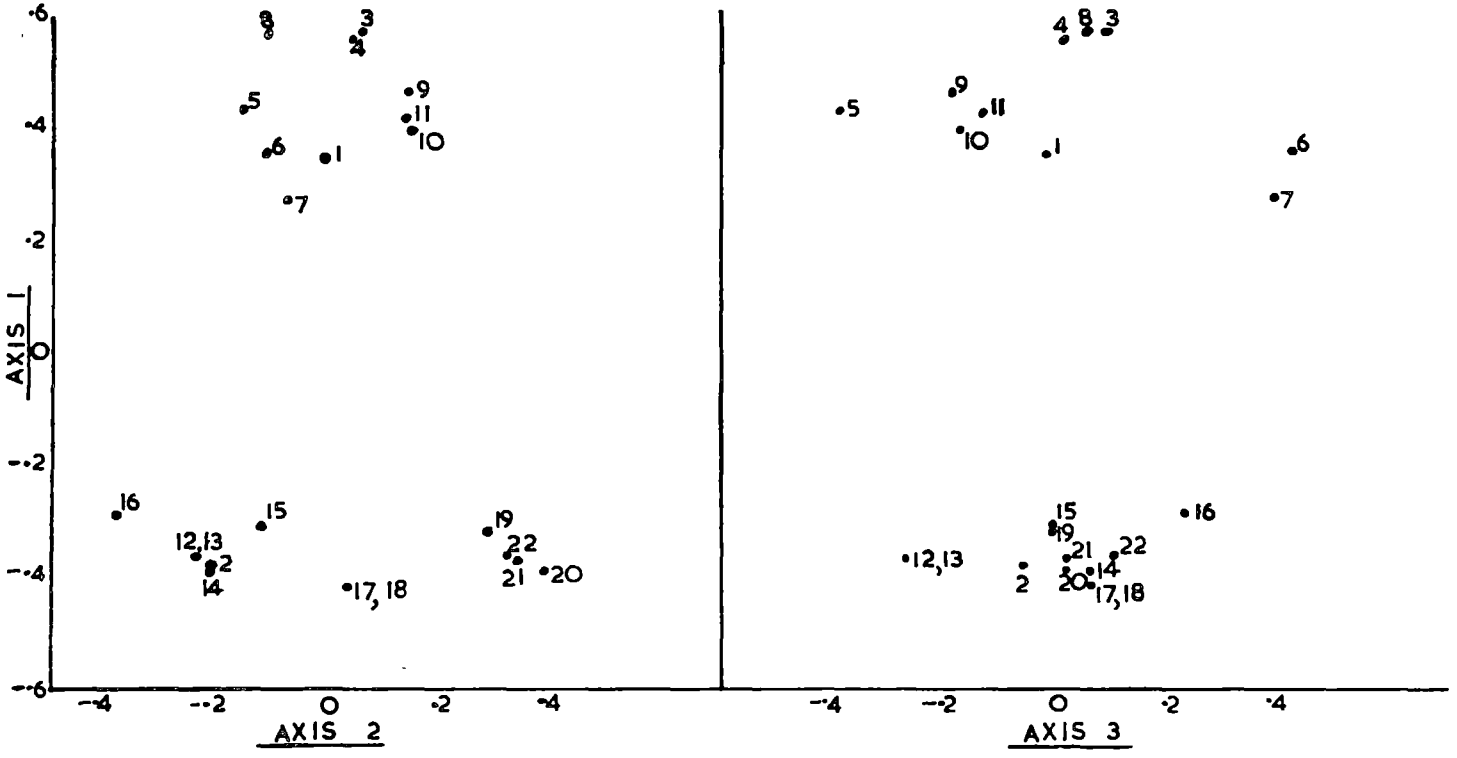


Fig. 4-2b: Co-ordination Plots using Total Protein Data.

Key to Species

1. E. ovata
2. E. aggregata
3. E. viminalis
4. E. rubida
5. E. dalrympleana
6. E. globulus
7. E. cordata
8. E. perriniana
9. E. urnigera
10. E. gunnii
11. E. johnstonii
12. E. obliqua
13. E. regnans
14. E. delegatensis
15. E. sieberi
16. E. amygdalina
17. E. linearis
18. E. simmondsii
19. E. coccifera
20. E. pauciflora
21. E. tasmanica
22. E. risdoni

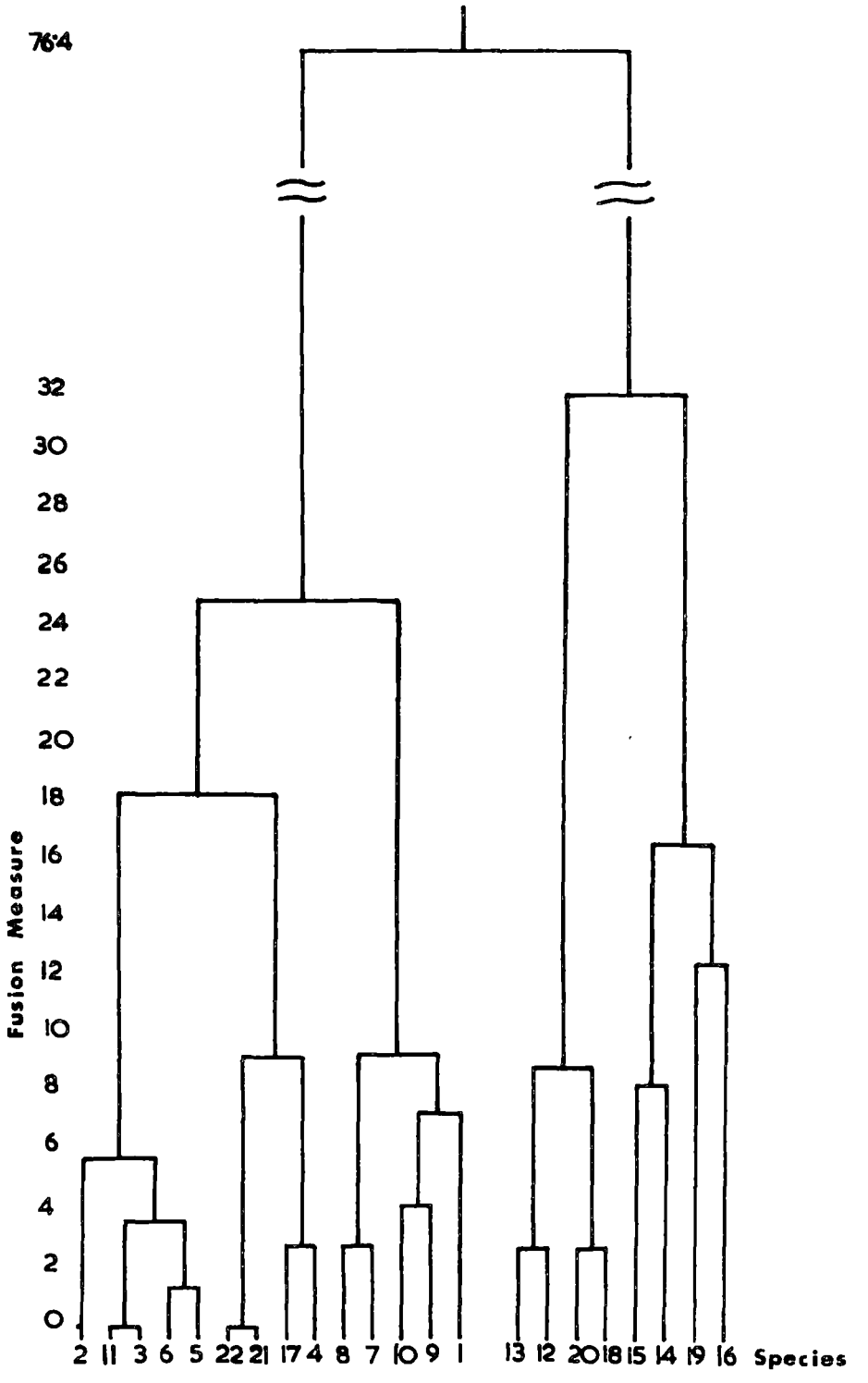


Fig. 4-3a Dendrogram of Classification based on Anthocyanin characters.

Key to Species

1. E. ovata
2. E. aggregata
3. E. viminalis
4. E. rubida
5. E. dalrympleana
6. E. gunnii
7. E. urnigera
8. E. perriniana
9. E. cordata
10. E. globulus
11. E. johnstonii
12. E. obliqua
13. E. regnans
14. E. delegatensis
15. E. sieberi
16. E. pauciflora
17. E. coccifera
18. E. linearis
19. E. amygdalina
20. E. simmondsii
21. E. risdoni
22. E. tasmanica

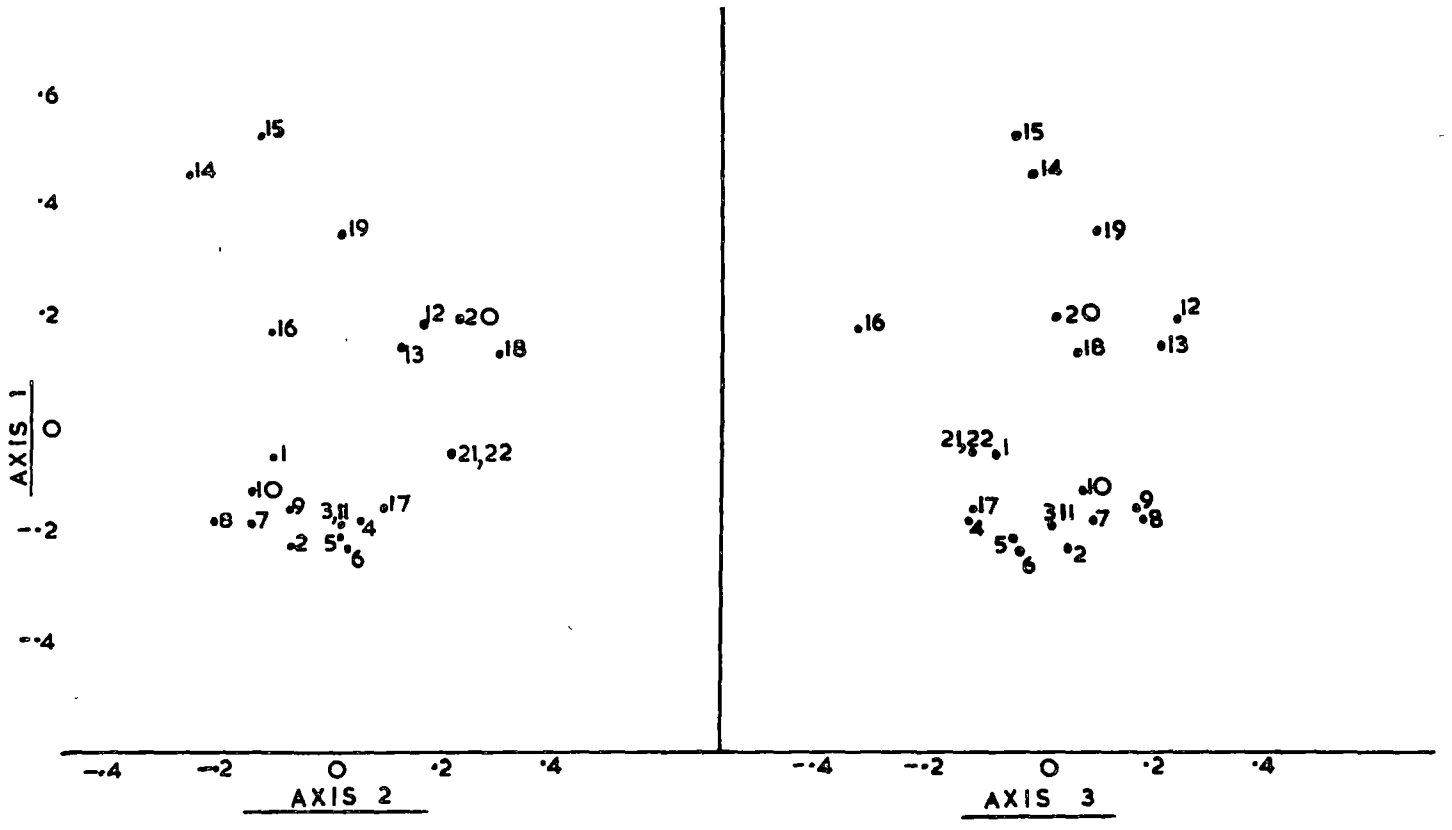


Fig. 4-3b: Co-ordinate Plots using Anthocyanin Data.

Key to Species

1. E. ovata
2. E. aggregata
3. E. viminalis
4. E. rubida
5. E. dalrympleana
6. E. gunnii
7. E. urnigera
8. E. perriniana
9. E. cordata
10. E. globulus
11. E. johnstonii
12. E. obliqua
13. E. regnans
14. E. delegatensis
15. E. sieberi
16. E. pauciflora
17. E. coccifera
18. E. linearis
19. E. anygdalina
20. E. simmondsii
21. E. risdoni
22. E. tasmanica

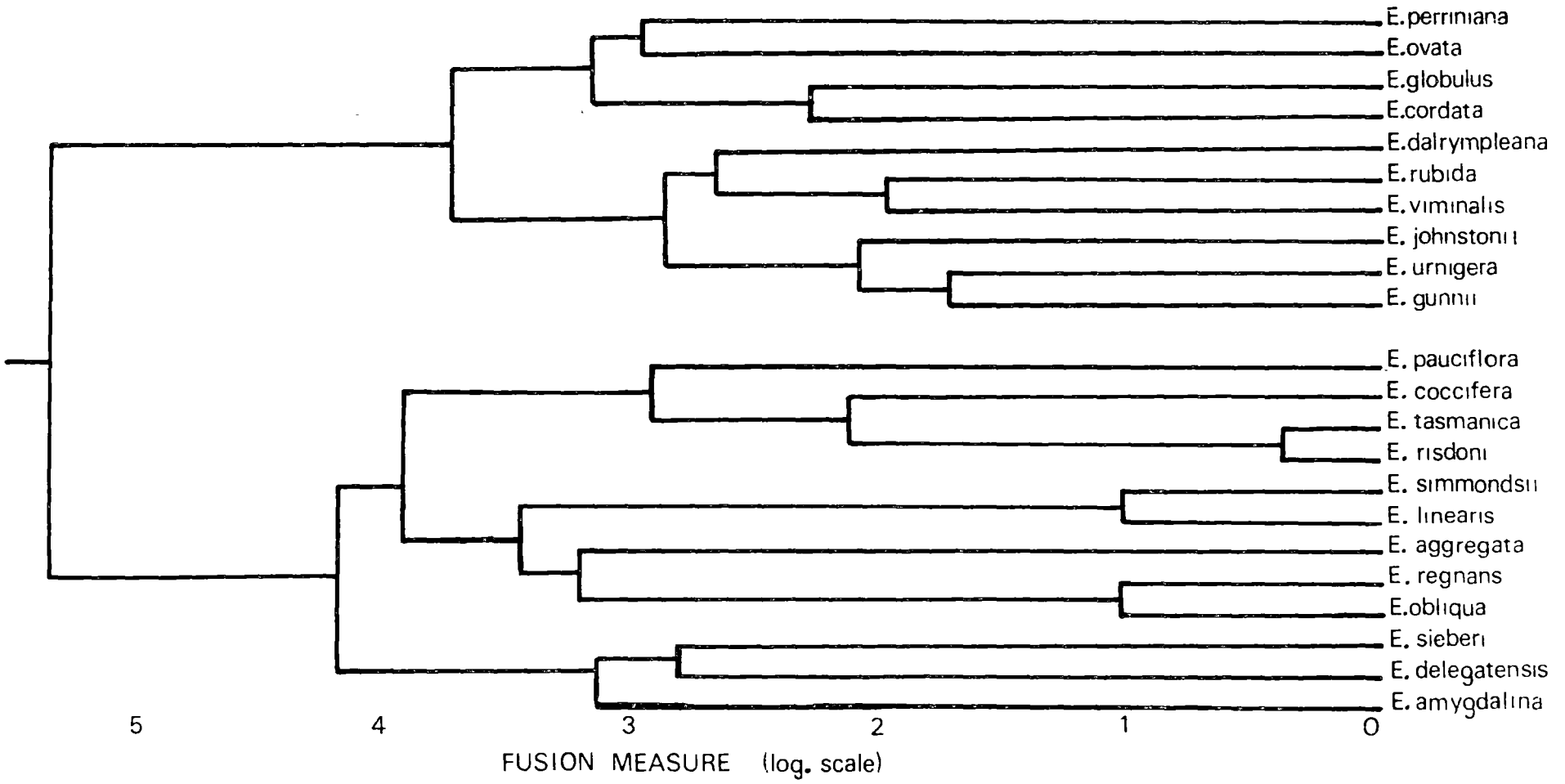


Fig. 4-4a Dendrogram of Classification based on all Chemical Data

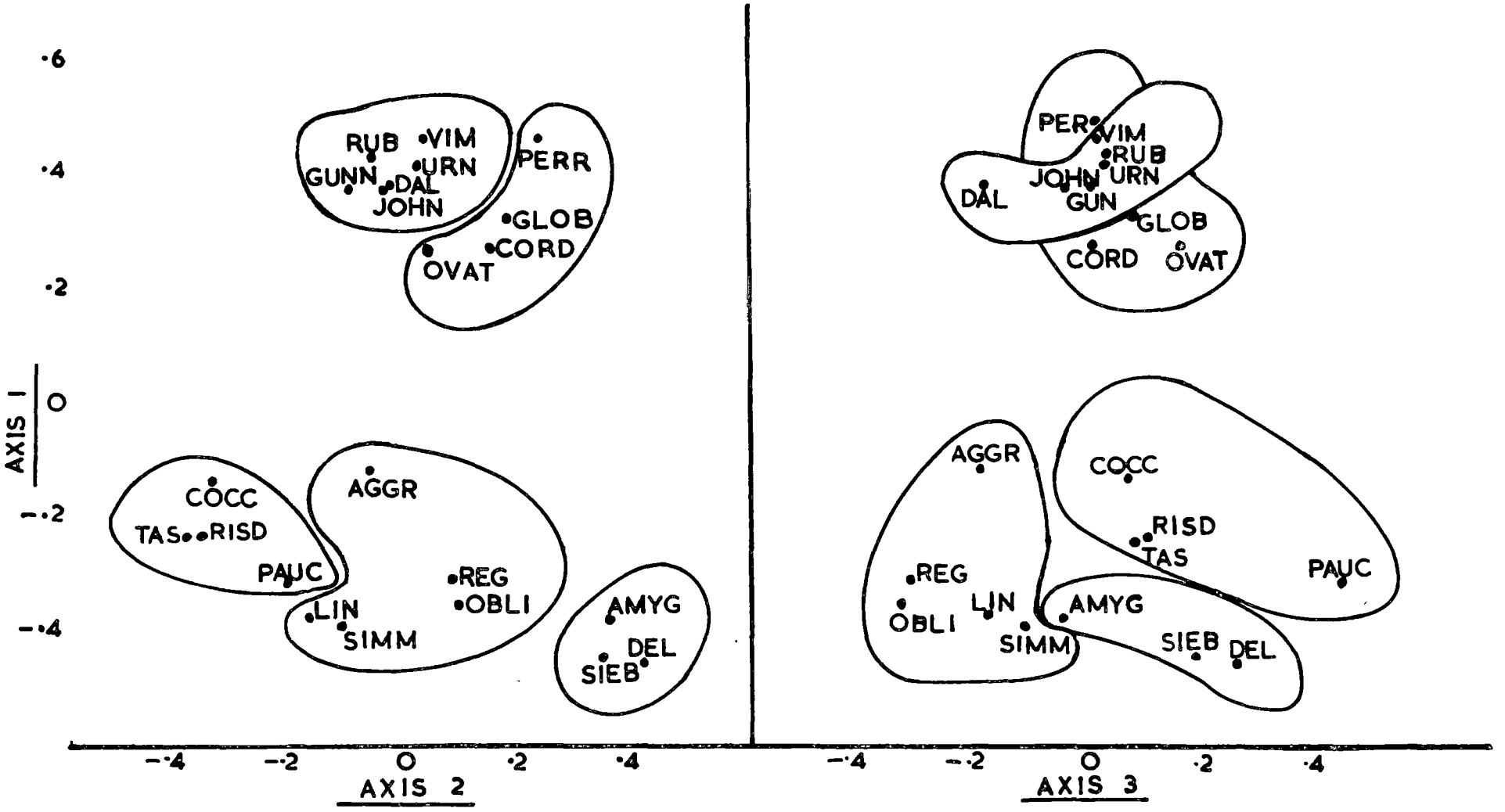


Fig. 4-4b: Co-ordinate Plots using all Chemical Data.

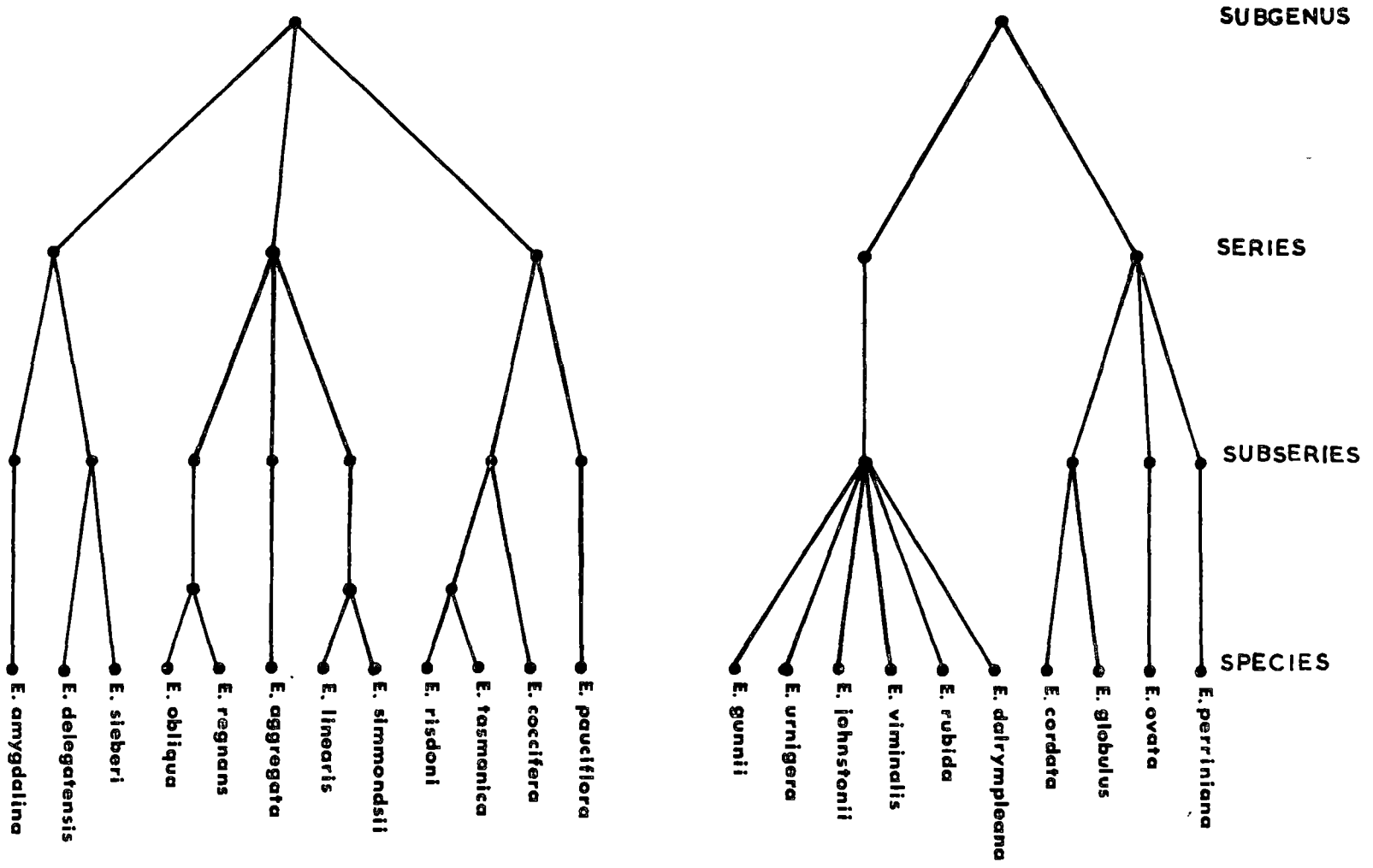


Fig. 4-5: Chemical Classification of Tasmanian Eucalypts.

5. E. VIMINALIS AND E. DALRYMPLEANA POPULATIONS IN TASMANIA

5-1 Introduction

The earlier survey of the phenolics of the Eucalypts (Sharma, 1970 - see appendix) revealed striking chemical differences between the Lowland White Gums, E. viminalis, E. dalrympleana and E. rubida. While chromatograms of the twig bark of these three species all showed, as major pigments, a number of purple fluorescing compounds (numbered 51, 52, 53, 61 and 65 in the appendix), these compounds were not found in the juvenile leaves of E. viminalis. However, they continued to occur as major pigments in those organs of E. dalrympleana and E. rubida. The altitudinal range of E. viminalis is 0 - 500 m; that of E. rubida is 300 - 700 m; and of E. dalrympleana up to 1000 m. Outside the Lowland White Gums, macrantherous species showing these compounds in either or both juvenile and adult foliage, were E. johnstonii, E. urnigera, E. archeri E. perriniana and E. gunnii - all high altitude species.

This suggests that ecological conditions could influence the biochemistry of these compounds. They could be the result of a response to the harsher ecological conditions in high altitudes, and have some selective value. If so, one would expect the compounds to be absent from high altitude species grown at, or near sea level. Again, the formation of these chemicals may be the result of genetic action which is now fixed in the species, even when that species is growing under conditions in which the compounds confer no selective value - thus, the chemicals will occur in all individuals of

these species, no matter what the ecological conditions. Or these diagnostic chemicals may have no selective value but simply be irrelevant by-products of the activity of important genes which are constant members of the genome of that species (Barber, 1955). If these important genes remain active under all conditions, the chemicals will always be present.

It is reasonable to suppose that these purple fluorescing compounds are the stilbenes described by Hillis (1966a and b). According to Hillis, the capacity to form stilbenes is a characteristic of many Series of the Macrantherae, but not of the Renantherae. He reports the occurrence of stilbene-containing and stilbene-lacking forms of some species and suggests that the variation could be ecologically directed, as nineteen of the twenty-eight species possessing stilbenoid chemotaxa were collected in low rainfall regions or areas of high evaporation rates. Furthermore, in taxa having both stilbene-forming and stilbene-lacking forms, the latter occurred in low rainfall areas, the former in high rainfall areas. A few exceptions weaken this 'water-stress and stilbenes' association, and it is difficult to apply it to the E. dalrympleana situation, for this species occurs in areas of high rainfall.

Barber (1955) correlated glaucousness with frosty localities, pointing out, however, that the development of glaucousness is not the only method of increasing frost resistance open to Eucalypts. It is of interest that an estimation of frost damage to E. viminalis (which is never glaucous) and E. dalrympleana (usually glaucous) seedlings after the severe winter of 1972 gave higher mortality and damage rates for those provenances from the lower altitude areas, e.g. those coded M1 - M4 (Orme, unpublished). The samples containing

stilbenes displayed a higher degree of frost resistance. Orme recommends that these results be treated with caution as much of the stock had been damaged prior to planting, causing a general lowering in the resistance of the plants. Measurements over a number of years are needed before worthwhile conclusions can be drawn. Furthermore, some estimate of the degree of glaucousness of the various stilbene-containing seedlings is required.

The extreme differences occurring in the chemicals of E. viminalis and E. dalrympleana juvenile leaves can be utilized in a study of populations of these species in Tasmania, and to clarify the position of the Tasmanian tree E. dalrympleana (as described by Curtis, 1956). This latter is described by Pryor and Johnson (1971) as a sub-species of E. viminalis in Tasmania, while Barber (1955) had coined the term "vim-dal" to represent those "clinal" White Gums occurring in the altitudinal range of 600 - 1000 m. He maintained that the populations are intermediate phenotypically between E. dalrympleana of the Australian mainland, and E. viminalis. Willis (1972) considers that E. dalrympleana (as found in Victoria) cannot be treated as a form of E. viminalis.

Morphologically, E. viminalis and E. dalrympleana differ most in the juvenile foliage. The juvenile leaves of E. viminalis are opposite, pale green, sessile, stem-clasping, ovate-lanceolate with an acute apex, and they measure 5-10 cm long, 1.5-3 cm broad. E. dalrympleana has opposite, sessile, broadly ovate to almost orbicular green or glaucous juvenile leaves; the apex is blunt or apiculate and the leaves measure 4-6 cm long, 4-5 cm broad, or larger (Curtis, 1956).

The leaf size and shape does vary, even between provenances of the same species growing in close proximity. This has led to the belief of some workers (e.g. Barber, 1955) that the range of these White Gums and their variants represents an altitudinal cline of the one species, E. viminalis. Ideally, if the extremes of the cline can be compared with each other, and with mainland samples of E. viminalis and E. dalrympleana, one can find a basic pattern of variation either within the single clinal species, or within each of the two separate species. Thus a measure can be made of the range of variation acceptable within groupings, and of the nature of individuals in 'hybrid' zones.

Mr. K. Orme of the Forestry Commission, Hobart, undertook a morphological investigation of the E. viminalis/ E. dalrympleana complex, in an attempt to deduce the true identity of the various White Gums. He collected seed from individual trees in a variety of localities. The locality and altitude are shown in table 5-1. To allow for a possible clinal basis to the phenotypic variation, many of the parent trees were in the one locality, the Mersey Valley, an area which offers an altitudinal range from sea level to 1000 m (see code M1 through M11 and Map). Orme (unpublished,1970) gives three main reasons for choice of this site:

i) the white Gums are continuous over the altitude range;

ii) there are no areas of hybridisation with other species

iii) the valley provides the change in altitude over small changes in latitude and longitude.

Parent trees from other localities in the State are also included, and to provide a comparison with mainland members

of the two species, seed of E. viminalis from a sea level region (Boydtown, N.S.W.) and from an area at altitude 1300 m (Nimmitabel, N.S.W.) was used. E. dalrympleana seedlings were raised from seed collected in the Brindabella Range, A.C.T. (altitude, 1700 m).

The seed from each of the parent trees was germinated and a maximum of 50 seedlings per parent tree was transplanted into plywood cylinders, and continued their development under uniform conditions in a glasshouse, until the seedlings were ready for forest plantation at varying altitudes to ascertain their degree of frost sensitivity. While in the seedling stage in the glasshouse, Orme sampled leaves from individuals of each of the 39 provenances. From these sampled leaves Orme will obtain a quantitative value for juvenile foliage - that is, he will measure leaf shape (using polar co-ordinate measurements) of particular parent trees and provenances in an attempt to obtain statistically significant differences.

It was decided that it would be useful to parallel the morphological investigation with a chemical one, which would include a comparison of both the phenolics of the juvenile foliage, and the peroxidase and esterase activities of the seedlings.

5-2 Phenolic Survey

(a) Experimental Methods

From each batch of seedlings of the 39 provenances, 10 seedlings were sampled at random. Nodes '0' and '5' were sampled, node '0' bearing the last leaf pair to have unfolded. Occasionally, due to stunted growth or disease of the leaves of node '5', another node was sampled, this was node '4', '6' or '7'. Leaves from nodes '0' and '4' (or more) were studied separately.

The two leaves per node sample were weighed and 1 ml absolute ethanol per 15 mgm leaf weight was added to the leaf sample in a test tube. The samples were placed in a boiling water bath for 5 minutes - a little water was added to the sample if evaporation was too great.

On removal from the water bath some distilled water was added to each sample to increase volume, and then the sample was shaken with 5 ml of petroleum ether. After settling, the lower layer, from which waxes and a certain amount of chlorophyll had been removed by the petroleum ether, was pipetted onto a watch glass. This was evaporated to dryness in a current of air. The dried sample was re-dissolved in absolute ethanol and spotted onto Whatman's No. 1 Chromatography paper. This paper was run overnight in BAW solvent. After drying, the paper was run at right angles to the original run, but in 5% acetic acid for approximately 4 hours. The resulting 2-dimensional chromatograms were compared under ultra-violet light, with and without ammonia fumes.

(b) Results

(i) General Results

The 780 chromatograms obtained in this survey fell into one or other of two basic patterns, which can be called 'viminalis' pattern, and the 'dalrympleana' pattern. All the spots of the 'viminalis' chromatograms are shown in Figure 5-1, and those of the 'dalrympleana' chromatograms in Figure 5-2 (see also photographs). The colour and R_f for each compound is presented in Table 5-2.

The 'dalrympleana' chromatograms are obviously dominated by the intense purple compounds, hereafter referred to

as 'stilbenes'. As all the leaves examined in this survey were from seedlings grown in the glasshouse, the differences between the two types cannot be described as plastic, and depending on varying environmental conditions. It is likely then, that the presence of these chemicals has resulted from some response to the environment, but that the biochemical events this response has initiated are now a fixed part of the genetic make-up of the species.

These differences have enabled each seedling examined to be placed as a 'viminalis type' or a 'dalrympleana type'. All ten samples from each of 17 provenances were 'viminalis type', and all ten samples from each of 12 other provenances were 'dalrympleana type'. These provenances are listed in Table 5-3, and Figures 5-3 and 5-4 are histograms showing the mean frequency and standard error of each spot for all of the 'viminalis' and dalrympleana' compounds occurring in node '4' leaves. Some of these spots are more variable than others, and have a high standard error (e.g. in E. viminalis, spots 16, 27, 28, 44, 45, 47, 50 and 51, and in E. dalrympleana, spots 27, 63, 65 and 67 all have a standard error greater than 0.5).

Often, a number of spots represents a complex - for example, spots 13, 14, 15, 45, 38 (see Table 5-2) - but not all members of the complex are always present. This effect could be due to isomerism of particular chemicals. The several blue → blue green spots may be isomers of chlorogenic acid. Swain (1962) reports the existance of 5 or more such compounds, and Hillis (1966b) has given account of two forms in the Eucalypts.

The possibility that spot 51 represents certain of the 'stilbenes' (namely, spots 53 - 57) is eliminated by the presence of spot 51, as well as spots 53 - 57 in a small number of 'dalrympleana' chromatograms (node '0' samples from Minnow Creek and Great Lake) in which the latter compounds are in smaller amount. The 'stilbenes' mask any other compounds in the same area, so although spots 1-5 are not scored as 'present' in the 'dalrympleana' chromatograms, this is only because they cannot be recognised. In a sufficiently large number of the node '0' chromatograms in which the 'stilbenes' are only present in small quantities, one or all of spots 1-5 are always present. Again, in the 'dalrympleana' chromatograms, other purple compounds (50, 59, 60 - 67) may mask spots 16-20, 25-31 and 35, so these compounds which are normally present in 'viminalis' chromatograms will not always be scored in the 'dalrympleana' chromatograms.

It seems then that all the compounds present in E. viminalis are present in E. dalrympleana, but may be masked by other compounds. Those spots shown hatched in Figure 5-2 are unique to E. dalrympleana. They may not be found in every sample of this species, but spots 53 - 59 are diagnostic for all members of the species. It is possible that more than one number has been assigned to the one purple-fluorescing compound, where it covers a large area of the chromatogram. For example, spot 67 may be an extension of spot 58 overlapped by spot 13, although it does appear as a discrete spot in one instance. Spot 67 varies slightly in colour from 58, but this may be because of the underlying weak spot 17. Alternatively, the one compound may be scored as two different spots (e.g.

spots 60 and 62).

The distribution of some individual compounds is of interest - the mean, standard deviation, value of t, and the probability for each is given in Table 5-4. In E. viminalis, the mean frequency of compound 37 was significantly low for the provenances of King Island a, Rocky Cape, Nimmitabel and Boydtown. Compound 49 is absent or low in all samples except M3a and M4c, in which it occurs in high frequency in both node '0' and node '4' leaves. Compounds 50 and 51 are present, if only at a small frequency in the majority of provenances, but they never occur in the mainland provenances of Boydtown, and Nimmitabel or in Kind Island a and b, Rocky Cape and Scamander samples. The mean frequency of the two compounds in the above provenances and the M1b sample, differs significantly from the frequency for all other samples. Finally, the high frequency of compound 52 in Mt. Foster and M4a samples is significantly different from the mean for other provenances.

Five compounds show particular distribution patterns within E. dalrympleana provenances. The frequency of spot 47 is lower in provenances from ANU, Great Lake and Minnow Creek, and that of spot 61 in M10, Minnow Creek, Great Lake and ANU samples. Spot 62 occurs at lower frequency in the M8b and 007-71 samples. Compound 28 occurs at high frequency in the Minnow Creek provenance, while compound 67 occurs in higher amount in the M8a, M9, M10 and ANU provenances.

(ii) Ontogeny

Some information as to when compounds appear is obtained from this study. The full chemical profiles were found in the older leaf, although in the majority of node '0' leaves it was also evident. Basically, the differences between

the younger and older leaves was the higher frequency of compounds in node '4' leaves - spot 37 in both E. viminalis and E. dalrympleana doubled in frequency (at least) between nodes '0' and '4', indicating it is one of the latest compounds formed. For this reason, only node '4' leaves were used to compile Figures 5-3 and 5-4. However, a limited number of compounds were more frequent in the node '0' leaves. In both E. viminalis and E. dalrympleana, spot 38 was present in significantly higher frequency in node '0' leaves ($t = 8.2$; probability < 0.01). This compound may be degraded or converted into other compounds in the older leaf. Spot 69 is only recorded, and then at a very low frequency (0.167) in node '0' leaves of E. darlympleana. In this species, the following compounds are in higher frequency in node '0' than in node '4' leaves : spots 33 and 36 ($t = 2.1$; probability < 0.05) and spots 45 and 49 ($t \geqslant 9$; probability < 0.01). However, this difference can be explained by the increase of stilbenes as the leaf ages - an increase which efficiently masks many other compounds.

Some of the node '0' leaves which had just unfolded permitted speculation about the sequence of formation of the compounds, and this is summarised in Figures 5-5 and 5-6. In E. dalrympleana, the main change after the very early stage is the tremendous increase in the production of the purple 'stilbenes' which tend to swamp the chromatograms. The spots shown in the figures are only the ones present at high frequency in all samples. In those samples in which they occur, spots 33, 36, 44 and 50 are detected at the earliest stage shown.

To obtain a clearer picture of the sequence of formation it would be necessary to sample the latest-formed

leaves from their very early development until they unfold, for by the time the two leaves expand they normally display the total chemical profile.

(iii) Mixed Provenances

In 10 provenances the seedlings were mixed - that is, some showed a typical 'viminalis' pattern, others a typical 'dalrympleana' pattern (see Table 5-3). The trees in these provenances were not hybrid trees, but it seems hybrid seed must have been formed. Histograms of the frequencies of compounds in the population of these provenances are given in Figures 5-7 through 5-16, and the number of 'viminalis' and 'dalrympleana' types within each provenance are given below:

Provenance	No. of 'viminalis type	No. of 'dalrympleana' type
E. viminalis 011-71	9	1
Leven Rd. (a)	1	9
Leven Rd. (b)	5	5
Beulah	5	5
E. dalrympleana Golden Valley	4	6
M5a	6	4
M5b	7	3
M6a	1	9
M6b	1	9
M7	7	3

(iv) Discussion

This survey confirms that although 'stilbenes' are synthesised in E. viminalis bark, their synthesis does not occur in E. viminalis young juvenile leaves. However, stilbenoid synthesis occurs in both organs of E. dalrympleana.

This supports Willis (1966b, 1967b), and offers a useful chemical character for delimiting the two species. It is yet to be seen if the morphological characters will support this. The mainland forms have similar chemical profiles to their Tasmanian counterparts, although there are significant differences between the means of a limited number of compounds (see Table 5-5), none of these being important compounds in the separation of the two species.

A number of compounds which occur in both the species did show significant differences in their means. Obviously this is often due to masking of the compound in E. dalrympleana chromatograms, but with the compounds listed in Table 5-6 such interference does not occur. Thus, the frequencies estimated would seem reliable for the species. This information could be of use in determining hybrid populations; these would show intermediate frequencies perhaps not significantly different from either parent population. As the survey has not included hybrid populations, the hypothesis cannot be tested.

In examining the mixed offspring from several provenances, it appears that except in the case of provenance 011-71, all parent trees were phenotypically E. dalrympleana species. One can suppose that these trees have been cross-pollinated by both E. viminalis and E. dalrympleana. It would then seem that the single gene difference resulting in stilbenoid synthesis is recessive, so that blocking of stilbenoid synthesis by a dominant gene could occur in E. viminalis. Thus, that seed which results from a cross between E. dalrympleana and E. viminalis will produce seedlings which show a typical E. viminalis pattern.

The E. dalrympleana seedlings will have come from seed pollinated by another E. dalrympleana tree.

Another hypothesis involves epistatic interactions. The stilbene-forming gene could be epistatic to a dominant gene possessed by E. viminalis. Hergert (1962) mentions the likelihood of stilbenes being polymerised to form tannins - genes responsible for this polymerisation may be dominant in E. viminalis, recessive in E. dalrympleana.

These hypotheses need be examined with reference to the 011-71 samples - these are seedlings from an E. viminalis tree - all seedlings would be expected to have the 'viminalis' pattern. However, one of the ten samples shows part of the 'dalrympleana' pattern, i.e. appears to be a true hybrid, having only spots 55 and 58 as well as spots possessed in common by the two species. Spot 58 occurs in node '0' leaves alone. Sampling from provenances 011-71 (E. viminalis), Golden Valley (E. dalrympleana) and 006-71 (E. dalrympleana)was done at the same time, and the anomaly found with the E. viminalis provenance may be due to sampling error. Alternatively, this result may be explained by applying a theory which involves a dominant gene responsible for polymerisation of stilbenes; such a gene could occur in high frequency in E. viminalis (i.e. with present data, in 179 out of 180 samples), while the recessive allele occurs in high frequency in E. dalrympleana. The data suggests the occurrence of a low frequency allele (in 1 sample of 180) in E. viminalis which prevents the complete polymerisation of stilbenes. In hybrids in which the dominant allele occurs, stilbenes are not found; but in the hybrid in which some evidence for their existence occurs, the recessive allele from the E. dalrympleana parent does not allow their polymerisation, while the low frequency allele from the E. viminalis parent

will not allow complete polymerisation. A very extensive survey of seedlings from E. viminalis parents in regions adjacent to E. dalrympleana stands would be required to substantiate the existence of this allele.

Those provenances which gave mixed seedlings occur in the area where there is a sharp crossover from E. viminalis to E. dalrympleana. Very strong selective pressures must be operating to allow the development of the E. dalrympleana seedlings at the expense of those of the E. viminalis type. Stilbenoid formation may be a factor in this selection pressure; however, it is unknown on just what aspect of seedling formation this pressure is acting. It is certainly absent under glasshouse conditions, which allow the survival of both types of seedlings from the one provenance.

5-3 Enzyme Study

(a) Experimental Methods

Table 5-1 indicates those provenances which were examined for enzyme action; seed stock from the ten provenances not studied had been exhausted. The seed was stratified and grown under glasshouse conditions as detailed in chapter 3.

Initially, it had been planned to work with single seedlings. However, many isoenzyme bands in individual seedlings could not be detected. After several trials it was decided that three batches, each of six randomly-selected seedlings, be used from each provenance. An attempt was made to keep the concentration of protein in each sample constant. Extraction of seedlings, gel polymerisation and other experimental details are given in chapter 3. A difference is,

that in this experiment, a thin layer template of 1.2 mm thickness was used.

The gels were stained for peroxidase and esterase activity; after the bands had developed, the gels were placed in fixative (see Appendix). The developed gels were scanned and traced with an Integrating Densitometer and the tracings were compared to ascertain site and concentration of each enzyme band.

(b) Results and Discussion

It was hoped to compare tracings within and between provenances, and thus gain an estimate of the correlation of samples within a provenance, and the correlation between provenances. These could be used to arrange a clustering pattern which would separate E. viminalis and E. dalrympleana, and perhaps indicate extreme closeness of some provenances. The correlation value would be found by comparing optical densities of two tracings at a specified number of migration points. This would not only take into account the migration velocity of a band, but the quantity of enzyme present - for direct comparison then, it is important to equalise the areas under the curves when taking the tracings.

Figure 5 - 17 gives a peroxidase band pattern and a simplified tracing. The bands for each of the samples were the same although sometimes band 8 could not be seen. This could be due to a concentration factor. When the tracings were compared it was obvious that correlation measurements were impractical. The amount of variation between the same sample in two slots on the same gel was very high, and often, greater variation occurred within provenances than between them. This is attributable to the heavy interference patterns obtained.

Unequal polymerisation of the cyanogum produced lines in the gel, which caused interference to the tracings. This also occurred when o-dianisidine crystals adhered onto and into a gel; even careful washing could not completely dislodge these.

In most of the gels treated with esterase stain the bands did not develop, and preliminary densitometer scanning of those gels in which the bands did develop, indicated similar problems as had occurred with the peroxidase bands. However, it did seem that there may be more variation in esterase than in peroxidase bands in E. viminalis and E. dalrympleana. The exact range of polymorphism of these in each species needs to be obtained; then, if interference problems in the gels could be overcome, correlations from esterase tracings could be of some value.

5-4 Conclusion

Paper chromatographic analysis has provided significant information in determining the species affinity of certain juvenile leaves. In these, both 'viminalis' types and 'dalrympleana' types can be recognised by the chemical spot patterns. The phenolic compounds are obviously selected under certain environmental pressures, as are morphological ones. At the moment it cannot be decided whether the accumulation of stilbenes in some way makes E. dalrympleana more fit than E. viminalis in the boundary areas, and it will be of interest to discover the mechanism which allows the accumulation of these compounds under particular conditions.

Some authors (e.g. Weimarck, 1972) consider unidentified chromatographic spots are not very suitable characteristics for evaluation between taxa, as spots which appear to be identical in some comparisons may turn out to represent different compounds. However, the use of the chromatograms as

'finger-prints' for comparisons is considered valid in many instances in separating taxa or indicating taxonomic relationships (Brehm and Ownbey, 1965; Asker and Frost, 1970). Naturally, the material used should be from corresponding parts of the compared plants at the same stage of development; environmental conditions also need to be stabilised. In the present study, these conditions were fulfilled. Obviously, separation, purification and identification of the many compounds in E. viminalis and E. dalrympleana species would involve an enormous amount of labour.

Although the species have characteristic band patterns, electrophoresis did not reveal any significant information of use in indicating either the E. viminalis or the E. dalrympleana affinity of the provenances. It would appear that the two species may be so closely related that available staining reactions are not sensitive enough to reveal differences - or again, the appropriate extracting procedure and stain may not be available. This certainly appears true for the peroxidase enzymes. It may be possible that esterases could be used in such population studies when techniques are better developed. However, it would first be necessary to fully investigate the range of polymorphism of the esterases within and between Eucalypt populations.

On the whole, then, it appears that macromolecular studies on Eucalypts will aid in separation of larger taxa (e.g. the two Sections in Tasmania), while micromolecular investigations appear useful in identifying species, and to a limited extent, populations within a species.

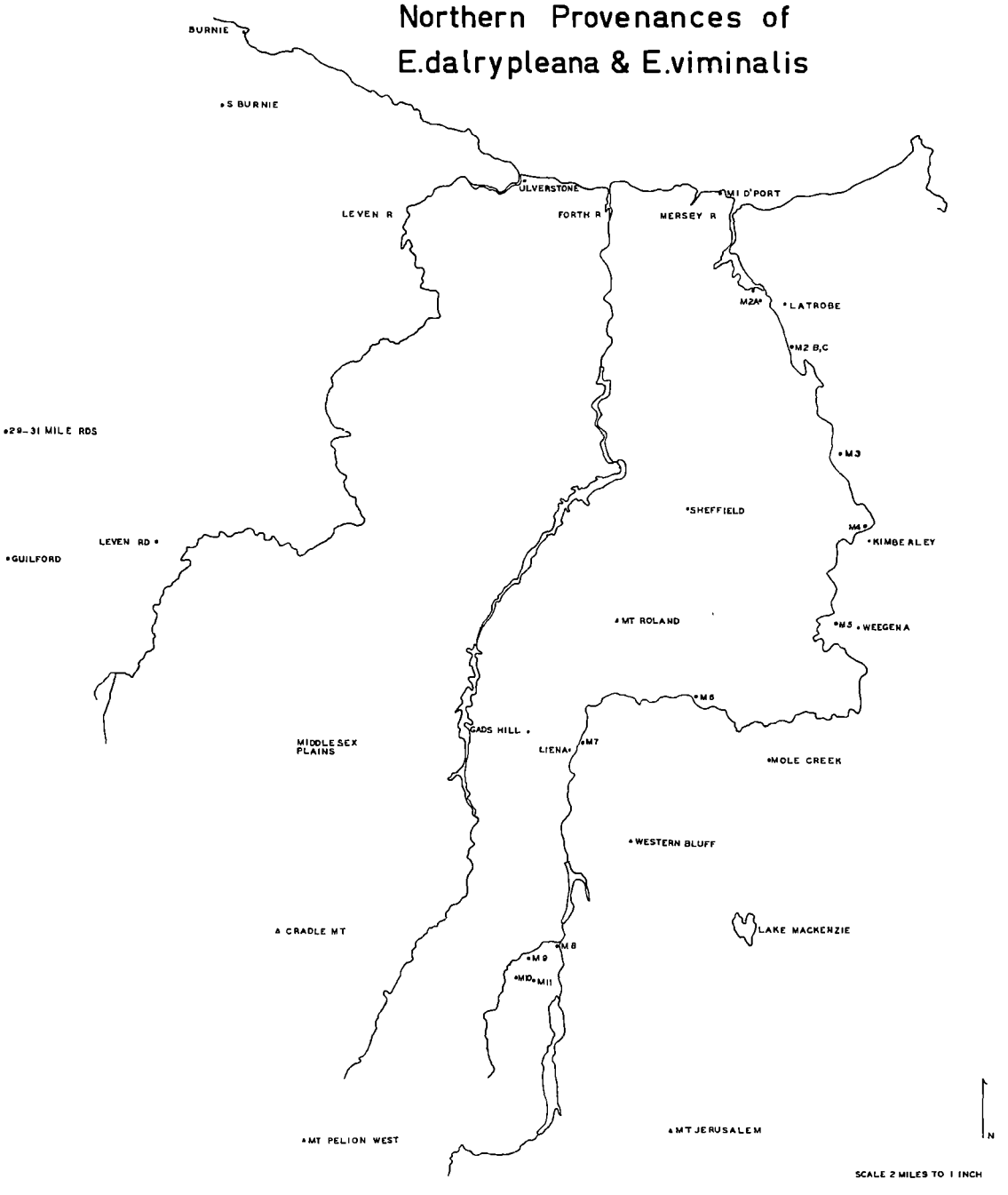

Northern Provenances of
E.dalrypleana & E.viminalis
BURNIE
S BURNIE
ULVERSTONE
LEVEN R
FORTH R
MERSEY R
MI D'PORT
M2A
LATROBE
M2 B,C
29-31 MILE RDS
M3
SHEFFIELD
M4
KIMBERLEY
LEVEN RD
GUILFORD
MT ROLAND
M5
WEEGENA
M6
GADS HILL
LIENA
M7
MIDDLESEX PLAINS
MOLE CREEK
WESTERN BLUFF
CRADLE MT
LAKE MACKENZIE
M8
M9
M10
M11
MT PELION WEST
MT JERUSALEM
N
SCALE 2 MILES TO 1 INCH

Table 5-1 Locality of Provenances

Code	Tree	Locality	Altitude (in metres)	Phenolic studies	Enzyme studies
M1	a	Mersey Bluff	0	Yes	No
	b	Latrobe	0	Yes	Yes
M2	a	Latrobe	0	Yes	Yes
	b	Shale Works, Latrobe	0	Yes	Yes
	c	Shale Works, Latrobe	0	Yes	Yes
M3	a	Merseylea	50	Yes	Yes
M4	a	Kimberley	70	Yes	No
	b	Kimberley	70	Yes	No
	c	Kimberley	70	Yes	Yes
M5	a	Weegena	150	Yes	Yes
	b	Weegena	150	Yes	Yes
M6	a	Union Bridge	230	Yes	Yes
	b	Union Bridge	230	Yes	Yes
M7		Liena	320	Yes	Yes
M8	a	Arm R./Mersey R. Junction	450	Yes	Yes
	b	Arm R./Mersey R. Junction	450	Yes	Yes
M9		Maggs Mt.	720	Yes	Yes
M10		Maggs Mt.	850	Yes	Yes
M11	a	Maggs Mt.	970	Yes	Yes
	b	Maggs Mt.	970	Yes	Yes

cont.

Table 5-1 continued.

N1M		Nimmitabel,N.S.W. (*E. viminalis*)	1300	Yes	Yes
MB		Beulah	200	Yes	Yes
SC		Scamander	6	Yes	No
KI	a	King Island	0	Yes	Yes
	b	King Island	0	Yes	Yes
LR	a	Leven Rd., Surrey Hills	700	Yes	Yes
	b	Leven Rd., Surrey Hills	700	Yes	Yes
ANU*		Brindabella Range, A.C.T.	1700	Yes	Yes
BT		Boydtown, Coastal N.S.W.	0	Yes	Yes
(010-71)		South Burnie	230	Yes	No
MF		Mt. Foster, Fingal	700	Yes	Yes
003-71		Bronte Area	820	Yes	Yes
005-71		Golden Valley	530	Yes	No
006-71		Bronte Area	920	Yes	No
011-71		Camden Area	1000	Yes	No
GL*		Wihareja	1000	Yes	Yes
RC		Rocky Cape, Dentention R.	0	Yes	Yes
007-71		North Bronte	820	Yes	No
MC		Minnow Creek	220	Yes	No

*Seedlings grown from seed mixture from several parent trees.

Table 5-2 Colours and R_f's of spots found in *E. viminalis* and *E. dalrympleana*

Spot	Colour	R_f BAW	R_f 5% Acetic Acid
1	blue	0.16	0.15
2	yellow	0.26	0.02
3	mauve	0.35	0.02
4	yellow	0.48	0.02
5	mauve ⟶ yellow	0.27	0.12
6	dark ⟶ yellow	0.42	0.22
7	yellow or dark ⟶ yellow	0.52	0.22
8	yellow or dark ⟶ yellow	0.66	0.17
9	yellow	0.70	0.30
10	yellow or dark ⟶ yellow	0.38	0.38
11	yellow	0.60	0.44
12	dark ⟶ yellow	0.51	0.41
13a,b	blue ⟶ blue green	0.52,0.49	0.64,0.68
14a,b	blue ⟶ blue green	0.61,0.53	0.73,0.75
15a,b,c	⟶ intense blue	0.63,0.63,0.75	0.78,0.87,0.65
16	blue or ⟶ blue (or blue green)	0.41	0.83
17	blue or ⟶ blue (or blue green)	0.55	0.74
18	blue or ⟶ blue (or blue green)	0.40	0.68

Table 5-2 continued

19	deep blue	0.46	0.77
20	⟶ purple	0.27	0.73
21	blue ⟶ blue green	0.40	0.52
22	blue ⟶ blue green	0.38	0.69
23	yellow	0.30	0.53
24	yellow	0.30	0.38
25	yellow	0.24	0.55
26	yellow	0.28	0.67
27	yellow	0.31	0.74
28	purple or blue ⟶ blue green	0.26	0.62
29	yellow	0.20	0.77
30	yellow	0.20	0.35
31	yellow	0.16	0.29
32	⟶ yellow	0.19	0.13
33	yellow	0.25	0.23
34	pale ⟶ blue	0.22	0.49
35	yellow	0.30	0.57
36	yellow	0.32	0.30
37	blue ⟶ mauve	0.65	0.43
38a,b	blue ⟶ blue green	0.73,0.72	0.49,0.64
39	yellow	0.76	0.80
40	yellow	0.74	0.56
41	blue/purple ⟶ blue green	0.50	0.09
42	blue	0.39	0.17

Table 5-2 continued

43	yellow	0.33	0.23
44	blue/purple → pale	0.55	0.30
45a,b,c	pale yellow	0.32,0.45,0.62	0.10,0.15,0.17
46	yellow	0.40	0.38
47	deep blue	0.25	0.32
48	blue	0.61	0.47
49	→ blue	0.62	0.34
50	purple	0.48	0.82
51	purple	0.26	0.11
52	purple	0.34	0.10
53	purple	0.22	0.07
54	purple → paler	0.33	0.12
55	purple	0.44	0.16
56	purple → pale	0.52	0.15
57	purple	0.60	0.17
58	purple	0.64	0.70
59	purple	0.35	0.70
60	blue/purple → blue	0.28	0.64
61	purple → pale	0.31	0.53
62	blue/purple → blue	0.29	0.75
63	purple	0.28	0.20
64	purple	0.22	0.18

Table 5-2 continued

65	purple	0.30	0.30
66	purple	0.22	0.22
67	blue/purple ⟶ blue	0.44	0.70
68	purple	0.64	0.08
69	yellow	0.50	0.77
70	yellow (could be part of spot 4)	0.52	0.19

Table 5-3

All 'Viminalis' type	All 'Dalrympleana' type	Mixed
Scamander	ANU	dal Golden Valley
Rocky Cape	006-71	M Beulah
Burnie 010-71	dal N. Bronte	Leven Road a
King Island a	Minnow Creek	Leven Road b
King Island b	Great Lake 1480	011-71
Boydtown	003-71	M5a
Nimnitabel	M11b	M5b
Mt. Foster	M11a	M6b
M1a	M10	M6a
M1b	M9	M7
M2a	M8b	
M2b	M8a	
M2c		
M3a		
M4a		
M4b		
M4c		

Table 5-4 Statistics for a comparison of some compounds in certain provenances.

	Mean	Standard Deviation	t	p
A) E. viminalis				
(1) Compound 37				
Provenances KIa, RC, NIM, BT:	3.0000	1.6300	7.3	< 0.01
Other provenances:	8.8462	1.3445		
(2) Compound 49				
Provenances M3a,M4c:	8.0000	0.0000	11.7	< 0.01
Other provenances:	0.3333	0.8997		
(3) Compound 50				
BT, NIM, KIa,KIb,M1b,SC:	0.7143	1.8898	12.9	< 0.01
Other provenances:	9.3000	0.8233		
(4) Compound 51				
BT,NIM,KIa,KIb,M1b,SC:	0.1429	0.3780	6.3	< 0.01
Other provenances:	6.4000	2.5906		
(5) Compound 52				
M4a, MF:	6.0000	0.0000	19.3	< 0.01
Other provenances:	0.2000	0.414039		

Continued

Table 5-4 continued.

B) E. dalrympleana

(1) Compound 28

All provenances except MC:	0.2727	0.4671	MC frequency: 5	
			t	p
(2) Compound 47				
GL,Mc,ANU:	6.0000	1.0000	8.2	< 0.01
Other provenances	9.5556	0.5270		
(3) Compound 61				
M10,MC,GL,ANU:	3.5000	0.5774	5.9	< 0.01
Other provenances:	7.6250	1.3025		
(4) Compound 62				
M8b,007-71:	5.5000	0.7071	6.3	< 0.01
Other provenances:	9.1000	0.7379		
(5) Compound 67				
M8a,M9,M10,ANU:	8.2500	0.9574	11.9	< 0.01
Other prevenances:	1.8750	0.8345		

Table 5-5 Comparison of Certain Compounds in Tasmanian and Mainland Samples.

A) E. viminalis

	Mean Tasmanian Samples	Standard Deviation	Frequency in Boydtown Sample	Frequency in Nimmitabel Sample
Cpd. 9	9.9333	0.2582	9	9
Cpd.11	9.6000	0.9103	7	7
Cpd.12	8.6000	2.0284	2	5
Cpd 21	0.4000	0.6325	7	5
Cpd.22	0.2000	0.4140	4	1
Cpd.29	9.4000	1.1212	9	5
Cpd.36	1.6667	1.1751	6	3
Cpd.46	0.3333	0.6172	3	0

B) E. dalrympleana

	Mean	Standard Deviation	Frequency in ANU Sample
Cpd. 9	9.8182	0.4045	8
Cpd.15	9.9091	0.3015	9
Cpd.33	1.1818	0.9816	4
Cpd.44	9.6364	0.5045	6
Cpd.67	3.5455	2.9787	9

Table 5-6 Comparison of Certain Compounds present in both E. viminalis and E. dalrympleana

Compound	E. viminalis Mean	E. viminalis Standard Error	E. dalrympleana Mean	E. dalrympleana Standard Error	t	p
7	9.5294	0.1259	9.9167	0.0069	3.1	0.01
9	9.8235	0.0091	9.6667	0.0354	4.2	0.01
10	9.2941	0.2777	9.9167	0.0069	2.4	0.05
11	9.2941	0.0865	10.0000	0.0000	8.2	0.01
12	8.0000	0.3971	9.5000	0.0682	3.7	0.01
37	7.4706	0.4935	9.4167	0.0676	3.9	0.01
39	0.4706	0.0597	0.1667	0.0278	4.6	0.01
44	4.7059	0.5792	9.3333	0.1111	7.8	0.01
46	0.4706	0.0450	0.9167	0.0827	4.8	0.01
47	4.5882	0.7284	8.6667	0.2475	5.3	0.01
48	1.8235	0.2811	0.0833	0.0069	6.2	0.01
50	5.7647	1.2171	9.8333	0.0278	3.3	0.01

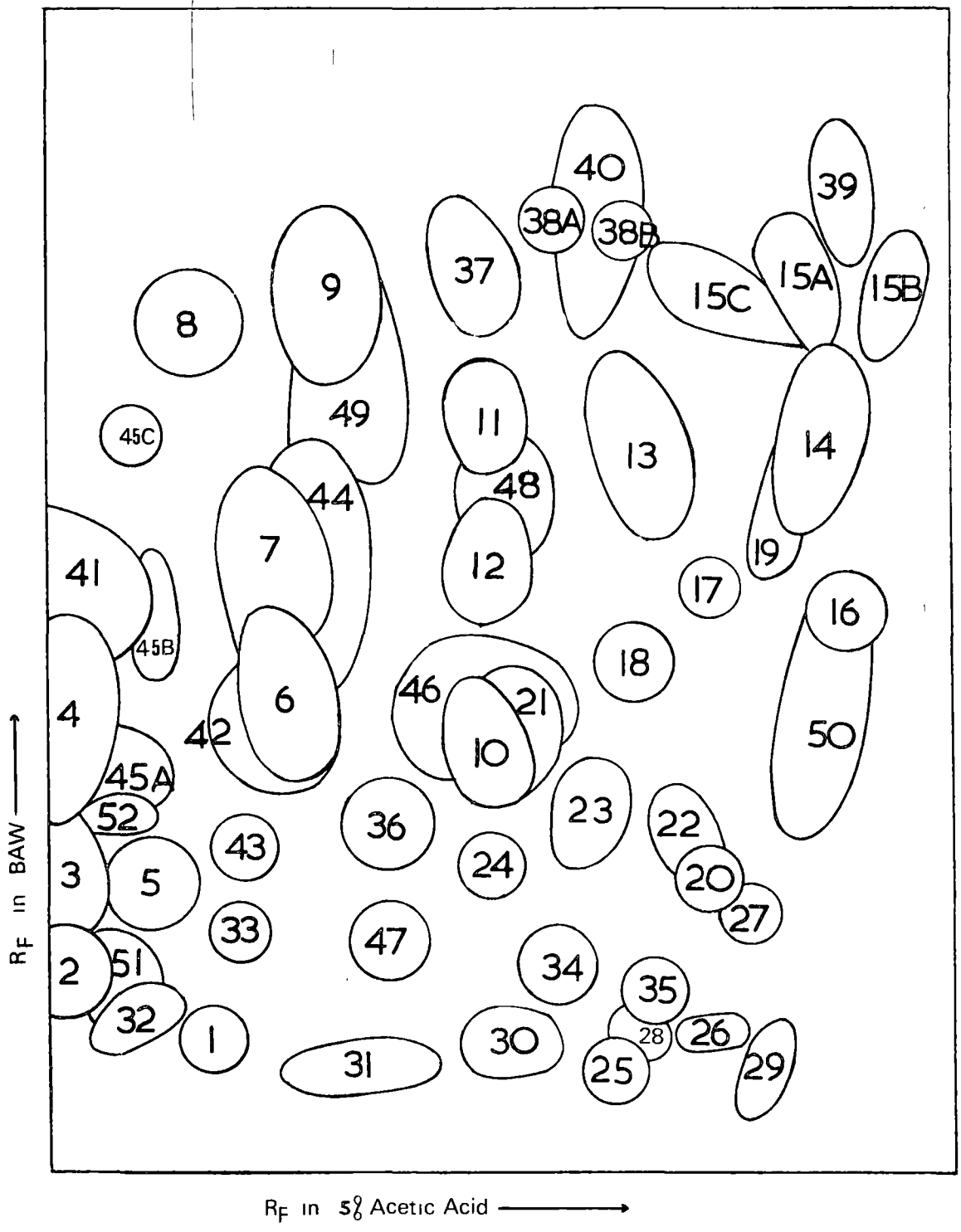


Fig. 5-1

Master chromatogram of the phenolic compounds in E. viminalis Provenances. Key - see Table 5-2.

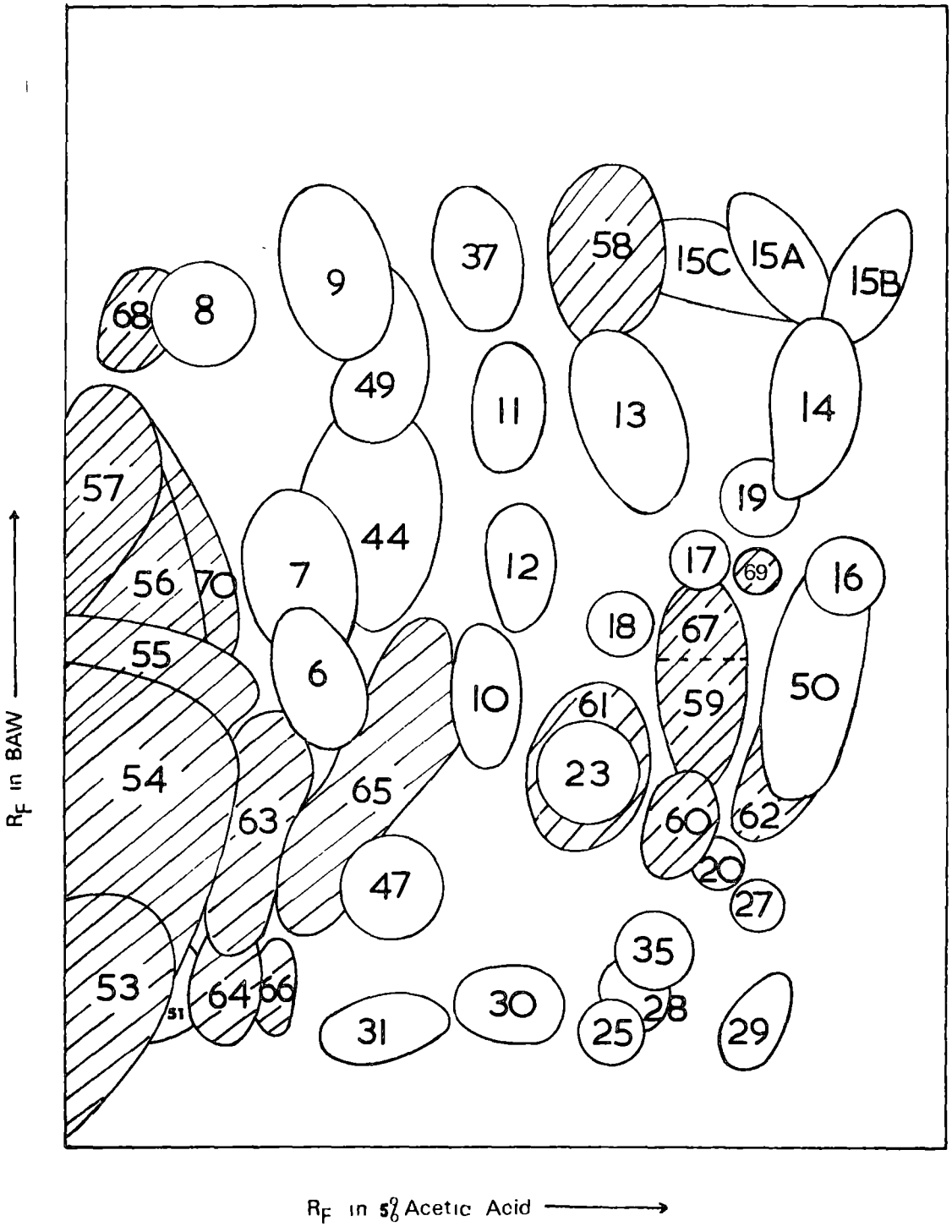


Fig. 5-2

Master chromatogram of the Phenolic Compounds in E. dalrympleana Provenances. Key - see Table 5-2.

Figure 5-3 E. viminalis provenances

Compound	Mean	Standard Error	Compound	Mean	Standard Error
1	8.5294	0.4347	27	6.4118	0.6622
2	9.8824	0.0138	28	4.8824	0.5506
3	9.9412	0.0035	29	9.1176	0.1315
4	9.9412	0.0035	30	9.7647	0.0333
5	9.3529	0.1687	31	9.6471	0.0583
6	9.9412	0.0035	32	1.1765	0.1414
7	9.5294	0.1259	33	6.2941	0.4836
8	8.3529	0.2202	34	1.6471	0.2863
9	9.8235	0.0091	35	9.1176	0.0947
10	9.2941	0.2777	36	2.0000	0.1397
11	9.2941	0.0865	37	7.4706	0.4935
12	8.0000	0.3971	38	0.6000	0.0992
13	10.0000	0.0000	39	0.4706	0.0597
14	10.0000	0.0000	40	0.0588	0.0035
15	9.8824	0.0065	41	2.0588	0.1799
16	6.9412	0.7020	42	3.7059	0.4983
17	5.1765	0.2517	43	1.7059	0.1674
18	8.4118	0.1401	44	4.7059	0.5792
19	4.7647	0.4083	45	4.8824	0.6977
20	8.0000	0.3382	46	0.4706	0.0450
21	1.0588	0.2314	47	4.5882	0.7284
22	0.4706	0.0597	48	1.8235	0.2811
23	8.8235	0.2811	49	1.2353	0.4230
24	0.7647	0.0921	50	5.7647	1.2171
25	9.9412	0.0035	51	3.8235	0.8179
26	4.2941	0.4247	52	0.8824	0.2271

Figure 5-4 E. dalrympleana provenances

Compound	Mean	Standard Error	Compound	Mean	Standard Error
1	0.0000	0.0000	36	0.7500	0.1231
2	0.0000	0.0000	37	9.4167	0.0676
3	0.0000	0.0000	38	0.0000	0.0000
4	0.0833	0.0069	39	0.1667	0.0278
5	0.0000	0.0000	40	0.0000	0.0000
6	9.9167	0.0069	41	0.0000	0.0000
7	9.9167	0.0069	42	0.1667	0.0278
8	8.8333	0.3460	43	0.0000	0.0000
9	9.6667	0.0354	44	9.3333	0.1111
10	9.9167	0.0069	45	0.0000	0.0000
11	10.0000	0.0000	46	0.9167	0.0827
12	9.5000	0.0682	47	8.6667	0.2475
13	10.0000	0.0000	48	0.0833	0.0069
14	10.0000	0.0000	49	0.0000	0.0000
15	9.8333	0.0126	50	9.8333	0.0278
16	0.3333	0.0354	51	0.0000	0.0000
17	1.7500	0.3504	52	0.0000	0.0000
18	0.2500	0.0322	53	9.9167	0.0069
19	0.2500	0.0322	54	10.0000	0.0000
20	0.1667	0.0278	55	10.0000	0.0000
21	0.0000	0.0000	56	9.9167	0.0069
22	0.0000	0.0000	57	10.0000	0.0000
23	9.0833	0.0676	58	9.9167	0.0069
24	0.0000	0.0000	59	10.0000	0.0000
25	9.9167	0.0069	60	9.3333	0.0354
26	1.4167	0.3251	61	6.2500	0.4413
27	3.0833	0.5827	62	8.5000	0.2045
28	0.6667	0.1717	63	5.9167	0.7797
29	8.6667	0.3081	64	2.1667	0.2247
30	9.4167	0.0524	65	4.0000	0.6515
31	9.4167	0.0524	66	1.5000	0.1288
32	0.0000	0.0000	67	4.0000	0.8788
33	1.4167	0.1282	68	0.3333	0.0657
24	0.0000	0.0000	69	0.0000	0.0000
35	6.5833	0.4615	70	0.0833	0.0069

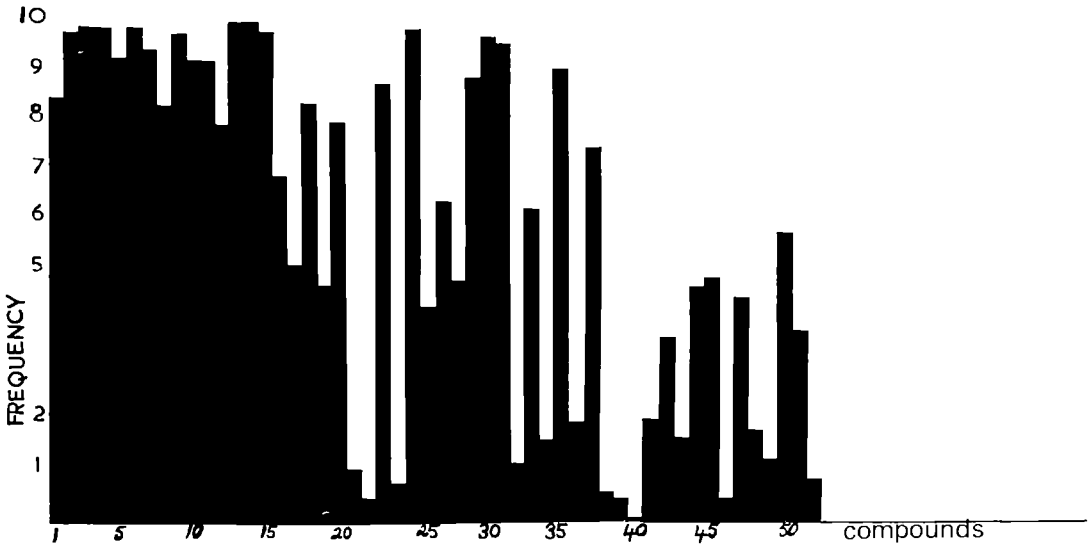


FIG. 5-3 : E. viminalis Provenances

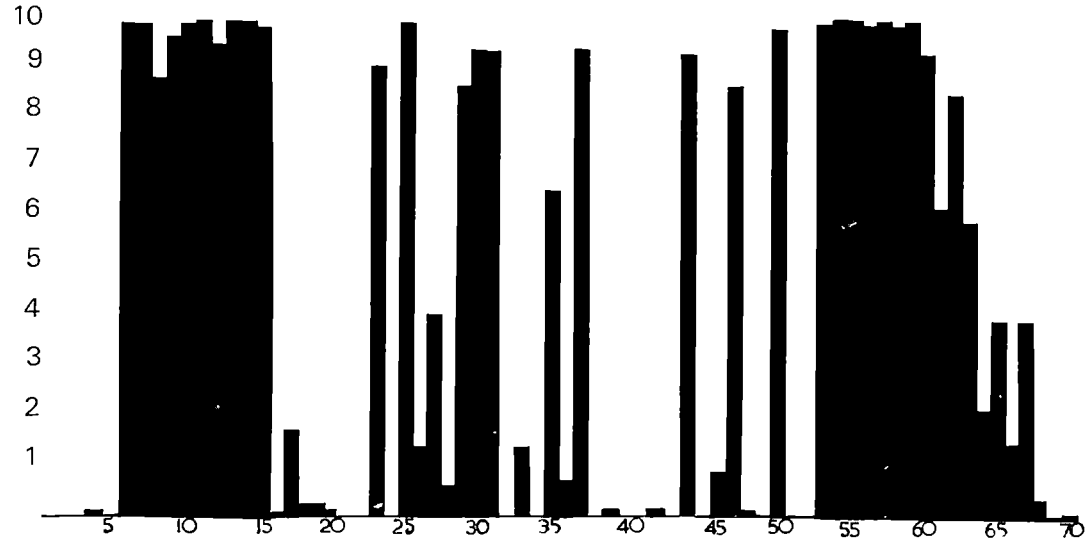


Fig 5-4 E dalrympleana Provenances

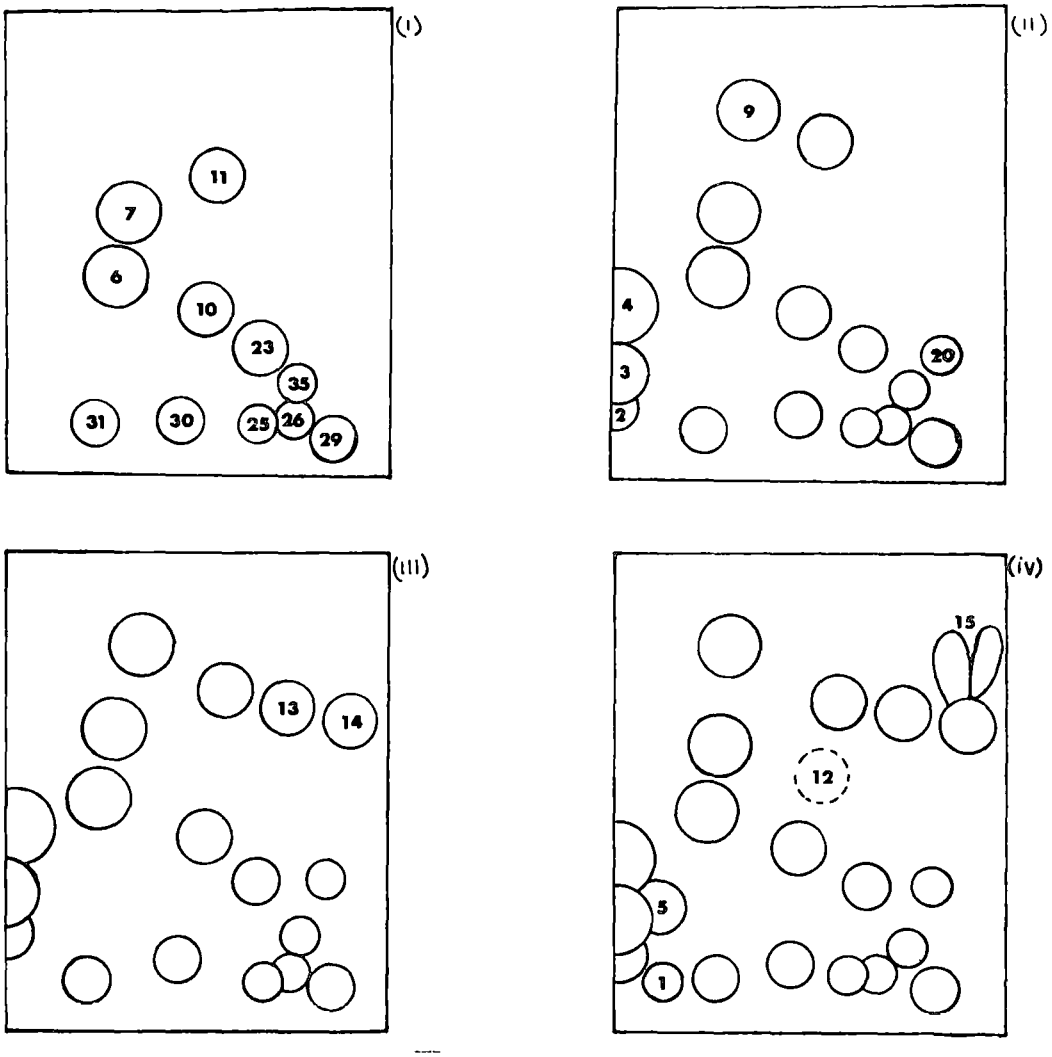


Fig. 5-5

The appearance of compounds at different stages of development in E. viminalis Provenances.

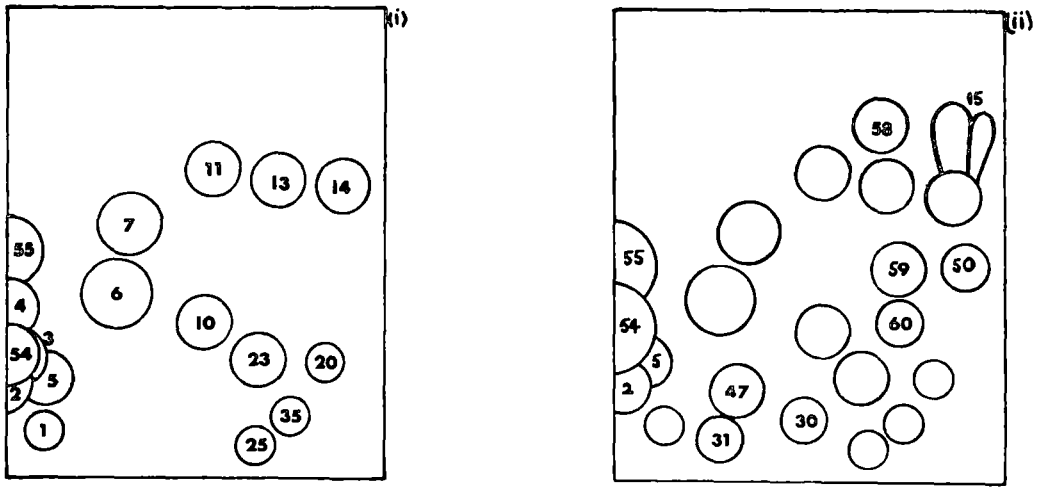


Fig. 5-6

The appearance of compounds at different stages of development in E. dalrympleana Provenances.

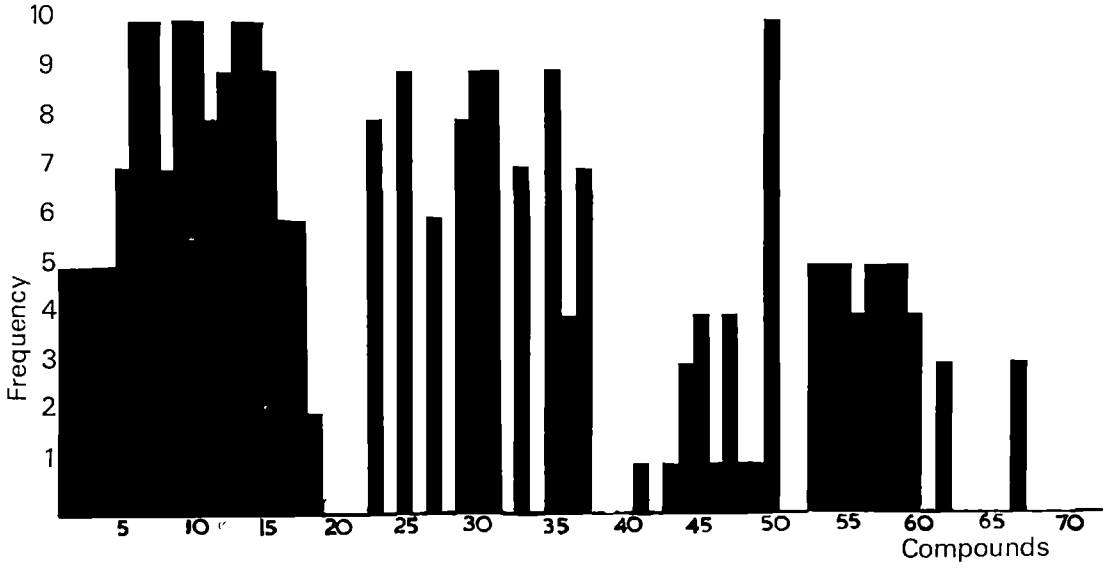


Fig. 5-7 Leven Rd.(b) Provenance

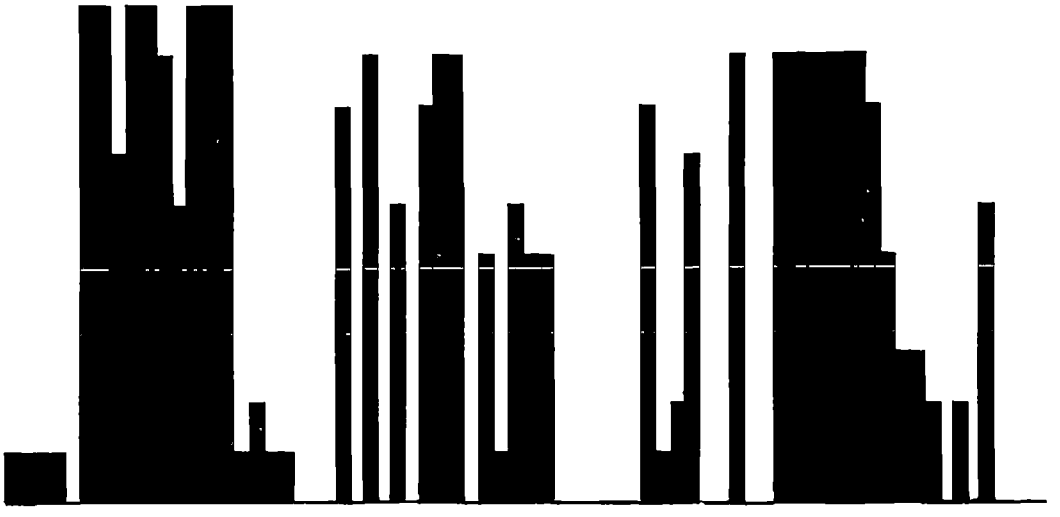


Fig. 5-8. Leven Rd.(a) Provenance

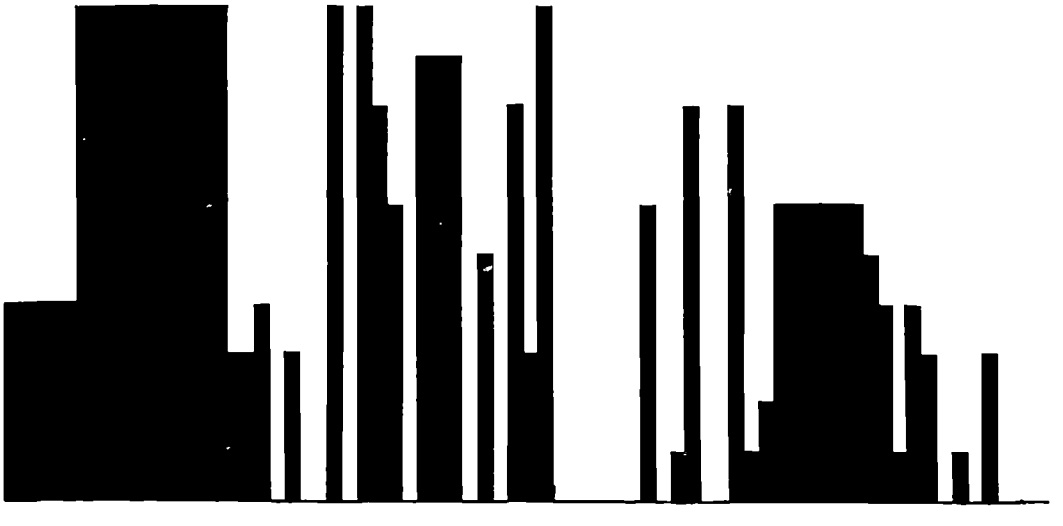


Fig. 5-9 Golden Valley Provenance

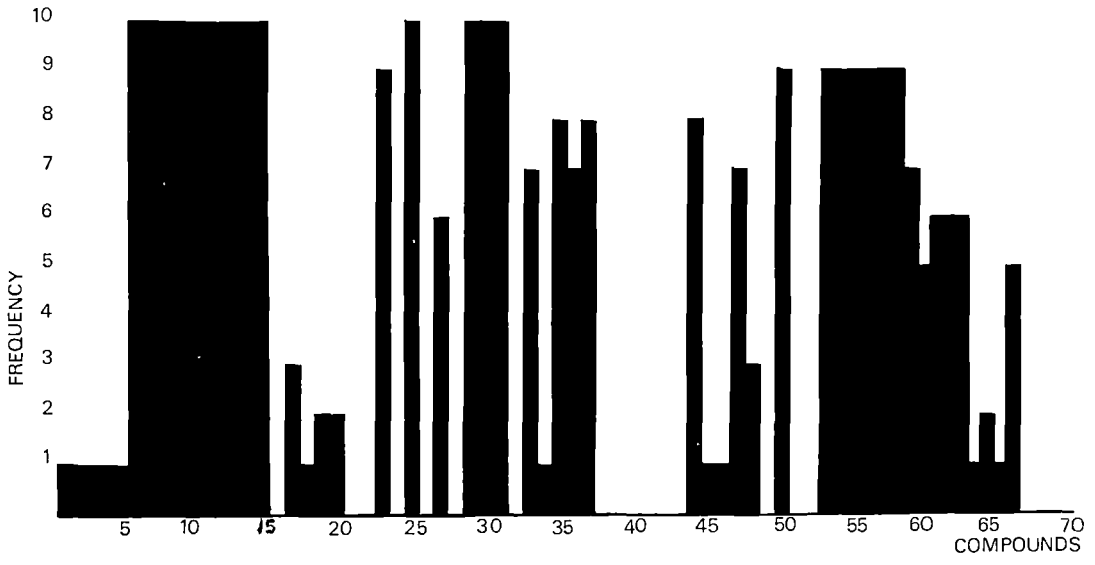


Fig. 5-10 M6 (a) Provenance

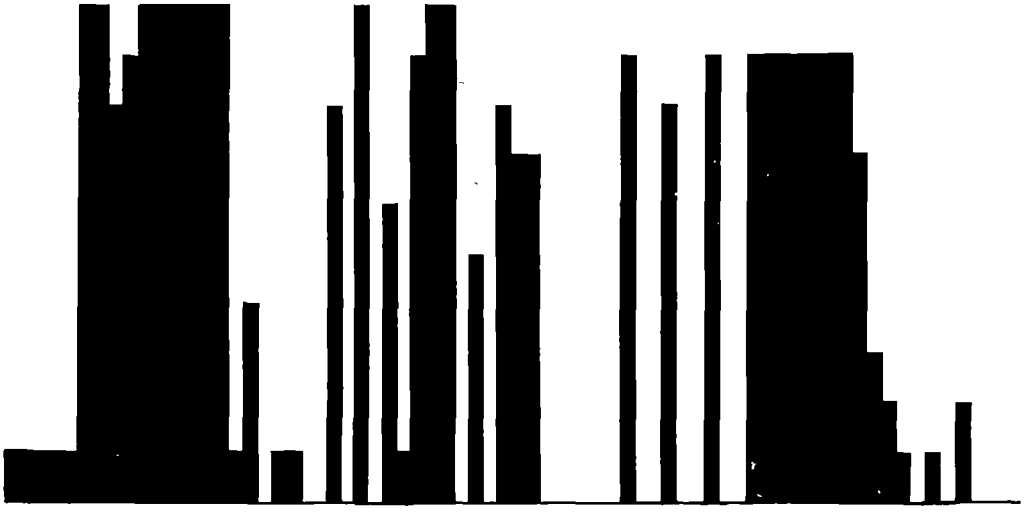


Fig. 5-11 M6(b) Provenance

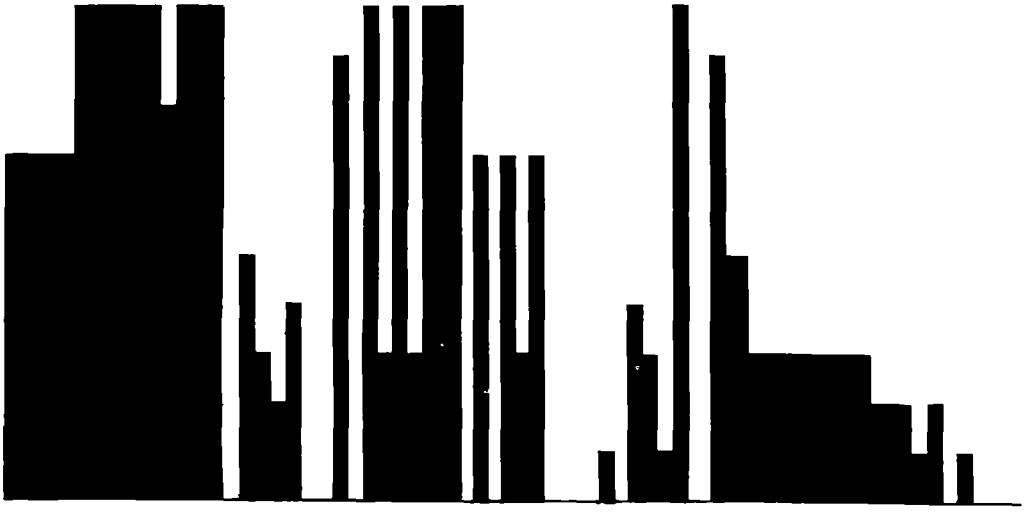


Fig. 5-12 M 7 Provenance

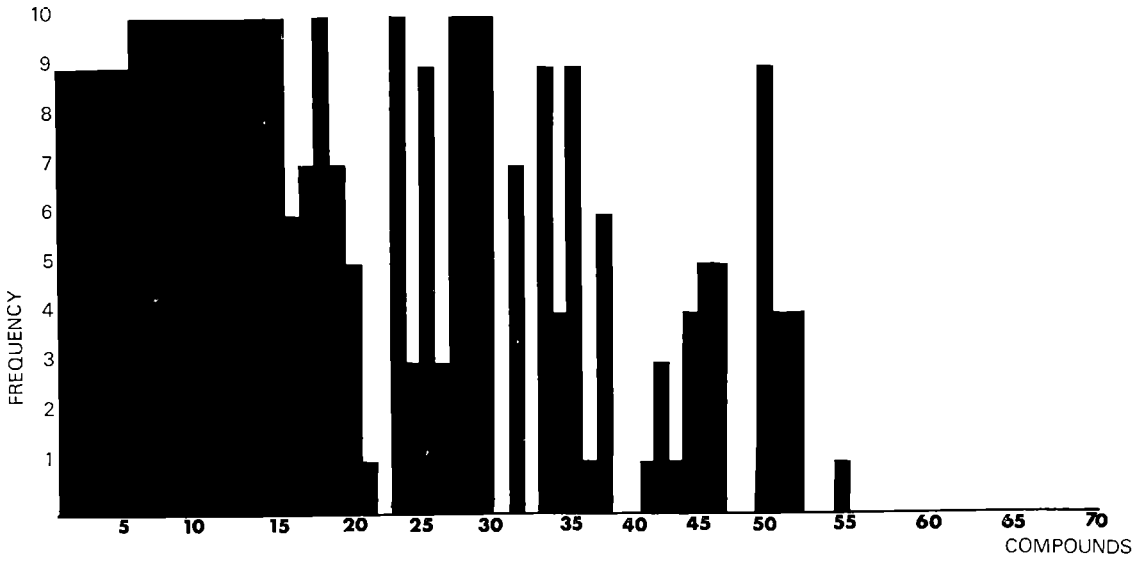


Fig 5-13 Provenance 011-71

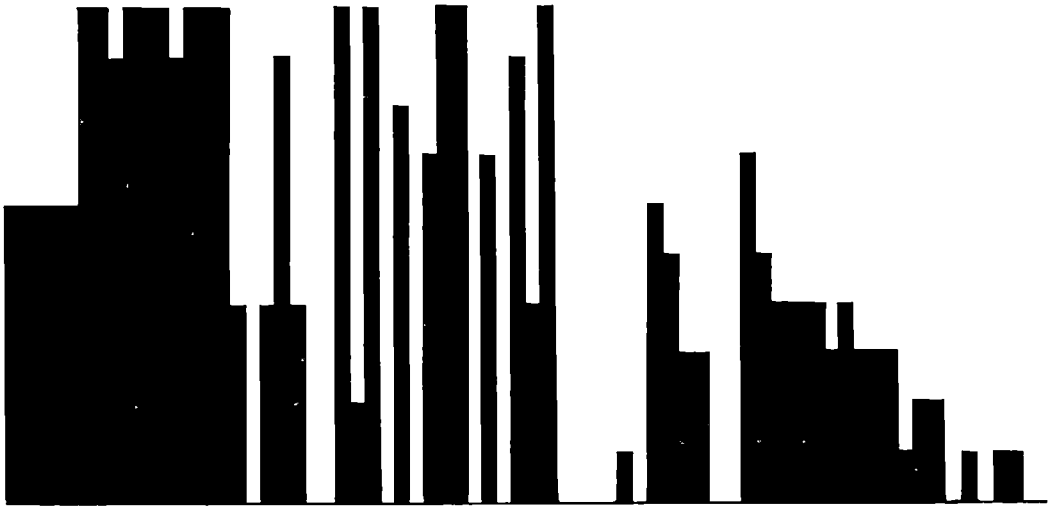


Fig 5-14 M 5 (a) Provenance

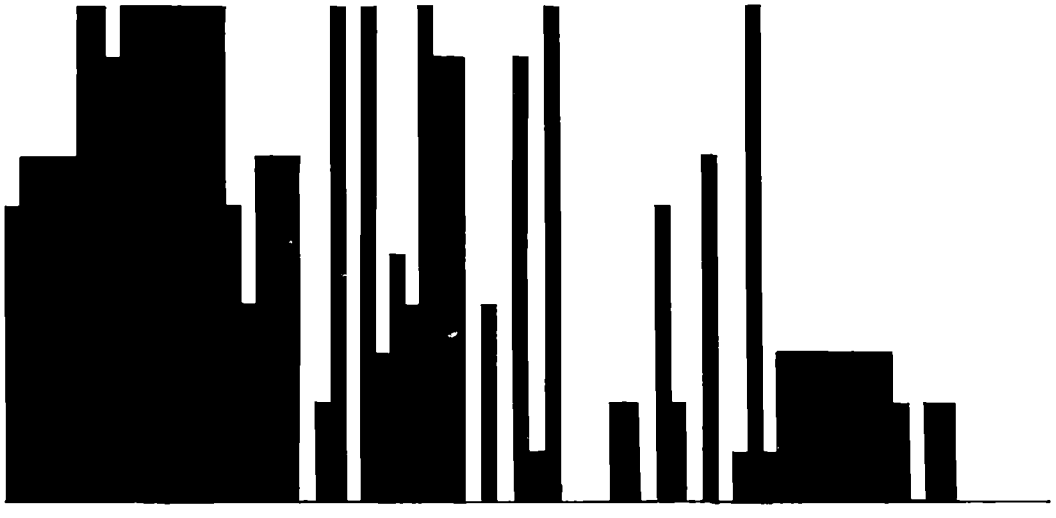


Fig 5-15 M 5 (b) Provenance

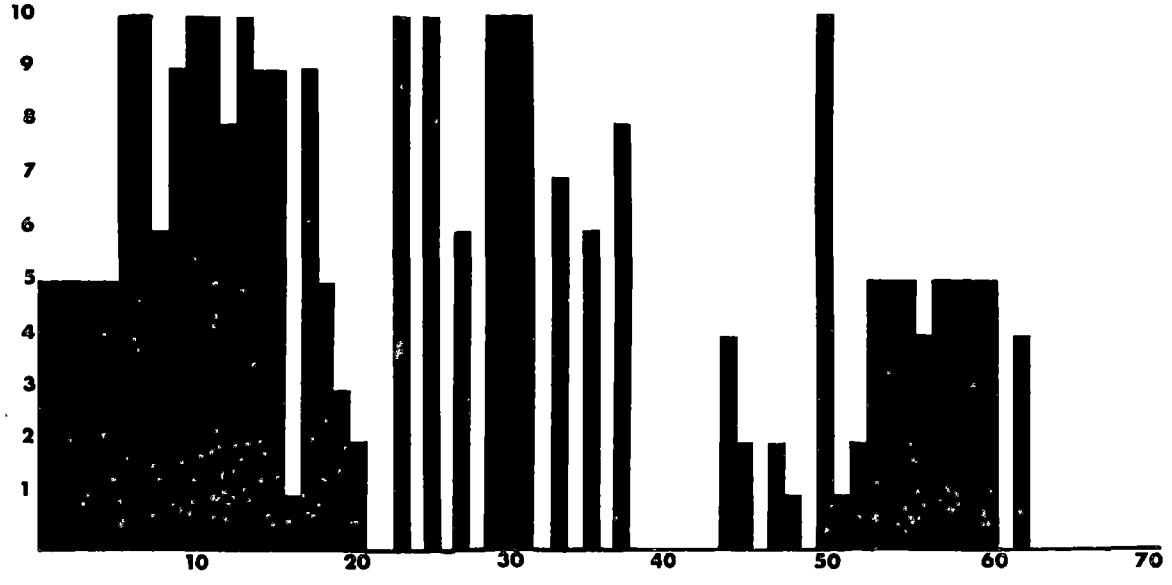


Fig.5-16· Beulah Provenance

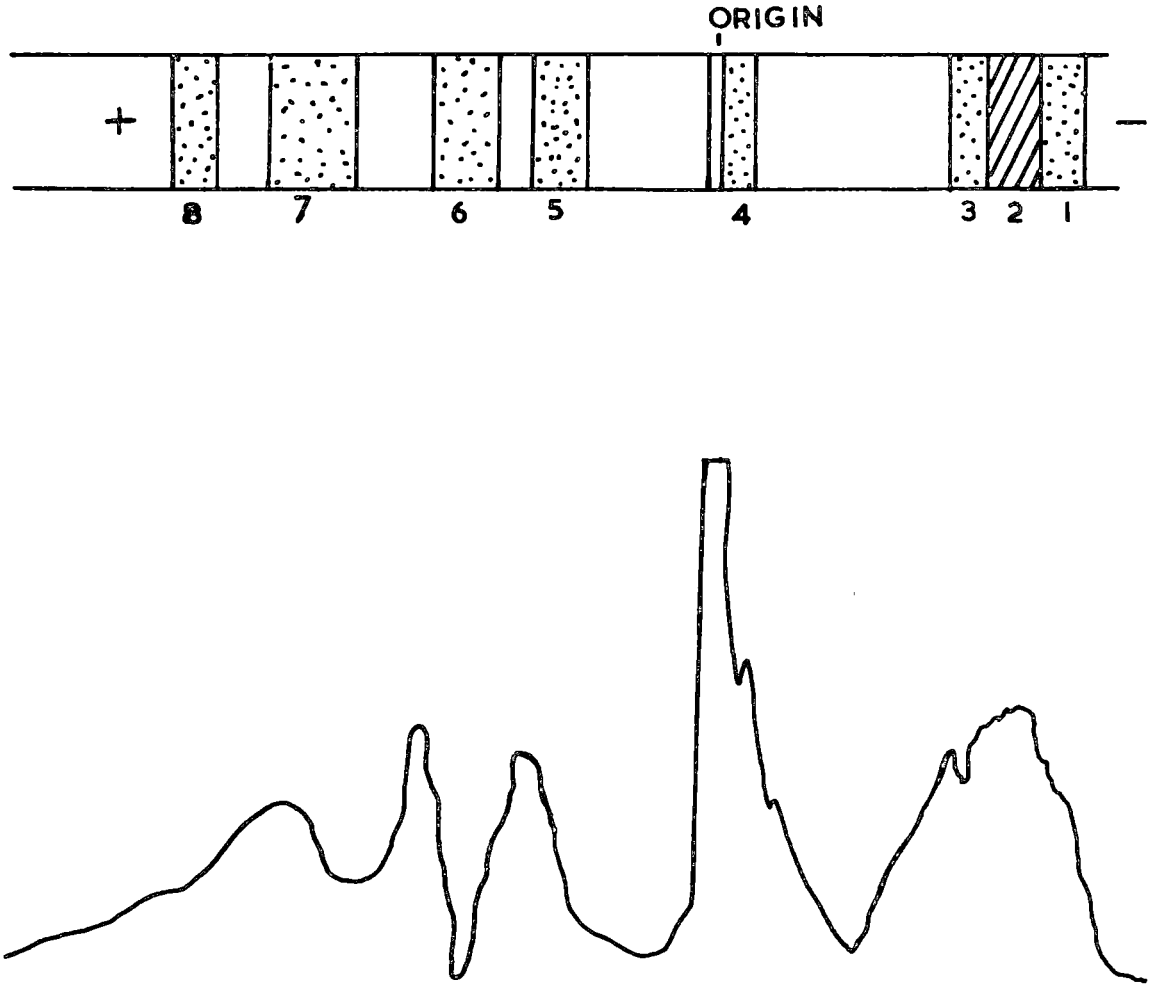


Fig. 5-17 Desitometer Tracing of Peroxidase Bands in E. viminalis and E. dalrympleana species.

Top Photograph: Chromatogram of E. dalrympleana under ultra-violet light.

Bottom Photograph: Chromatogram of E. viminalis under ultra-violet light.

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APPENDIX

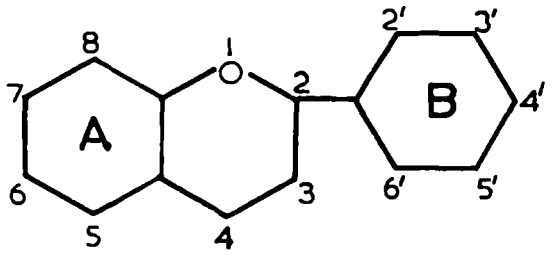
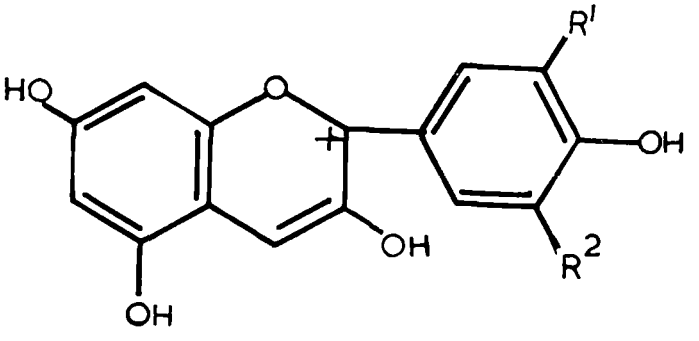


FIG. 1: Flavylium Skeleton



$R^1 = R^2 = H$: pelargonidin;

$R^1 = OCH_3$; $R^2 = H$: peonidin

$R^1 = OH$; $R^2 = H$: cyanidin;

$R^1 = OCH_3$; $R^2 = OH$: petunidin

$R^1 = R^2 = OH$: delphinidin;

$R^1 = R^2 = OCH_3$: malvidin

FIG. 2 Common Anthocyanidins

Table 3: Grouping of Tasmanian Eucalyptus for comparison in Taxonomic work (after Prof. W. D. Jackson).

Section Macrantherae

- *E. bicostata*, *E. globulus*, *E. cordata* — Blue Gums
- *E. rubida*, *E. viminalis*, *E. dalrympleana* — Lowland White Gums
- *E. johnstoni*, *E. vernicosa*, *E. subcrenulata* — Yellow Gums
- *E. gunnii*, *E. archerii*, *E. urnigera*, *E. morrisbyi*, *E. perriniana* — Alpine White Gums
- *E. ovata*, *E. aggregata*, species nova — Ovata Group

Section Renantherae

- *E. coccifera*, *E. simmondsi*, *E. linearis*, *E. amygdalina*, *E. robertsonii*, *E. tasmanica*, *E. risdoni* — Pepper-mints
- *E. pauciflora* — Snow Gum
- *E. regnans*, *E. obliqua* — Stringy Barks
- *E. delegatensis*, *E. sieberiana* — Ashes

Table 10: Characterisation of phenolic acids and aglycones other than anthocyanidins which are found in Eucalyptus species.

Spot	Colour	R_f Forestal	R_f 50% Acetic Acid	R_f BAW	R_f 5% Acetic Acid	Identity
1	dk→Y→I	0.60	0.40	0.90	0.00	kaempferol
2	dk→Y→I	0.44	0.34	0.69	0.00	quercetin
3	dk→Y→I	0.26	0.25	0.50	0.00	myricetin
4	bl→blgr	0.80	0.66	0.85	0.60	chlorogenic acid
5	pu→Y	0.33	0.27	0.34	0.00	ellagic acid
6	grey→pale Y	0.88	0.85	0.92	0.74	gentisic acid
7	dk	0.66	0.61	-	-	-
8	dk	-	0.74	-	-	-
9	I pu	0.55	0.43	-	-	-
10	I white	-	0.65	0.43	-	-
11	I white	-	-	0.19	-	-
12	dk	-	-	0.09	-	-
13	bl pu	0.54	0.64	-	0.40	-
14	pale bl/pu	0.45	0.55	-	0.28	-
15	bl	-	-	-	0.65	-
16	I pu	0.15	0.18	-	-	-
17	pu	0.62	0.41	0.57	-	gallic acid
18	I Y	-	0.53	-	-	-

Table 10: (cont.)

Spot	Colour	R_f Forestal	R_f 50% Acetic Acid	R_f BAW	R_f 5% Acetic Acid	Identity
19	green	-	-	-	0.83	-
20	bl/pu	-	-	-	0.69	-
21	green	0.72 0.83	0.18 0.34	-	0.60	-
22	dk	-	0.69 0.74	0.86	0.65	-
23	dk	-	0.24	-	-	-

KEY: dk - dark
Y - yellow
I - intense
bl - blue
blgr - blue-green
pu - purple
The arrow indicates the addition of ammonia.

Table 11: Distribution of aglycones and phenolic acids in Eucalyptus species.

Bark

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
E. viminalis	+	3+	-	2+	+	2+	-	-	-	-	-	-	2+	3+	2+	-	-	-	-	-	-	-	-
E. rubida	t	+	t	2+	3+	2+	-	-	-	-	-	-	2+	3+	2+	-	-	2+	-	t	-	-	-
E. dalrympleana	-	+	-	2+	2+	+	t	+	+	-	-	-	-	-	-	-	t	-	-	-	-	-	-
E. globulus	t	3+	-	3+	2+	+	-	-	-	-	-	-	-	3+	+	-	t	-	-	-	-	-	-
E. cordata	t	2+	2+	2+	2+	2+	-	-	-	-	-	-	-	3+	2+	-	2+	+	-	-	-	-	-
E. johnstoni	2+	3++	2+	+	2+	+	+	+	-	-	-	-	-	2+	-	-	-	-	-	-	-	-	-
E. gunnii	-	t	-	2+	3+	t	-	-	-	-	-	-	-	2+	+	-	-	-	-	-	-	-	-
E. perriniana	-	2+	+	3+	+	+	-	+	-	-	2+	-	4+	4+	4+	3+	-	-	-	-	-	-	-
E. ovata	+	+	+	t	2+	2+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. aggregata	2+	3+	2+	+	2+	2+	2+	2+	-	-	-	-	+	t	-	-	-	-	-	-	-	-	-
E. obliqua	t	+	+	2+	2+	+	-	-	-	-	-	-	2+	t	-	-	-	2+	2+	-	-	-	-
E. delegatensis	+	2+	t	+	2+	+	-	+	-	-	-	-	t	+	-	-	-	-	-	-	-	-	-
E. coccifera	t	t	+	2+	2+	+	+	+	-	-	-	-	2+	3+	2+	2+	-	-	-	-	2+	2+	-
E. simmondsi	-	+	+	t	+	+	-	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. linearis	+	3+	3+	t	2+	2+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. amygdalina	-	+	2+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. tasmanica	+	2+	2+	+	2+	+	-	-	-	-	-	-	+	2+	-	-	-	-	-	-	-	-	-
E. risdoni	-	2+	2+	+	+	+	+	+	2+	-	-	-	-	+	-	-	-	-	-	-	-	-	-
E. pauciflora	t	2+	2+	+	2+	2+	-	-	-	-	-	-	t	+	-	-	-	-	-	-	-	-	-

Table 11, cont.

Young Juvenile Leaves

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
E. viminalis	+	3+	-	2+	2+	2+	-	-	-	-	-	-	-	3+	-	-	-	2+	-	-	-	-	-
E. globulus	+	3+	-	3+	2+	+	-	-	-	-	-	-	2+	3+	-	-	t	-	-	-	-	-	-
E. urnigera (a)	t	2+	t	2+	+	+	?	?	-	-	-	-	-	2+	2+	-	-	2+	-	-	-	-	-
E. urnigera (b)	t	4+	2+	2+	2+	+	+	+	-	-	-	-	-	2+	t	-	-	-	-	-	-	-	-
E. coccifera	t	2+	+	2+	t	+	+	+	-	-	-	-	-	+	2+	-	-	+	-	-	2+	2+	2+
E. amygdalina	t	+	3+	+	t	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. tasmanica	t	4+	4+	2+	2+	+	3+	3+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-

Juvenile Leaves

E. viminalis	+	3+	-	3+	2+	2+	-	-	-	-	-	-	-	3+	-	-	-	2+	-	-	-	-	-
E. rubida	t	2+	t	2+	3+	2+	-	-	-	-	-	-	2+	2+	2+	-	2+	2+	-	2+	-	-	-
E. dalrympleana	-	+	-	t	2+	+	+	+	4+	-	-	-	-	-	-	-	t	-	-	-	-	-	-
E. globulus	+	3+	-	3+	2+	+	-	-	-	-	2+	-	?	3+	2+	-	+	-	-	-	-	-	-
E. cordata	t	3+	+	2+	2+	2+	-	+	-	-	-	-	-	4+	2+	-	-	+	-	2+	-	-	-
E. johnstoni	2+	3+	2+	+	2+	+	+	-	-	-	-	-	-	2+	-	-	-	-	-	-	-	-	-
E. urnigera (a)	t	2+	t	2+	2+	+	+	+	-	-	-	-	-	3+	t	-	-	-	-	-	-	-	-
E. urn-igera (b)	t	2+	2+	2+	3+	+	+	+	-	-	-	-	-	3+	2+	-	-	-	-	-	-	-	-
E. perriniana	t	2+	t	3+	+	2+	-	-	-	-	-	-	4+	3+	3+	-	-	-	-	-	-	-	-
E. ovata	-	+	+	3+	2+	2+	-	-	-	-	-	-	-	2+	2+	-	t	-	-	2+	-	-	-
E. obliqua	+	2+	2+	2+	2+	+	-	-	-	-	2+	-	2+	2+	-	-	-	2+	2+	-	-	-	-
E. delegatensis	+	2+	+	+	2+	+	-	+	-	2+	2+	+	t	+	-	-	-	-	-	-	-	-	-

Table 11, cont.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
E. coccifera	t	2+	+	?	2+	+	+	+	-	-	-	-	-	-	t	+	-	+	-	-	2+	2+	2+
E. linearis	+	3+	3+	2+	2+	2+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. amygdalina	t	+	3+	+	t	t	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. tasmanica	+	2+	2+	+	2+	+	3+	3+	-	-	3+	-=	+	-	t	-	-	-	-	-	-	-	-
E. risdoni	-	2+	3+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. pauciflora	+	2+	2+	2+	2+	2+	-	-	-	-	-	-	t	+	-	-	-	-	-	-	-	-	-
Young Adult Leaves																							
E. viminalis	+	3+	-	2+	+	2+	-	-	-	-	-	-	-	2+	-	-	-	2+	-	-	-	-	-
E. amygdalina	t	+	3+	+	-	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Adult Leaves																							
E. viminalis	+	3+	?	4+	3+	2+	-	-	-	-	-	-	-	3+	-	-	-	-	-	-	-	-	-
E. rubida	t	3+	t	3+	3+	2+	-	-	-	-	-	-	2+	3+	2+	-	-	2+	-	2+	-	-	-
E. johnstoni	t	3+	+	3+	3+	2+	+	-	-	-	-	-	-	3+	2+	-	2+	-	-	-	-	-	-
E. gunnii	t	2+	-	2+	5+	2+	-	2+	-	-	+	-	2+	2+	t	-	2+	2+	-	-	-	-	-
E. urnigera	t	2+	2+	3+	2+	+	+	+	-	-	-	-	-	4+	2+	-	-	-	-	-	-	-	-
E. perriniana	-	2+	t	3+	+	2+	-	-	-	-	2+	-	4+	4+	3+	-	-	-	-	-	-	-	-
E. ovata	+	2+	2+	2+	2+	2+	-	-	-	-	-	-	-	2+	-	-	t	-	-	2+	-	-	-
E. aggregata	2+	3+	2+	+	2+	2+	2+	2+	-	-	-	-	+	2+	-	-	-	-	-	-	-	-	-
E. coccifera	-	2+	-	2+	2+	2+	-	-	-	-	-	-	-	3+	-	-	-	-	-	-	2+	2+	-
E. simmondsi	+	+	+	t	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. linearis	+	3+	3+	2+	3+	2+	+	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

Table 11, cont.

Species	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23
E. amygdalina	t	+	3+	4+	+	+	+	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
E. pauciflora	+	2+	2+	3+	2+	2+	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-

KEY: E. urnigera (a) - E. urnigera with glaucous foliage.
E. urnigera (b) - E. urnigera with green foliage.
t - compound present in trace amounts.
? - presence of compound suspected only
The number before the + sign indicates the proportional quantity of the compound present.

Table 14: Colours and R_f values of phenolic compounds found in the genus Eucalyptus.

Spot	Colour	R_f BAW	R_f 5% Acetic Acid
1	yellow	0.44	0.04
2	yellow	0.28	0.00
3	pale purple	0.36	0.02
4	pale	0.35	0.08
5	blue	0.16	0.06
6	dark → yellow	0.70	0.29
7	dark → yellow	0.56	0.23
8	dark → yellow	0.44	0.23
9	yellow	0.42	0.49
10	blue → blue-green	0.57	0.60
11	→ intense blue	0.70	0.74
12	→ intense blue	0.71	0.83
13	blue → blue-green	0.60	0.75
14	pale blue	0.83	0.67
15	→ blue	0.38	0.80
16	pale	0.35	0.52
17	pale	0.28	0.62
18	pale → blue-green	0.28	0.60
19	yellow	0.17	0.70
20	pale purple → pale blue	0.45	0.20
21	dark → yellow	0.53	0.54
22	pale purple → pale blue	0.66	0.57
23	pale → intense blue	0.65	0.89
24	yellow	0.42	0.85
25	→ purple	0.27	0.74
26	pale	0.17	0.40
27	→ purple	0.28	0.40
28	pale	0.23	0.22

Table 14 (cont.)

Spot	Colour	R_f BAW	R_f 5% Acetic Acid
29	pale	0.14	0.25
30	→ pale blue	0.54	0.09
31	blue	0.44	0.54
32	→ brown	0.20	0.54
33	→ brown	0.29	0.46
34	pale	0.34	0.13
35	pale → yellow	0.64	0.91
36	pale yellow	0.57	0.40
37	→ faint blue	0.49	0.25
38	yellow	0.56	0.11
39	blue	0.47	0.46
40	→ yellow	0.11	0.53
41	white → intensifies	0.09	0.88
42	brown	0.31	0.53
43	yellow	0.87	0.46
44	blue-purple	0.53	0.76
45	blue-purple	0.27	0.73
46	blue	0.12	0.68
47	pale blue	0.16	0.41
48	→ pale	0.05	0.36
49	pale	0.13	0.22
50	pale	0.12	0.15
51	light purple	0.50	0.05
52	deeper purple	0.40	0.08
53	purple	0.30	0.05
54	blue	0.36	0.82
55	yellow	0.20	0.43
56	pale	0.09	0.27
57	→ purple	0.16	0.65
58	purple	0.04	0.05

Table 14 (cont.)

Spot	Colour	R_f BAW	R_f 5% Acetic Acid
59	yellow	0.02	0.57
60	→ yellow	0.42	0.21
61	purple → whitish-purple	0.26	0.10
62	→ yellow	0.14	0.30
63	purple	0.18	0.59
64	purple	0.45	0.67
65	purple → paler	0.60	0.09
66	pale yellow	0.18	0.62
67	blue	0.50	0.37
68	yellow	0.29	0.81
69	blue	0.78	0.60
70	yellow	0.39	0.60
71	yellow	0.33	0.55
72	yellow	0.74	0.86
73	→ purple	0.24	0.68
74	green	0.46	0.06
75	pale → blue-green	0.66	0.44
76	purple	0.69	0.66
77	blue → blue-green	0.40	0.03
78	blue → blue-green	0.61	0.05
79	blue	0.39	0.67
80	intense purple	0.67	0.39
81	intense purple	0.42	0.28
82	pale → purple	0.35	0.80
83	pale → purple	0.29	0.84

The arrow indicates the addition of ammonia.

Table 18: The occurrence of species-specific compounds and of compounds of restricted distribution.

Species specific compounds

Spot	72	- E. obliqua
"	74	- E. coccifera
"	75	- E. tasmanica
"	76	- E. perrineana
"	77	- E. amygdalina
"	80	- E. pauciflora
"	81	- E. pauciflora
"	82	- E. archeri
"	83	- E. archeri

Compounds of restricted distribution

Spot	73	- E. archeri, E. risdoni, E. coccifera, E. perrineana
Spot	78	- E. linearis, E. amygdalina
Spot	79	- E. amygdalina, E. pauciflora

Chromatography Solvents:

BAW - n-butanol, glacial acetic acid, water (4:1:5). Upper phase.

5% Acetic acid - acetic acid, water (5:95).

3% Hydrochloric acid (v/v) - hydrochloric acid, water (3:97).

WAH - water, glacial acetic acid, conc. hydrochloric acid (82:15:3).

Forestal - acetic acid, water, conc. hydrochloric acid (30:10:3).

Formic acid - formic acid, water, conc. hydrochloric acid (5:3:2).

BBPW - n-butanol, benzene, pyridine, water (5:1:3:3).

Other Chemicals:

MAW - methanol, acetic acid, water (85:5:10).

Neutralising agent - N, N di-n-octylmethylamine, chloroform (10:90).

Spray reagent (aniline hydrogen phthalate):

aniline	9.15 ml
phthalic acid	16 gm
n-butanol	490 ml
ether	490 ml
water	20 ml

Electrophoresis Solutions:

Extracting Solution

Gel buffer	100 ml
Tween 80	1 ml
Soluble polyvinyl Pyrrolidine	1 gm
β-mercaptoethanol	0.3 ml

Borate Tank Buffer

7.22 g/litre boric acid and 15.75 g/l sodium tetraborate

Gel mixture

130 ml gel buffer

10.4 gm cyanogum 41

0.13 ml DMAEC (2-dimethyl-amino-ethyl cyanide)

130 mg ammonium persulphate

Gel buffer

4.598 g/l Tris (Tri-(hydroxymethyl) Methylamine)
0.525 g/l citric acid
} gives pH of 8.7 at 25°C

Acetate buffer

Solution A: 11.55 ml acetic acid in 1000 ml

Solution B: 27.20 gm $CH_3COONa.3H_2O$ in 1000 ml

0.2M solution at pH = 5

14.8 ml A + 35.2 ml B diluted to a total of 100 ml

0.2M solution at 5.4 pH

8.8 ml A and 41.2 ml of B diluted to 100 ml

Phosphate buffer

Solution A: 31.202 gm monobasic sodium phosphate . $2H_2O$ in 1000 ml

Solution B: 28.39 gm anhydrous dibasic sodium phosphate in 1000 ml

0.2M solution at pH 7.5

16 ml A + 84 ml B diluted to a total of 200 ml

0.2M solution at pH 6.5

68.5 ml A and 31.5 ml B diluted to 200 ml

Fixative solution

45 ml methanol

45 ml water

10 ml acetic acid

Basis of Staining Reactions

Peroxidase:

The sites of peroxidase isozymes are stained by the following reaction:

$$2H_2O_2 \xrightarrow{\text{peroxidase}} 2H_2O + O_2$$

The liberated oxygen then oxidizes o-dianisidine to a coloured compound.

Protein:

Amido-black stain combines directly with the phenol groups on the protein.

Esterase:

The sites of esterase isozymes are stained when a diazonium salt (Fast Blue BB) couples with α-naphthol after the α-naphthol is liberated from α-naphthyl acetate by the esterase activity.

Acid Phosphatase:

Acid phosphatase activity liberates α-naphthol from α-naphthol phosphate. The α-naphthol is then stained by a coupling reaction with the diazonium salt.