STUDIES OF VARIATION IN SOME TASMANIAN SPECIES OF *PLANTAGO* L.

by

Michael James Brown

B.Sc. (Hons.) Tas.

Submitted in fulfilment of the requirements for the degree of

Doctor of Philosophy

UNIVERSITY OF TASMANIA

AUGUST 1979

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, the thesis contains no copy or paraphrase of material previously published or written by another person, except where due reference is made in the text of the thesis.

M. J. Brown

ACKNOWLEDGEMENTS

I wish to thank my supervisors, Dr. R.K. Crowden and Professor W.D. Jackson for their advice and interest in this project. Dr. D. Ratkowsky of the C.S.I.R.O. provided many helpful discussions of the multivariate analytic techniques.

I acknowledge with thanks the assistance of Dr. B. Briggs of the National Herbarium of New South Wales and Dr. H. Eichler of the Herbarium Australiense for loans of herbarium material.

Thanks are also due to Mrs. E. Gourlay who provided invaluable assistance in the glasshouse, and to Mrs. S. Jones who typed this thesis. I am grateful to Drs. S.J. Jarman and J.B. Reid for many amicable debates during the course of the study.

To Deirdre and Rebecca I am ever in debt.

SUMMARY

An experimental investigation of the variation exhibited by some Tasmanian species of *Plantago* has been conducted. The study combines field, glasshouse and herbarium investigations with multivariate analytic techniques to examine adaptive strategies within the genus.

The major portion of the study concerns leaf-shape variation in *P. glabrata* and *P. paradoxa*. The leaf-shape of these species is divergent in summer but converges under winter conditions. The degree of convergence is limited by habitat. Transplant experiments show a marked convergence in the shapes of winter-leaves when plants are grown in the same environment. The characters measured were the width, the position of the widest part and the petiole length of the leaves relative to their total length. The field analyses indicate that these characters vary independently. When grown in the glasshouse, both species respond to changes of temperature, light intensity, photoperiod and inundation, factors which differentially affect the various leaf characters. Progeny testing shows that only the relative leaf-width is strongly heritable in *P. glabrata*, whereas in *P. paradoxa* the position of the widest part of the leaf is also strongly and independently heritable. The implications of the studies are discussed in relation to the maintenance of adaptive variation in the species with respect to season.

A second part of the study is concerned with intra- and inter-specific variation in some *Plantago* species. Variation in leaf-shape is correlated with microhabitat in two populations of *P. paradoxa*. Progeny testing demonstrates that differences of leaf shape between

populations are masked by canalization within populations. In one of the populations, the differences in leaf-shape are phenotypic modifications, whilst one of the leaf-forms occurring in the second population is genetically fixed. Gibberellic acid is able to induce similar changes in leaf-form. A possible model for the control of leaf-shape in the species is presented.

Some populations of *P. glabrata* are intermediate in their morphology between *P. glabrata* and *P. antarctica*. Morphometric analysis reveals three distinct aspects of variation in the complex. The first differentiates *P. glabrata* from *P. antarctica* at low altitudes. The second aspect demonstrates ecoclinal variation in *P. glabrata* and is correlated with the severity of climate. The third aspect involves a genetically maintained bimodal distribution of leaf-shape within populations. The alternatives of introgressive hybridization and disruptive selection as originating mechanisms are discussed.

An experimental taxonomic investigation of the *P. tasmanica* - *P. daltonii* complex is made. Morphometric analyses suggest that the taxa are largely distinct, although it is probable that introgression has occurred.

Finally, a brief investigation of other taxa belonging to *Plantago* section *Mesembrynia* is made. The study provides an estimate of the level of diversification in the section, and clarifies some aspects of the established taxonomic relationships within the section.

TABLE OF CONTENTS

Introduction

Differences of shape of plant parts are characteristics which can be used at most levels of experimental-taxonomic investigation. The differences can result from purely plastic (i.e. direct environmental) modification, or they may arise through directly or indirectly determined genetic differences. In each case the differences may be exhibited by the plants of a single population or among different populations of a species. The differences may be sufficiently distinct and constant that they provide useful characters for distinguishing between species. The work reported in this thesis was undertaken to assess how shape (mainly of leaves) varies at these levels using species of *Plantago*.

The objectives of the thesis are:-

1. To describe some of the methods which can be used to quantify shape;
2. To assess the suitability of these morphometric methods for genecological and experimental-taxonomic studies in *Plantago*;
3. To examine some of the factors which influence the variability of leaf-shape in selected species of *Plantago*, and to investigate the degree to which this variability is adaptive.

The thesis is divided into three parts. The first part gives a brief review of the principles of genecology and provides a general background to the application of these principles in studies of leaf-shape variability. A review is then given of the ways in which the description of leaf-shape has been treated in some biological studies, and the multivariate analytical

techniques which are used in the subsequent experimental studies are described.

The second part provides a brief background to the taxonomy of the Plantaginaceae and gives a descriptive taxonomic account of the genus *Plantago* within Tasmania.

The third part deals with experimental studies on selected *Plantago* species. The major portion of the work is concerned with the seasonal response of leaf-shape to season shown by two species, *P. paradoxa* and *P. glabrata*. This study is then extended to examine inter- and intra-population variability of leaf-shape in each of the species. The role of leaf-shape in interspecific differentiation is examined using *P. tasmanica* and *P. daltonii*. The experimental part of the thesis concludes with a multivariate distance analysis among some species of *Plantago* sect. *Mesembrynia*. This analysis is undertaken to assess the degree to which morphometry can be used to clarify and complement established taxonomic relationships within *Plantago* at the sub-generic level.

1. Genetic Variability, Phenotypic Plasticity and Leaf-Shape

Introduction

The observed phenotype of a plant is the result of the interaction between its genetic constitution and the environmental influences experienced during growth. Thus the differences observed between plants of a single species may arise from genetic differences, differences due to the environment in which they grow, or from the interaction of these factors at any stage in their ontogeny. The interaction therefore has a temporal as well as a spatial aspect.

The implications of the above for the erection of taxonomic classifications usually are accepted tacitly. However, modern experimental-taxonomic studies have formalised the nature of population variation into a number of concepts which attempt to rationalize its complexity.

Population, Variation and Adaptation

In this thesis the term population refers to a group of individuals which are spatially related, i.e. occur together in a local geographic situation (the 'topodeme' of Gilmour and Gregor [Heslop-Harrison (1953)]) and which are potentially capable of interbreeding to produce fertile offspring. However, the potential for interbreeding among all individuals may not necessarily be realised. For example, the population may be subdivided into intrabreeding groups (gamodemes) which normally do not interbreed because of differences in flowering time. Alternatively, the population might contain plants which have cleistogamous flowers. The population may be subdivided into groups of individuals (ecodemes) which occupy distinct habitats within the area. These sub-populations of plants may have distinct characteristics

which are non-heritable (phenecodemes) or which are maintained genetically (genecodemes).

The differences which are observed in a characteristic between plants can be effectively continuous or they may be discontinuous. In either case, the differences can have a genotypic or phenotypic origin, or result from the interaction of genotype with phenotype (G x E). Different genotypes arise within a population through the processes of mutation, migration or recombination. Genetically maintained differences between populations result from the processes of natural selection, genetic drift or from founder effects.

The differences which arise both within and between populations may or may not be adaptive. The concept of adaptation has two interdependent meanings in an evolutionary sense. It may refer to the fitness of an individual, i.e. its ability to survive and reproduce in a given environment for a given (long) number of generations (Thoday, 1953). Alternatively, a characteristic of a plant may be said to represent an adaptation to a particular environment in the sense that it provides"fitness for a particular function in a particular environment" (Cain and Shepherd, 1954).

In either case the concept of adaptation is comparative, a comparison of the relative performance of different forms in a particular environment, or a comparison of the relative performance of a particular form in a range of different environments is implicit in the concept. In order to make experimental comparisons, an estimate of performance or fitness is required. This assessment involves the estimation of a characteristic which indicates the relative potential to survive and reproduce. Characteristics such as seed yield, capsule number, ramet production or differential mortality are normally used for this purpose.

The adaptive variation which occurs between plants may arise in two ways. Either it has a direct genetic origin and is maintained by natural selection favouring one or other form (or both), or phenotypic modifications may be evoked by differing environments to produce forms which better enable the plant to survive in the different conditions. The ability to produce this latter type of adaptive phenotypic response is itself genetically determined, and therefore probably results from past selection. In any event it is the phenotype upon which selection acts. Ultimately, the genotype determines the survival of the organism, but survival is regulated by the developmental pattern exhibited by the organism in each environment (Dobzhansky, 1951). The review which follows is concerned mainly with those aspects of variation which can be inferred to be adaptive. In the case of genetic variation, this is taken to mean that the variation previously has shown (or presently is showing) a response to selection. Aspects of genetic variation which are neutral to selection, e.g. which result from random effects (genetic drift) or from an initially small population size (founder effects), will not be considered.

Genetic Variability between Populations

Whilst plant species traditionally have been classified on the basis of their morphological similarities or their putative phylogenetic affinities, they also present an ecological array which depends on their relative abilities to grow in diverse habitats. Thus, some species are capable of existence in a variety of ecological situations, whilst others are limited in occurrence to a particular habitat. Species from the former group were studied by Turesson (1922), who demonstrated the

genetic origin of much of the intraspecific variation accompanying the habitat differentiation in plant species having wide ranges.

The concept of the ecotype, developed by Turesson, was extended by Clausen, Keck and Hiesey (1939, 1940) and later workers (Clausen and Hiesey 1958, 1960; Hiesey and Nobs, 1970; Hiesey, Nobs and Bjorkman, 1971; Cline and Agatep, 1970a, b, etc.). This work provided an elegant description of the adaptive nature of much of the genetically determined differentiation between the populations of a single species. Hiesey and Milner (1965) have reviewed the physiological implications of genetic differentiation of ecological races within species. The ecotypes of many species have been shown to respond differentially to a wide range of ecological factors. These factors may affect, for example, the photosynthetic and respiratory rates of the ecotypes and the differential response can usually be inferred as adaptive. The authors point out that there is multigenic inheritance of most characters distinguishing ecological races. Furthermore, the distinguishing characters are genetically coherent in the sense that they tend to be partially linked in groups in the hybrid F_2 and F_3 generations. Wilkins (1968) states that in such cases, the mutual relationships of the character differences must be considered in relation to the environment, and eventually with the intercorrelations between the habitat factors themselves.

Clinal variation in the characteristics of a species was first described by Huxley (1938). The term is used to describe the continuous gradation which may occur in some character of the species and which is correlated with the geographical or ecological distribution of the species. The term is applicable

equally to quantitative characteristics and to qualitative characteristics in which the frequency of occurrence of a particular form varies quantitatively from place to place. A mathematical basis for the role of selection in the maintenance of this type of variation has been provided by Haldane (1948) and Fisher (1950). An example of the way in which natural selection maintains clinal variation is provided by Barber and Jackson's (1957) study of the frequency of occurrence of green and/or glaucous leaves in populations of *Eucalyptus urnigera*. The frequency of occurrence of the leaf-types is correlated with altitude and the authors show that the selection coefficients may be large when there is a steep ecological gradient. Plants with glaucous leaves are most prevalent in upland areas occupying exposed habitats, and Thomas and Barber (1974) have argued that there is a selective advantage accruing from this in the protection of leaves from freezing, and in the reduction of absorbed radiation.

Ecotype and cline were combined by Gregor (1939), and Gregor and Watson (1961) in the description of ecoclinal variation in species of *Plantago*. Thus genetically different plants of a single species might result from selection within a polygenic system in a gradational manner. This differentiation reflected ecological gradients and did not necessarily result in disjunct ecotypes. The effects of multigenic selection along ecological gradients to determine clinal variation have been discussed by Barber (1965), who considered that this may provide a mechanism for the origin of sympatric speciation.

The overall diversity of genetically determined adaptive strategies adopted by plant species is witnessed by the evidence for ecoclinal variation on the one hand, and the evidence of

Clausen *et al.* (*loc. cit.*) and Böcher (1967) on the other, who demonstrate that for many species, there is no discernible gradient of characters between populations.

Genetic Variability within Populations

Evidence for the occurrence of genetically based polymorphism within a single population of a species was reviewed by Ford (1975) who showed that the relative frequencies of the different morphs may shift both within and between populations. The role of disruptive selection in the maintenance of genetic polymorphism within a population has been described by Thoday (1959).

In situations where markedly different environments are contiguous, or form a mosaic, a single population may become genetically differentiated over very short distances. This may or may not be accompanied by the erection of barriers to gene flow among the different types. The majority of cases in which such differentiation has been reported are the result of strongly disruptive forces being established across relatively sharp ecological boundaries. For example, Gregor (1930) described differences in vegetative characters of *Plantago maritima* plants from habitats of differing salinity within a single population. The plants maintained their differences in the experimental garden, and the differences reflected ecological gradients which were not necessarily spatially separated.

In a study of the intrapopulation differentiation in *Veronica peregrina*, Linhart (1974) found that plants growing in 'vernal pools' were distinct from those growing on the margins of the same pools. The differences apparently are adaptive. Thus seed from the pool populations germinated significantly faster than the

peripheral populations, and this was correlated with the predictability of moisture availability. The peripheral plants grew much larger than those of the pools, and this was presumed to be in response to interspecific competition from associated tall grasses, a factor not pertaining within the pools.

Habitat-related intra-population differentiation frequently has been demonstrated across man-made ecological boundaries. Thus changes in edaphic factors such as altered fertilizer levels may act as the component of selection (e.g. Snaydon and Davies, 1972). Bradshaw and other workers have demonstrated the occurrence of differential tolerance to heavy metals within interbreeding populations of a single species (Bradshaw, 1952; Wilkins, 1960; Jain and Bradshaw, 1966; Antonovics *et al.*, 1972; Bradshaw, 1974). A recent example serves to show both the nature of this variation, and the unconscious role man may play in plant evolution:- plants of *Plantago lanceolata* which were located on roadside verges and in adjacent fields were found to exhibit differential tolerance to poisoning by lead (Wu and Antonovics, 1976). The differences in lead tolerance were found to be heritable, and are presumed to be maintained by selection. The agent of selection appears to be the exhaust emissions of passing cars.

Phenotypic Plasticity

The variation observed between plants of a single species may not be solely the result of directly determined genetic differences. Other ways in which plants may differ include:-

i. phenotypic plasticity - in which the phenotype of a plant may vary according to the environment in which it grows.

ii. developmental changes of form during ontogeny.

iii. epigenetic effects which result in minor fluctuations of phenotype - the so-called 'developmental noise' of Waddington (1957).

The last of these is of minor importance in population differentiation and is usually incorporated in the 'error' terms of experimental studies of populations. It will not be considered further in this discussion.

Whilst (i) and (ii) may represent fundamentally different responses, in practice their distinction is blurred. Thus developmental changes are frequently tied to changes in the external environment, e.g. the light requirement of some species for seed germination, and the photoperiodic control of flowering in others. Also, there is abundant evidence that environmentally controlled phenotypic plasticity is itself under genetic control (Bradshaw, 1965), and is amenable to selection (Heslop-Harrison, 1953).

Jones and Wilkins (1971) have defined phenotypic plasticity as 'the ability of an individual to change the developmental course of some characteristic in response to environmental pressure'. The degree of the response is a measure of its plasticity (Cook, 1968), and Bradshaw (1965) states that plastic response is specific both for the environmental influence and for the particular character under study.

The ability to exhibit such a response may or may not confer adaptive advantage upon the organism (Heslop-Harrison, 1969; Daubenmire, 1974; Jones and Wilkins, 1971). The evolutionary significance of plasticity has been reviewed by Bradshaw (1965), who cites considerable evidence for the adaptive nature of many such responses. This is generally supported by other workers

(Baker, 1965; Mulligan, 1965; Cook, 1968, 1969; Falconer, 1970; Helsop-Harrison, 1964; Jain, 1969; Jones and Wilkins, 1971; Snaydon, 1973; Daubenmire, 1974). The relative adaptive advantages accruing to species which maintain high levels of plasticity are manifold. Cook (1968) has shown that populations of *Ranunculus aquatilis* which are subject to unpredictable changes in water levels contain genotypes which are markedly plastic. This contrasts with populations from terrestrial habitats which are composed of genotypes with less plasticity, but greater genetic variability. Mulligan (1965) and Baker (1965), in comparisons of closely related species, have shown that there is an overall increased plasticity in weedy compared with non-weedy species. Baker considers that the capacity for increased levels of plastic response gives rise to 'general purpose' genotypes which confer a relatively greater range of environmental tolerance.

Westerman and Lawrence (1970) have reviewed the terminology associated with the developmental responses exhibited by plants which grow in a variable environment. These authors have proposed the term developmental stability to describe genotypes in which development is buffered against environmental variation. Developmentally flexible genotypes are those which can give rise to different phenotypes in different environments. In this case the phenotype evoked by the particular environment is adapted better to that environment than to any other. Thus developmental flexibility is equivalent to adaptive phenotypic plasticity. The authors agree with Lewontin (1957) that developmental stability and developmental flexibility are the developmental analogues of the population concepts of mono- and poly-morphism and may represent alternative

evolutionary strategies.

Whilst adaptive phenotypic plasticity and genetic variability of species are to some extent alternative strategies, a character under study may exhibit both responses within a single population. Snaydon (1973) considers that the distinctions between genetic and environmental influences on the phenotype are artifical. Similar arguments have been advanced by Davis and Heywood (1963) who feel that the term ecotype should be used in a general sense, so that ecotypic differentiation can then be qualified as phenecotypic or genecotypic depending on the control of the observed differentiation.

Gregor and Watson (1961) have demonstrated the advantages of phenotypic plasticity in providing a buffer for genetic variability in populations. Thus by adopting a 'general purpose' genotype, the underlying genetic variability of a population may be masked by plastic responses super-imposed by the environment. The adaptive advantages of such a mechanism become obvious in situations where there is recurrent variation of selective forces occurring over time periods which are less than the reproductive cycle of the plant, e.g. seasonal variation in temperature, etc. (Bradshaw, 1965). In this situation a species which is able to 'anticipate' the environmental variation will be at a selective advantage since there will normally be a time lag between the causative factor and the production of organs modified to suit the changed conditions.

The process of genetic assimilation described by Waddington (1953, 1957) provides a mechanism whereby plastic modification of a character which confers adaptive advantage may become genetically fixed (Heslop-Harrison, 1964). The mechanism could be invoked to describe genetically controlled differentiation which may orignally

have been ecologically induced (Davis and Heywood, 1963) and also to explain the apparently 'pre-adaptive' or autoregulatory responses due to indirect environmental control of a factor, such as those observed in species of *Ranunculus* (Cook, 1968).

Ecotypic Differentiation and Leaf-Shape

Developmental Changes in Leaf-Shape

In many species, changes in the shape of leaves occur during ontogeny, and this heteroblastic development may or may not be linked to obvious external environmental factors. The factors effecting alterations in leaf-shape within a plant during development have been discussed at length by Ashby and later workers (Ashby, 1948 a, b; Ashby *et al.* 1949; Njoku, 1956 a, b; Njoku, 1957). This work shows that the final leaf-shape adopted at any stage of development is a resultant of the shape of its primordium, the number, distribution and orientation of cell divisions, and of the amount and distribution of cell enlargement. Ashby (1948a) provides a large number of references which demonstrate the interactions of these determinants with the internal and external environments of the plant. The major conclusions that may be drawn from the morphogenetic studies are:-

(i) Leaf-shape changes during ontogeny are effected indirectly through the action of such factors as light intensity, photoperiod, mineral nutrition, temperature and water availability. In some cases the changes wrought by these variables have been shown to be mediated by phytohormones.

(ii) In some species leaf-shape is a function of the age

of the plant and is not obviously correlated with the prevailing external conditions. Such cases possibly reflect the changing carbohydrate nutritional status of the plant with age.

(iii) Leaf-shape, and the rate of heteroblastic development, is at least partly under genetic control.

The Genetic Control of Leaf-Shape

Notwithstanding the variation of leaf-shape apparent in heteroblastic and heterophyllous species, one of the most immediate differences which strikes the casual observer is the overall similarity of leaf-shape within species compared to the great diversity of leaf-shapes displayed between species. This in itself strongly infers that leaf-shape is under genetic control. Surprisingly, there appears to be comparatively few formal genetic analyses of leaf-shape differences within species. Monogenic and digenic inheritance of leaf-shape have been demonstrated in celery (Bouwkamp and Honma, 1970) and tobacco (Humphrey *et al.*, 1964) respectively. Jindla and Singh (1970) have shown that the hastate leaf-shape in the cowpea is controlled by four genes. Silow (1969) has found that sinus length and lobe width, the two primary components of leaf-shape in *Gossypium*, are independently controlled.

There is a considerably body of genecological evidence which supports the heritable nature of leaf-shape within species. Genetically controlled intraspecific differentiation of leaf-shape has been demonstrated in a number of species (Lewis, 1969; Woodell and Koatin-Sanwu, 1971; Siddiqui and Swaminathan, 1971; Wilcock, 1974) and clinal variation in leaf-shape is known to occur in species of *Eucalyptus* (Jackson, 1960; Ladiges and Ashton, 1974).

Within the genus *Plantago*, Day and Fisher (1937) have reanalysed Gregor's data to demonstrate population differentiation of leaf-shape in *P. maritima*, and Böcher *et al.* (1955) and Groot and Boschhuizen (1970) found genecological leaf-forms in plants of other *Plantago* species.

The Environmental Control of Leaf-Shape

'The leaf is probably the most plastic single organ that the plant body possesses' (Stebbins, 1974). There is a wealth of information in support of this contention, and in most cases the plasticity is derived, directly or indirectly, in response to shifts in the level of environmental factors. Thus phenotypic polymorphisms arise which are habitat correlated. Factors known to influence leaf-shape include light quality (Sanchez, 1967), quantity (Sanchez, 1967; Scmidt and Millington, 1968; Fisher, 1960a, b) and duration (Heslop-Harrison, 1958; Cook, 1968), soil moisture (Cook, 1968; Wilcock, 1974; Quinn and Fairbrothers, 1971), soil nutrition (Njoku, 1957; Sarruge and Malavolta, 1970), soil salinity (McCully and Dale, 1961), and temperature (Fisher, 1960).

The frequently complex interactions between these variables can be illustrated by two examples from heterophyllous species.

The three habitats occupied by the leaves of the amphibious *Ranunculus aquatilis* are markedly disruptive, and heterophylly in the species is pronounced (Cook, 1969). The leaves can be submerged, terrestrial or occupy the air-water interface. Leaf-shapes in the three environments are markedly different. Cook's experiments conducted in controlled growth chambers showed that the submerged leaf-form develops in both terrestrial and submerged conditions under short photoperiods, but under long days it is only

produced on submergence. The terrestrial leaf-form develops only under long photoperiods in terrestrial conditions, whilst the leaf-form typical of air-water interface develops only under long day-lengths in submerged conditions. Cook has demonstrated that photoperiod is the basic mechanism of control of the different leaf-shapes, but that submergence regulates their phenotypic expression. He found that temperature and light intensities in the normal ranges did not affect leaf shape.

A somewhat different mechanism of control was found in *Proserpinaca* (Schmidt and Millington, 1968). Submerged shoots of this species maintained dissected leaves under short and long photoperiods, whilst leaves of aerial shoots are dissected in short days, but expanded-lanceolate in long days. Elevated temperatures and light intensities were found to induce aerial leaf-shapes on long day submerged plants.

The expression of phenotypic plasticity of leaf-shape has been shown in some cases to be mediated by growth regulating substances and by phytohormones. The action of these chemicals produces effects which mimic responses otherwise obtained by environmental change. Haccius and Garrecht (1963) have used phenylboric acid to induce lanceolate leaves in *Solanum*. Thorpe and Hield (1970) altered the leaf-shape of *Citrus* by foliar application of gibberellic acid. Heslop-Harrison (1958) found that the exogenous application of gibberellin to *Cannabis* grown under long photoperiods resulted in leaves having shapes similar to those occurring on plants grown under short days.

Physiological Implications of Leaf-Shape Variability

The evidence presented in the previous sections suggests that many of the plastic responses of leaf-form are adaptive. In fact it has been shown that environmentally induced changes in leaf-form may frequently be accompanied by changes which appear to represent physiological adaptations to the new situation. This interrelationship has been demonstrated by Raschke (1960) and Holmgren *et al.* (1965) with respect to heat transfer and gaseous exchange across the leaf-air interface. The derived equations of aerodynamic fluxes used in physiological ecological studies incorporate an empirical constant to allow for differences of leaf-shape (Rose, 1966; Slatyer, 1967).

Lobed leaves of *Ranunculus hirtus* are associated with open areas of forest margins, whilst entire leaves occur in sheltered regions within the forest (Fisher, 1960). Fisher points out the damage which may accrue to the marginal areas of leaves which are at a distance from major veins, compared with dissected leaves when subject to excessive transpiration. A similar situation prevails in *Geranium sanguineum* (Lewis, 1968; Wilkins and Lewis, 1969). The species exhibits ecoclinal variation in leaf-form, there being an increased degree of leaf dissection which is associated with a vegetation gradient toward more open communities. The implication here is that the more highly dissected leaves are relatively more efficient in maintaining lower internal temperatures under ambient conditions which favour increased transpirational stress. This type of response has also been observed in *Nigella* (Waisel, 1959) and *Clarkia* (Vasek, 1964).

Alterations of leaf-form may also affect the relative photosynthetic efficiency of the plant (Holmgren, 1968). Vasek (1968) points out that under conditions of high light intensity,

CO_2 availability becomes limiting. Resistance to CO_2 diffusion will be lowered if there is a reduction in the distance between the palisade cells and the stomata. This can be achieved by an increased leaf dissection or by a reduction in the relative width of leaves. At lower light intensities when CO_2 is not limiting, the relative photosynthetic efficiency would be enhanced by larger or broader and thinner leaves.

The Description of Form in Biological Studies

There are two aspects to the form of any planar object. The first of these aspects is size, which is equivalent to area. The second aspect is the shape of the object, i.e. circular, triangular, etc. In this thesis each of these terms is used in a relative sense, i.e. comparisons are made between objects which may differ in their size and shape. Two objects which differ only in the proportional magnitudes of their linear dimensions have the same shape but differ in size. Thus a requisite amount of equal relative dimensional 'growth' of the smaller object will result in an object which is identical in all respects with the larger object. If differential 'growth' in the linear dimensions of one object is required to make it equivalent to the second, then they differ in shape. One of the major problems of the study of morphological form has been to find ways of treating these two aspects.

The differences in shape of a set of objects are readily perceived by the human eye, and these differences provide a useful basis for the classification of the objects. The problem for the biologist lies in the logical description and communication of the different patterns. There is now a standardised taxonomic nomenclature which aids in the description of shape (Systematics Association Committee, 1962). This

nomenclature is useful for the erection of taxonomic keys, and for indicating the limits of variation to be observed in an organ of a particular species. However, the great diversity to be found in the size and shape of organs such as leaves, and the almost universal occurrence of quantitative variation of form, both within and between taxa, has led to a search for numerical means of specifying form.

Studies on the variation in leaf-shape to date have centred on the more startling contrasts of form exhibited by markedly heteroblastic species, or by plants exhibiting heterophylly due to strongly disruptive environments. Thus many species of *Eucalyptus*, for example, exhibit a sharp switch in leaf-shape between the juvenile and adult plants. Heterophylly has been most extensively studied in semi-aquatic species such as *Ranunculus* spp. (Cook, 1969). In such typical cases, the change in leaf-shape is so pronounced that there is little need to undertake a detailed analysis. The alterations in leaf-shape are shown either by silhouettes or by photographs, with an accompanying verbal description, or by the use of a readily obtained index of leaf-shape such as the degree of dissection. The qualitative nature of the changes almost obviates the necessity of statistical analysis. A similar situation obtains in genetical studies where marked differences of leaf-shape between genotypes result from the presence of one of a few alleles at a single locus (e.g. Silow, 1939).

In attempting to study the morphological variation of shapes which form a continuum, more detailed consideration must be given to the quantification of shape. Thus the variables must afford some means of quantifying the apparent variation and be capable of accurately distinguishing between aspects of variation which

are size-related and those which can be ascribed to shape. The variables must also be capable of statistical manipulation, so that the degree of difference or similarity between samples can be assessed. The variables should also be capable of biological interpretation in both field and controlled environment studies, and in tests of heritability.

The resolution of the problem in the various studies has been largely *ad hoc*, so that a diversity of methods for describing shape has arisen. Methods of shape description which have been used include indices, and simple compounded characters (Njoku, 1956; Ashby, 1948; Silow, 1939; Sanchez, 1967; Lewis, 1969), polar coordinates (Fisher, 1960; Woodson, 1947), allometry and multiple regression methods (Pearsall, 1927; Groot and Boschhuizen, 1970; Day and Fisher, 1937; Sinnot, 1958), rectangular coordinates (Melville, 1937), non-parametric methods (Meltzer *et al.*, 1967), and, most recently, multivariate methods.

The various multivariate approaches to the study of shape have included principal components analysis (Seal, 1964; Blackith and Reyment, 1971; Joliceur, 1963; Sprent, 1972), multivariate distance methods (Fisher, 1936; Humphrey *et al.*, 1964; Dale *et al.*, 1971), canonical variates analysis (Blackith and Reyment, 1971; Dancik and Barnes, 1973) and factor analysis (Hopkins, 1966; Atchley, 1971).

It can be seen from this brief outline that the different methods used in the description of shape have increasingly involved the cooperation of the mathematician and the biologist. This creates some problems for the biologist. Thus, whilst the initial choice of characters for measurement is the province of the biologist, the subsequent analytic methods will be dictated to a

degree by the biometrician. Every care must therefore be taken to select mathematical techniques which both optimise the biological information and satisfy the mathematical assumptions of the particular method.

Anderson (1954) questioned the development and use of regression methods in the specification of form, preferring graphical or other semi-pictorial methods to the mathematical techniques then available. Beals (1973) argues against the use of principal components analysis in phytosociological studies where the known distributions of plant species within and between communities do not meet the requirements of the mathematical model. Atchley and Hensleigh (1974) question the value of some multivariate techniques as morphometric tools arguing that their use will normally either confound aspects of size and shape variation (canonical variates) or artificially separate what are in fact related patterns of growth (principal components). These authors then proceed to deal with contrasts of form using a factor analytic approach, a technique which has been criticised by Seal (1964) and Blackith and Reyment (1971). Furthermore, in referring to the supposed independence of size and shape characteristics obtained from principal components analysis, Sprent (1972) argues that there is no anomaly in cases where these are known to be dependent, e.g. size dependent variation of leaf-shape in heteroblastic studies. He states that the shape components extracted will usually have a component of size associated with them. This was found to be so in simulation studies conducted during the present work (Appendix A.1, p.266).

Blackith and Reyment (1971) maintain that the multivariate techniques of principal components and canonical variates are particularly robust to departures from their underlying assumptions, and point to the abundant empirical evidence that the techniques produce results which have useful biological interpretations. The techniques are frequently capable of detecting multivariate structure both within and among populations, even though the mathematical bases for testing the significance of such differences are not well established.

Analytical Methods Used in this Thesis

It is apparent that there are many different ways of quantifying shape and some of the methods involve complicated mathematical analysis. A number of procedures will be used in this thesis. Firstly, the linear dimensions of the leaf are measured, i.e. length *l*, length to widest part *lwp*, width *w*, and length of lamina *ll*. It is obvious that these measures *per se* are of little use in estimating differences of shape between leaves. For example, two leaves may have different widths because their shapes differ, or one leaf may simply be larger than the other, but have the same shape. This effect can be removed by scaling the width of the leaves according to their areas or to their largest dimension, i.e. length. In many cases, the ratio of *w/l* may give an adequate description of the shapes of the leaves. The differences between leaves can then be tested by the appropriate univariate analyses. On the other hand, if, for example, the *ll* and *w* are covariates, the independent estimations of the *ll/l* and *w/l* ratios will over-emphasise the differences between sets of leaves. Since most of the variability examined for this thesis involved simultaneous variation in a number of characters, the techniques of multivariate analysis were considered most suitable. The multivariate

techniques used are principal components analysis (PCA), canonical variates analysis (CVA), canonical correlations and the generalised distance (D^2) of Mahalonobis. PCA is used mainly to find sets of mutually independent inter-relationships among the variables. CVA is used to maximise the differences between groups of individuals. The overall distances between groups are estimated by D^2. Canonical correlations are used to estimate the relationships between paired sets of data. A fuller discussion of the different techniques, and an appraisal of their relative advantages and disadvantages in the numerical specification of form, is given in Appendix A.2, p.270.

Terminology

Some of the concepts discussed above require qualification for this thesis. The concept of genetic polymorphism as defined by Ford (1975) is strictly applicable only to genetically determined discontinuous differences between the members of a single population. This view has been supported by Mayr (1965) who coined the term polyphenism for purely phenotypic differences. However, polymorphism has come to be used in a much broader sense to cover qualitative differences between the morphs of a single population, whether these are of genotypic or phenotypic origin (Stebbins, 1965; Davis and Heywood, 1963). The term is used here in the latter sense.

Similar arguments apply to the concept of the ecotype. Thus ecotypic differentiation is defined as interpopulation differentiation which is maintained genetically, whilst the term ecad has been used by Clements (Heslop-Harrison, 1953) to describe populations which are different by virtue of plastic modification. However, in view of the frequently adaptive nature of such plastic modification, there appears to be no *a priori* reason for the artificial distinction of the two types of variation. For this reason, the terminology of Davis and Heywood (1963) is adopted in this thesis. Thus ecotypic differentiation is given the broader meaning, whilst genecotype and phenecotype are used to describe ecologically distinct populations which differ genetically or phenotypically respectively.

The concept of adaptation requires some qualification. In this thesis, the characteristics of a plant are termed adaptive if it can be inferred that the particular plant is able to persist in situations which otherwise might not be exploited by the taxon.

For several reasons, the functional advantage which is endowed on the particular morph may be obscure. For example, a habitat-correlated occurrence of two different leaf-forms within a single population might reflect past selection for differing physiological status of the morphs in a heterogeneous environment. Alternatively, the primary adaptive advantage may in fact be conferred by the differential expression of some other characteristic which is controlled by the same gene, i.e. pleiotropy.

The use of the terms "size" and "shape" also need clarification. In the present studies, the terms "size component" and "size vector" refer to vectors which result from a multivariate analysis and which contain coefficients representing equal relative proportions among the linear dimensions which have been measured. This means that the individual scores calculated from the coefficients of the vector are correlated with the areas of the individuals. A "shape vector" is one in which the coefficients of the vector represent a contrast between the relative proportions of the measured individuals. Appendix A.1 (p.266) sets out three examples of the correspondence that obtains between the intuitive concepts of size and shape and the vectors which result from multivariate analysis.

2. The Taxonomy of the Plantaginaceae

The Plantaginaceae

A brief description of the classification and associated fields of study within the Plantaginaceae is given here to provide a background for experimental studies which follow.

The family is composed largely of rosulate annual or perennial herbs and contains three genera:- *Bougueria*, a monotypic genus, *Littorella*, a genus of three species, and *Plantago*, a genus of about 260 species. The flowers are usually in heads or spikes, bracteate, hermaphrodite, regular and predominantly anemophilous. The floral structure described below is after Curtis (1967), Willis (1973) and Briggs *et al.* (1977):- Sepals 4, free (or sometimes the lower pair ± fused), imbricate, diagonal, equal or unequal, persistent in the fruiting stage. Corolla gamopetalous, lobes 3 or 4, imbricate and membranous. Stamens (1-2-)4, epipetalous, hypogynous or free. Ovary superior, 2-carpellary (sometimes 1-carpellary), (1-)2-locular, sometimes up to 4-locular by the development of false septa. Placentation axile or basal or free central, ovules semi-anatropous. Style simple, terminal. Fruit a membraneous capsule with circumscissile dehiscence (*Plantago*) or an indehiscent, 1-seeded nut (*Littorella*, *Bougueria*).

The family occurs throughout the temperate and cold temperate regions of the world. It is represented in the tropics only by adventive species. The genus *Littorella* has one species in each of North America, South America and Europe. *Bougueria* is found in the Andes. The genus *Plantago* contains over 200 species which can be considered as endemic, i.e. occupying a limited range.

Only 29 species have a wide or discontinuous range (Good 1965). The genus therefore exhibits a fairly high degree of speciation apparently arising from geographical isolation.

Classification of the Plantaginaceae

The affinities of the Plantaginaceae are not clear, and on the basis of morphological characters, the family has been placed variously in the Tubiflorae near the Scrophulariaceae (Lawrence 1951; DeWit 1965; Takhatjan 1969; Willis 1973), in the Primulales (Bessey 1897) and in the Bignoniales (Thorne 1968). On the other hand, a number of authors consider that it belongs in a separate order (Plantaginales), e.g. Bentham and Hooker (1862-83), Engler (1954), Benson (1957), Hutchinson (1959, 1969), Cronquist (1968).

Cytological studies (e.g. McCullagh 1934) have been of little assistance in demonstrating affinities at the family level, although they have been valuable in suggesting probable lines of diversification within the genus *Plantago*.

A systematic classification of the family was published first by Barneoud (1845), who recognized three genera and subdivided the genus *Plantago* into six sections. Decaisne (1852) subsequently divided this genus into seventeen sections. Engler and Prantl (1897) recognized twelve sections within *Plantago*; these were divided among two sub-genera, one of which contained a single section.

The most recent monograph of the family is that of Pilger in das Pflanzenreich (1937). Pilger's classification recognizes two sub-genera within *Plantago*;- *Euplantago* with 247 species divided among 18 sections and *Psyllium* containing 13 species in a single section (Table 2.1).

Table 2.1. The Taxonomic Classification of the Plantaginaceae (after Pilger 1937). The numbers in brackets are the numbers of species in each group recognized by Pilger.

Family: PLANTAGINACEAE

Genus: *Bougueria* (1)

Genus: *Littorella* (3)

Genus: *Plantago* (260)

Sub-genus I *Euplantago* (247)

Section 1. *Polyneuron* (19)

2. *Micropsyllium* (6)

3. *Palaeopsyllium* (28)

4. *Holopsyllium* (1)

5. *Oliganthos* (16)

6. *Microcalyx* (2)

7. *Coronopus* (19)

8. *Oreophytum* (1)

9. *Novorbis* (47)

10. *Mesembrynia* (22)

11. *Lamprosantha* (3)

12. *Eremopsyllium* (1)

13. *Oreades* (3)

14. *Gentianoides* (1)

15. *Bauphula* (1)

16. *Arnoglossum* (8)

17. *Leucopsyllium* (66)

18. *Hymenopsyllium* (3)

Sub-genus II *Psyllium* (13)

Section 19. *Psyllium* (13)

Pilger's classification of *Plantago* is currently under review. American taxa are being examined using cytology, ecology, morphology, pollen morphology (Bassett 1966, 1967; Bassett and Crompton 1968; Rahn 1974) and controlled growth studies in conjunction with taximetric procedures (Rahn 1974). Rahn has completely overhauled the relevant American sections recognized by Pilger. Thus all of Pilger's sections *Novorbis* and *Oreophytum* have been included in Barneoud's section *Virginica* and two species from section *Leucopsyllium* and one from section *Palaeopsyllium* have been transferred to *Virginica*.

In Australia, only two of Pilger's sections (*Oliganthos*, *Mesembrynia*) are represented by native species. Briggs, Carolin and Pulley (1973, 1977) have reviewed the taxonomy of some of these species using cytological and morphological criteria. They state that considerable revision of Pilger's classification is necessary, and in particular they query the division of the Australian species into two sections. Examples of species which combine "diagnostic" characteristics (see p. 37) of both sections are not infrequent and as a typical example they cite *P. cladarophylla*, a new species which exhibits considerable similarity to some members of section *Oliganthos*, whilst having fruit characters typical of *Mesembrynia*.

Cytology of *Plantago*

There appear to be at least three series of multiple chromosome numbers in the genus *Plantago*. The probable primitive base number for the genus is x = 6 giving rise to the polyploid series 12, 24, 36, 48 and 72. The other two series appear to

derive from aneuploids, with bases x = 5 (2n = 20, 20, 30) and x = 4 (2n = 8, 16) (McCullagh 1934; Maude 1939; Jackson unpubl.; Rahn 1957; Bassett 1967; Groves and Hair 1971; Briggs 1973).

Polyploid races are known to occur within some species of the genus, but the occurrence of polyploidy does not appear to be well correlated with morphological variation at either the intra- or inter-specific level. For example, Rahn (1974) found that polyploid races of *P. australis* (2n = 24, 48) did not exhibit obvious morphological differences, and a similar result was found by Briggs (1973) for *P. gaudichaudii* (2n = 12, 24, 36). Bassett and Crompton (1968) found no overall correlation between chromosome number and pollen size of N.American taxa. Böcher *et al.* (1953, 1955), in an investigation of the cytogeographic variation of *P. coronopus*, found diploid and hexaploid races within the species, but found that the morphological differences between the ploidy levels were less than those observed among races of the diploid type. McCullagh (1934) found some agreement between chromosome number and Decaisne's division of the genus.

The consensus of these reports has been put succinctly by Briggs (1973): "Polyploidy is fairly common, but the primary diversification within the genus has apparently not been accompanied by change of either base number or level of ploidy."

Breeding Systems within *Plantago*

The genus has hermaphrodite flowers which are protogynous, largely wind-pollinated and outbreeding, although exceptions do occur. Thus *P. media* L. produces flowers which are scented, have coloured stamens and are at least partly insect pollinated. Members of the section *Novorbis* produce cleistogamous flowers

and these species are held by Pilger to be apomictic, although no evidence for this was found by Rahn (1974). Pilger reports dioecy as occurring in *P. tubulosa* Decne., and *P. rigida* Kunth., and gynodioecy in *P. lanceolata* L.

The occurrence of protogyny provides a measure of facultative outbreeding, at least in the early part of the season, but unless self-incompatibility mechanisms are present, selfing can occur by fertilization of younger inflorescences of the same plant. In a survey of the breeding systems of eleven species of *Plantago*, Ross (1970) found a diversity of breeding systems to be present, from facultative inbreeding to obligate outbreeding. Of particular interest was the occurrence of both self-incompatibility mechanisms and gynodioecy in populations of three species. Even more remarkably, it was found that whilst both self-incompatibility and gynodioecy occurred in populations of *P. maritima* located in Europe, the American populations of the same species were self-compatible and not gynodioecious.

Phenotypic Modification in *Plantago*

Many of the species exhibit extensive phenotypic plasticity (Marsden-Jones and Turrill 1930-1945; Salisbury 1951; Gregor and Watson 1961), and some of the habitat induced modifications have been shown to correspond closely to previously described infra-specific taxa. Thus Marsden-Jones and Turrill (*loc. cit.*) were able to demonstrate the correspondence between edaphically induced modifications of *P. major* and varieties described by Pilger (1937). Similarly, Salisbury (1952) showed that the form adopted by plants described as *P. coronopus* var. *pygmaea* was habitat induced: on transplanting, the plants assumed the 'typical' form of *P. coronopus*.

However, Böcher *et al.* (1955) have also demonstrated that in some cases this dwarf form is genetically fixed.

Ecotypic and ecoclinal variation in a variety of characters has been demonstrated for a number of species. Much of this variation is genetically based. Gregor (1930) found quantitative differences in the spike:scape length ratio in *P. maritima* from different habitats and these were under genetic control. Groot and Boschhuizen (1970) have found heritable differences of leaf-form among populations of *P. major* and Böcher *et al.* (1955) found that geographically distinct races of *P. coronopus* are maintained genetically.

From the examples given here it can be seen that within a single species of the genus there are characters capable of exhibiting morphological differences which are habitat correlated, and which may simultaneously be genetic in origin and yet exhibit plastic modification.

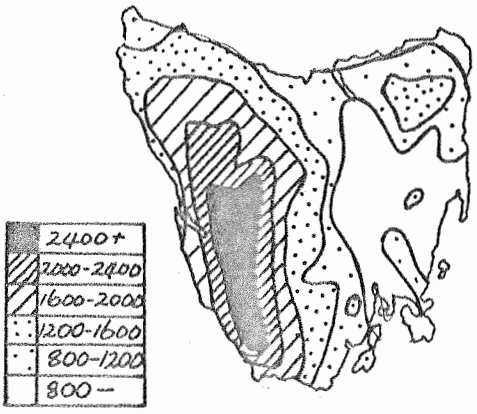
The Genus *Plantago* in Tasmania

Tasmania: A Descriptive Background

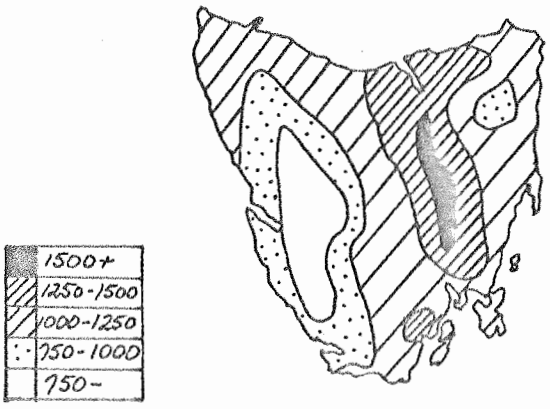
Climate

Tasmania is an island of approximately 6.5 million hectares lying between 40 and 43.5^{o}S. Its climate is classified as temperate maritime (Bur. Met. 1976). The island lies within the influence of the belt of westerly winds known as the "Roaring Forties" and this combines with the mountainous terrain of the western half of the state to produce a pronounced west-east variation of climate, especially of rainfall (Figs. 2.1 to 2.5).

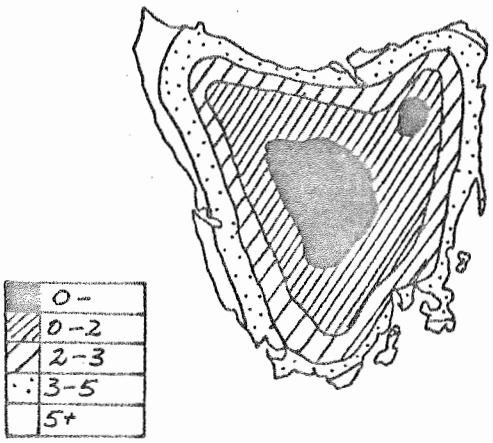
2.1 RAINFALL (mm.)



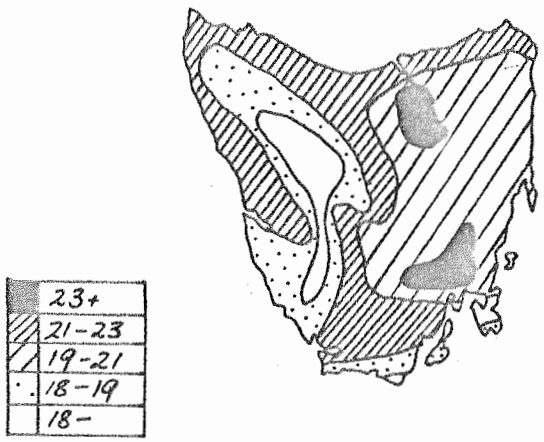
2.2 EVAPORATION (mm)



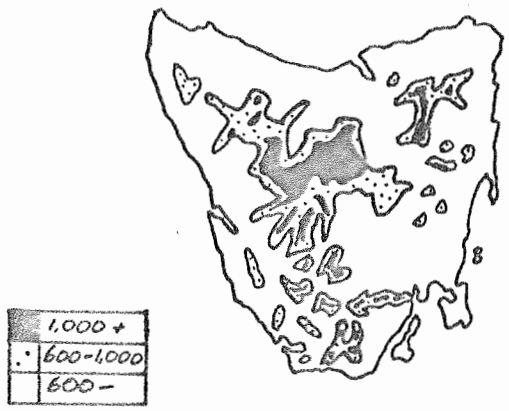
2.3 MINIMUM TEMPERATURE (JULY)(°C)



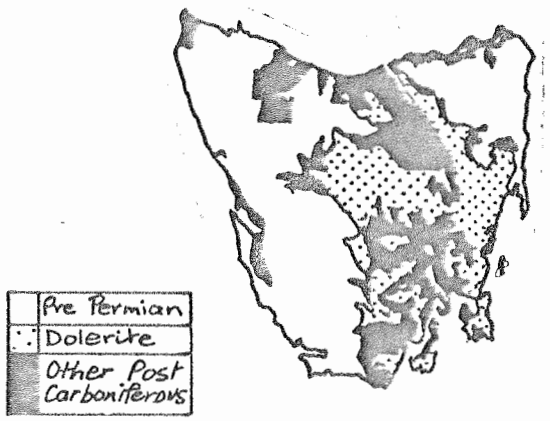
2.4 MAXIMUM TEMPERATURE (JAN.)(°C)



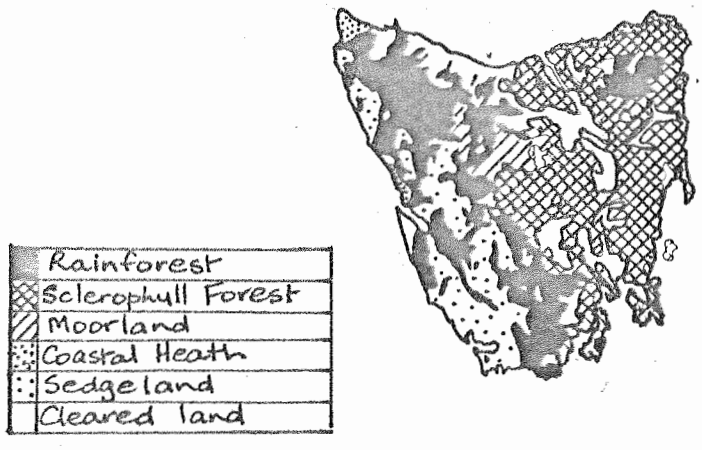
2.5 ALTITUDE (METRES)



2.6 GEOLOGY



2.7 VEGETATION



In the west, annual rainfall averages from 1300 mm at the coast to 3600 mm inland; compared with from 550 mm at the coast to 1300 mm on the highlands in the north east. Effective rainfall occurs from May to September in all centres, but the probability of effective rainfall decreases from October to January, except in the west.

Summers are mild, characterised by 15-15.5 hours of daylight, compared with winter daylengths of 9 hours. In winter the westerly winds reach their greatest strength and persistence.

The generally high relief of the western half of Tasmania (Fig. 2.5), and the greater cloud cover, has a pronounced influence on temperature (Figs. 2.2 - 2.4). Inland areas are frost-free only in summer. On the coast, the daily temperature range averages $8^{o}C$, rising to $12^{o}C$ inland, indicating a slight continental effect. Mean air temperatures in July vary from 0 to $10^{o}C$, and those in January from 10-$18^{o}C$.

Geology

The western and eastern halves of the state exhibit a major disjunction in geology. The western half of Tasmania is dominated by metamorphosed rock of pre-Permian age, which contrasts with the Central Plateau and eastern region of the state which are dominated by Jurassic dolerite and other Post Carboniferous rocks (Fig. 2.6) (Buckney and Tyler 1973).

Vegetation

The climax vegetation of Tasmania consists of three formations (Fig. 2.7). Temperate rainforest occurs in areas where summer rainfall exceeds 50 mm per month. Sclerophyll

forest dominated by *Eucalyptus* species occurs in lower rainfall areas. The third formation is 'austral montane' an alpine formation comprised of shrubland, sedgeland and grassland-herbfield alliances. The interaction of fire and physical determinants (climate, geology, aspect, topography and edaphic factors) with the vegetation has resulted in the establishment of a dynamic equilibrium between the various plant communities (Jackson 1968). Thus the occurrence of ecotones or transition zones and disclimax communities is common, both within and between the three major formations. This is particularly in evidence in the disclimax sedgelands of the west and south west, and the coastal heaths of the north. The climatic treeline in Tasmania occurs at approximately 915 m, and the subalpine zone occurs 300-400 m below this (Costin 1972). However, the effects of cold air drainage in upland areas such as the Central Plateau, and the increasingly harsh environments experienced in a westerly direction, result in the occurrence of many 'alpine' species at much lower altitudes.

The plant communities of Tasmania are usually classified on a structural basis (e.g. Jackson 1974). Plant communities referred to in the present study were similarly defined, by appending the name of the dominant species in the uppermost stratum to the associated structural alliance. Detailed information about their ecology is given by Jackson (1972).

Plantago in Tasmania

Eighteen species and two varieties have been recorded for Tasmania at various times, but of these I have not been able to confirm the presence of three species (*P. gaudichaudii, P. glacialis,*

P. muelleri). Four species are introduced; *P. lanceolata, P. major* and *P. coronopus* are of Eurasian origin whilst *P. australis* is from South America. Five of the native species are endemic to Tasmania (*P. glabrata, P. daltonii, P. paradoxa, P. bellidioides* and *P. gunnii*). Five of the species occur also on the Australian mainland (*P. varia, P. hispida, P. debilis, P. antarctica* and *P. tasmanica*). *P. triantha* has a disjunct distribution, being found in Tasmania and on some of the offshore islands of New Zealand.

The native species are divided into two sections (*Mesembrynia* and *Oliganthos*) according to Pilger's classification (Appendix B.2, p.280). Species of the section *Oliganthos* are characterised by a bilocular ovary which has four to many ovules per loculus. Members of section *Mesembrynia* have a trilocular ovary which is formed by the development of a false septum. Some discrepancies from this classification have been found in the Tasmanian species, both by Curtis (1967) and during the present study. For example, *P. debilis, P. varia, P. bellidioides* and *P. antarctica* are assigned to section *Mesembrynia* by Pilger, but Curtis (1967) states that *P. debilis* has a bilocular ovary, and that the presence of a trilocular ovary is not constant in the other three species. These observations have been confirmed in the present study. However, in spite of these problems, the classification of Pilger is convenient for the purposes of this study, and it has been retained throughout the thesis.

The nomenclature used here for the Tasmanian species is largely that of Curtis (1967). However, the review of New South Wales representatives by Briggs, Carolin and Pulley (1973, 1977) and field and herbarium investigations during the present study, have resulted in some changes to the treatment by Curtis (see

Appendix B.1,p.276). The relevant alterations are as follows:-

1. *P. glabrata* Hook.f.

This species typically shows the development of a trilocular ovary and should be referred to section *Mesembrynia* pending a review of Pilger's classification.

Some forms of *P. glabrata* may be mistaken for *P. antarctica* if the key characters given by Curtis (1967) are used to identify them, *viz.* "leaves with 3-7 almost equal parallel veins" (*P. antarctica*) or "leaves with midrib distinct but lateral veins not, or scarcely conspicuous" (*P. glabrata*). Consequently, some common variants of *P. glabrata* which have broad leaves would key to *P. antarctica* on the basis of the above characteristics. However, the two species have different chromosome numbers (*P. antarctica*, 2n = 12; *P. glabrata*, 2n = 24, see Table 2.2, p. 42), and have quite distinct morphological features. A more satisfactory way of distinguishing the two species is as follows. *P. glabrata* has leaves with an elongate, narrow petiole and floral bracts which have fimbriate-ciliate margins and which are as long or longer than the calyces, while *P. antarctica* has leaves which narrow gradually to a short, broad petiole and floralbracts with entire margins and which are only $^1/_2$ to $^3/_4$ as long as the calyces. These characteristics can readily be used to distinguish plants in the field, because the bracts and sepals are persistent in both species and old scapes remain on the plants for most of the year. In this thesis, *P. glabrata* and *P. antarctica* have been defined according to the above features.

2. *P. muelleri* Pilger

I have not seen any of Curtis' material, but her description of *P. muelleri* differs from typical *P. muelleri* in having scapes which are elongate at anthesis. It appears to be nearer to *P. glacialis*, a species formerly included in *P. muelleri*. However, both *P. muelleri* and *P. glacialis* have a tuft of golden-brown hairs in the leaf axils, a character not mentioned by Curtis. *P. muelleri sensu* Curtis has bracts "about as long as the calyces, elliptical, mucronulate," whereas *P. glacialis* has bracts "triangular, acute, 1.5-3mm long" and sepals" 2-2.2 mm long" (Briggs *et al.* 1977).

The description given by Curtis is most similar to depauperate specimens of *P. daltonii*. In the field, such plants are characterised by short scapes with flowers frequently reduced to 1(-4) to a head, but on transplantation assume the typical *P. daltonii* form. These plants occur in alpine areas, frequently forming mats in habitats similar to those described by Curtis for *P. muelleri*. The situation is further confused by the occurrence of forms of *P. paradoxa* which provide an excellent match to the vegetative description of *P. muelleri sensu* Curtis, but which, in respect of floral characters, and on transplantation, are 'good' *P. paradoxa*. Clearly the status of *P. muelleri sensu* Curtis requires clarification. Detailed investigations were not practicable in this study, and because of the confusion surrounding its taxonomic status, *P. muelleri sensu* Curtis has been excluded from this account.

3. *P. daltonii* Hook.f.

Although not listed as such by Curtis (1967), this species appears to be endemic to Tasmania. Reports of its occurrence in Victoria (Curtis 1967; Willis 1973) probably have resulted from confusion with *P. alpestris* (Briggs, *pers. comm.*).

4. *P. hispida* R.Br.

Since the work by Curtis (1967), this species has been returned to specific rank by Briggs *et al.* In Tasmania, the species is very much habitat limited, and shows consistent morphological variation from its nearest ally *P. varia*. The differentiation is maintained when plants of the two species are grown in a common environment.

5. *P. gaudichaudii* Decne.

This species is not included by Curtis (1967). There is only one record of this species from Tasmania; a specimen collected by Rodway in 1928 from Blackmans Bay, 10 km S of Hobart. This locality is now densely populated and I have been unable to re-locate the plant in the area.

6. *P. australis* Lamk.

An adventive species of South American origin, this taxon has recently been found naturalized in disturbed coastal areas of high rainfall in Western Tasmania.

A key to the Tasmanian representatives of the genus *Plantago* is given in Appendix B.1 (p.276).

The distribution of the species within Tasmania is shown in Figs. 1-16 of Appendix B.2 (p.280). A comparison of the distribution maps with the meteorological and physiographic maps of Figs. 2.1-2.7 shows that the species fall fairly naturally into altitudinal groupings, and to some extent reflect differences in effective rainfall. Thus the coastal species *P.triantha*, is restricted to the west and south-east coasts, and is replaced in similar habitats on the north and east coasts by *P. hispida*. With the possible exception of the introduced *P. major*, which preferentially grows in fertilizer enriched pastures and lawns, the species do not appear to be specifically associated either with soil types or geological substrate. For example, *P. tasmanica* is restricted to the upland areas of Tasmania, and grows on sedimentary rocks of Permian age on Mt. Maurice in the north east, on the Jurassic dolerite of the Central Plateau and southern mountains, and on Precambrian metaquartzites on Frenchman's Cap in the west.

Chromosome Numbers and Breeding Systems of Tasmanian *Plantago*

The chromosome numbers of the Tasmanian representatives of the genus *Plantago* are given in Table 2.2. The authorities for these numbers are Briggs (1973), Curtis (1967), Jackson (unpubl.) and Rahn (1974). The chromosome numbers credited to Brown and Jackson were obtained during the course of this project. A comprehensive cytological study was outside the scope of the present work and only brief experimental details are given (see p. 50). Furthermore, the results must be regarded as tentative, because only 3-4 plants have been used to obtain the counts for each species.

Table 2.2. Chromosome numbers of Tasmanian *Plantago* species.

Chromosome number		Species (Authority[+])
n	2n	
-	10	*P. coronopus* (2, 4[*])
-	12	*P. major* (2, 4[*]); *P. lanceolata* (2, 4[*]); *P. tasmanica* var. *tasmanica* (4[*]); *P. daltonii* (4[*]); *P. triantha* (4[*]); *P. glacialis* (2, 4); *P. debilis* (2, 4[*]); *P. bellidioides* (5[*]); *P.hispida* (2, 5[*]); *P. antarctica* (2, 5[*]).
-	24	*P. varia* (2, 4[*], 5[*]); *P. glabrata* (4[*]).
-	36	*P. gunnii* (4[*]); *P. muelleri* (2).
-	48	*P. paradoxa* (4[*]).
-	12, 24	*P. varia* (1[*]).
-	12, 24, 36	*P. gaudichaudii* (2)
-	24, 48	*P. australis* (3).
12	24	*P. glabrata* (narrow-leaf-form) (5[*]).
12	24	*P. glabrata* (broad-leaf-form) (5[*]).

[+]Authority - 1. Curtis (1967)
2. Briggs (1973)
3. Rahn (1974)
4. Jackson (unpubl.)
5. Brown and Jackson (this study)

[*]Determinations made on Tasmanian material.

It is apparent from Table 2.2, that polyploids occur in both sections of the genus native to Tasmania. In section *Oliganthos*, reported chromosome numbers are 2n = 12 (*P. tasmanica*, *P. daltonii*, *P. triantha*), 2n = 36 (*P. gunnii*) and 2n = 48 (*P. paradoxa*). In section *Mesembrynia*, the species are either 2n = 12 (*P. debilis*, *P. bellidioides*, *P. hispida*, *P. antarctica*) or 2n = 24 (*P. varia*, *P. glabrata*). Curtis (1967) reports diploid (2n = 12) and tetraploid (2n = 24) populations of *P. varia* in Tasmania, but Briggs (1973) found that all of the Australian mainland plants studied were tetraploid. Jackson (unpubl.) and Brown and Jackson (this study) have found only 2n = 24 in Tasmania.

No detailed studies of the breeding systems of the native Tasmanian species have been reported in the literature, but my own field observations suggest that, with the possible exceptions of *P. paradoxa* and *P. gunnii*, all of the species are protogynous, outbreeding and wind-pollinated. *P. paradoxa* and *P. gunnii* are protogynous, but produce flowers on scapes which are (sub-)sessile at anthesis, so that self-pollination may be more common in these species. However, both species probably are capable of outbreeding to some degree:- *P. paradoxa* occupies wet sites where cross-pollination could occur via water-borne pollen; plants of *P. gunnii* occur within "cushion plants" where they are elevated above the ground surface. Their stamens have versatile anthers on very long filaments so that some wind-pollination is likely.

All of the known native Tasmanian species have been grown in isolation at some stage during the course of this study, and all appear to be fully self-compatible. No evidence for gynodioecy was found in any of the species. Populations of some members of

section *Mesembrynia* (*P. glabrata, P. varia, P. hispida, P. debilis, P. bellidioides*) appear to show a polymorphism in flowering time (p. 185) but this does not occur in species from section *Oliganthos* (*P. paradoxa, P. triantha, P. daltonii*) or in the introduced species (*P. lanceolata, P. major* and *P. australis*).

In the field, flowers are produced during spring-summer (September-January) in all species, and seed-set occurs from late spring to the end of summer (November-February). It is probable that all species produce only one generation per year, although it may be possible for an early flowering phenotype to produce plants which set seed prior to the onset of winter. This aspect of the life cycle of the species requires further study.

In summary the chromosome numbers and breeding systems of species used in the experimental studies reported in this thesis are as follows:-

1. *P. paradoxa*: 2n = 48; protogynous. Scapes sessile at anthesis, plants self-compatible and probably self-fertilizing in most cases, but outcrossing can occur via water-borne pollen.
2. *P. glabrata*: 2n = 24; protogynous. Scapes elongate at anthesis, plants self-compatible, but also outbreeding and wind-pollinated. Self-pollination can occur by fertilization of flowers produced on younger inflorescences.
3. *P. antarctica, P. tasmanica, P. daltonii*: 2n = 12; breeding details similar to *P. glabrata*.

3. General Materials and Methods

Introduction

The work to be described involved some studies in which a number of the experimental and analytical techniques were the same. In order to avoid repetition, these general points are outlined here. They will not be mentioned specifically in the materials and methods sections of the individual experiments.

Techniques of Cultivation

(a) Seed Germination

Seeds were germinated routinely in the laboratory under ambient temperatures (20 ± 2°C) and lighting. The seeds were sown in lots of 25 or 50 onto germination pads in petri dishes. The pads were supported above a supply of water by filter paper wicks. In the case of *P. paradoxa* and *P. glabrata*, 90-100% germination of seeds occurred within two weeks. The sources of seeds used are given in the individual experiments.

(b) Transplants

Transplants were collected in the field and sealed into labelled plastic bags for removal to the glasshouse. The roots of the transplanted material were washed free of soil prior to planting. The transplants were given a period of from 6 to 8 weeks conditioning prior to the commencement of any experiment. After this time, the plants had produced a healthy 'crop' of new leaves and were considered to be equilibrated to the glasshouse environment.

(c) Cloned Material

Clones were obtained by cutting plants into 4 more or less equal sections and placing these in sand in fibre-glass trays under a misting machine. New leaves began to form within 2-3 weeks, and plants were usually well established after 8-10 weeks. The clones were then planted out into the selected growth medium and allowed to grow in the glasshouse for 4-6 weeks prior to use.

(d) Growing Conditions

Plants were grown in a 1:1 vermiculite-gravel mixture. They were watered once daily and supplied once weekly with a modified Hoagland's nutrient solution. The plants were grown either individually in 2.7 kg tin cans or grown together in plastic tote boxes. The number of plants per box varied, and is specified in each experiment.

Ambient temperatures in the glasshouse average from 23±4^{o}C in summer to 20±3^{o}C in winter. Unless otherwise stated, the plants were grown under natural daylight without supplementary lighting. Average daylength at Hobart is 15.5 hours in summer and 9 hours in winter.

(e) Daylength Studies

To study the effects of photoperiod, plants were placed on trucks in the Botany Department phytotron. The phytotron allows strict control of daylength under conditions of high light intensity at ambient temperature. Photoperiods used in the studies were 8 hours light (short days) or 24 hours light (long days). Some preliminary studies were conducted using

8, 16 and 24 hour daylengths, and these indicated that some of the responses to be reported are related quantitatively to daylength. The 24 hour daylength was used in the experiments reported here to maximize the difference in response between plants under the long and short photoperiods (*cf.* Murfet, 1977). During the daylight hours, plants under both photoperiods received natural light which was extended when necessary by banks of mixed fluorescent and incandescent lights.

(f) Temperature-Light Interactions

Experiments on temperature effects were conducted in growth cabinets designed within the Botany Department. These provide temperature control to within $\pm$ 0.5^{o}C, control of daylength and limited control of light intensity. The facilities are restricted since only small numbers of plants can be grown, light intensities are sub-optimal, and it is not possible to obtain independent diurnal control of both temperature and daylength.

Experimental Layout and Design

The amount of space available in the glasshouse is very limited at any one time. Therefore the experiments were designed to minimize the amount of space required. Wherever possible completely randomised designs were used. Inevitably, some deficiencies in design resulted from the need to compromise between the space available and the need for analytical efficiency.

In general, guard plants were not used in the glasshouse. The individual plants are rosettes, and except for temperature, all of the treatments (light duration, light intensity, submergence)

were imposed vertically, so that there was no between plant interference above ground, however, the possibility of edge effects remained. The plants were all grown in a homogeneous rooting medium and water and nutrient supply was non-limiting. When the plants were grown individually in cans, edge effects would be minimal. When the plants were grown in the phytotron, they were planted in tote boxes on, for example, a 5 x 4 spacing (i.e. 20 plants per box). The trucks are only wide enough to support one box, so that edge rows were impractical, otherwise *ca.* 70% of all plants grown would be guard plants. The same situation applied in the growth cabinets. However, the absence of guards increases the risk of a type II error in the subsequent analysis (Snedecor and Cochran, 1967), because within treatment variances will be increased if there are edge effects. It was thought preferable to accept this risk, rather than decrease the number of plants available for the estimation of errors in each experiment.

For much the same reason, clones were not replicated within treatments. In the glasshouse experiments on *P. glabrata* and *P. paradoxa*, the primary concern was to determine the averaged response of the species across genotypes to changes in the level of the different environmental factors. At this level of investigation, the response of individual genotypes within each species was not of major interest. Therefore a sampling of genotypes of each species were cloned and used in the different treatments. This means that essentially the same genotypes are subjected to the different treatments, but the response of each individual cannot be estimated.

Analysis of Results

Most of the analyses of variance (ANOVA) and all of the multivariate analyses were conducted using the GENSTAT programme sequence available on the CSIRO CYBER 76 computer. If missing values were present, calculated values were interpolated automatically into each treatment set by the computer. In cases where the number of replicates were uneven, the ANOVA's were calculated on a desk machine using a programme which makes a primary correction for non-orthogonality. Fixed effects (i.e. Model I) were assumed in all analyses, for reasons which are explained in the individual experiments.

One advantage of GENSTAT is that any out-lying values which may occur in the data set are indicated automatically. This situation occurred only once (Ch.12,p.231), and subsequent transformation of the data was necessary. In most other cases the data were not transformed. This may appear surprising because ratios of dimensions were used as the primary variables, and ratios might be expected to be distributed binomially. However, frequency histograms were plotted for many of the data sets and were found to be approximately normal so that angular transformations of the data were not appropriate.

Chromosome Counts

The chromosome counts were obtained from root tips (2n) or from pollen mother cells (n) at a magnification of x500.

1. Mitotic Divisions

Newly germinated seedlings were immersed in an aerated solution of colchicine (0.04%) for 2 hours at room temperature, and then given a 6-8 hour recovery period in aerated distilled water. The root-tips of the plants were excised and heated at $60^{o}C$ for five minutes in acid aceto-orcein. The root-tips were then macerated in aceto-orcein stain and examined under the microscope.

2. Meiotic Divisions

Chromosome counts were obtained from the pollen mother cells of plants grown in the glasshouse. Anthers were excised from young flowers, squashed in aceto-orcein and examined under the microscope.

4. *P. paradoxa* and *P. glabrata*: A Descriptive Background

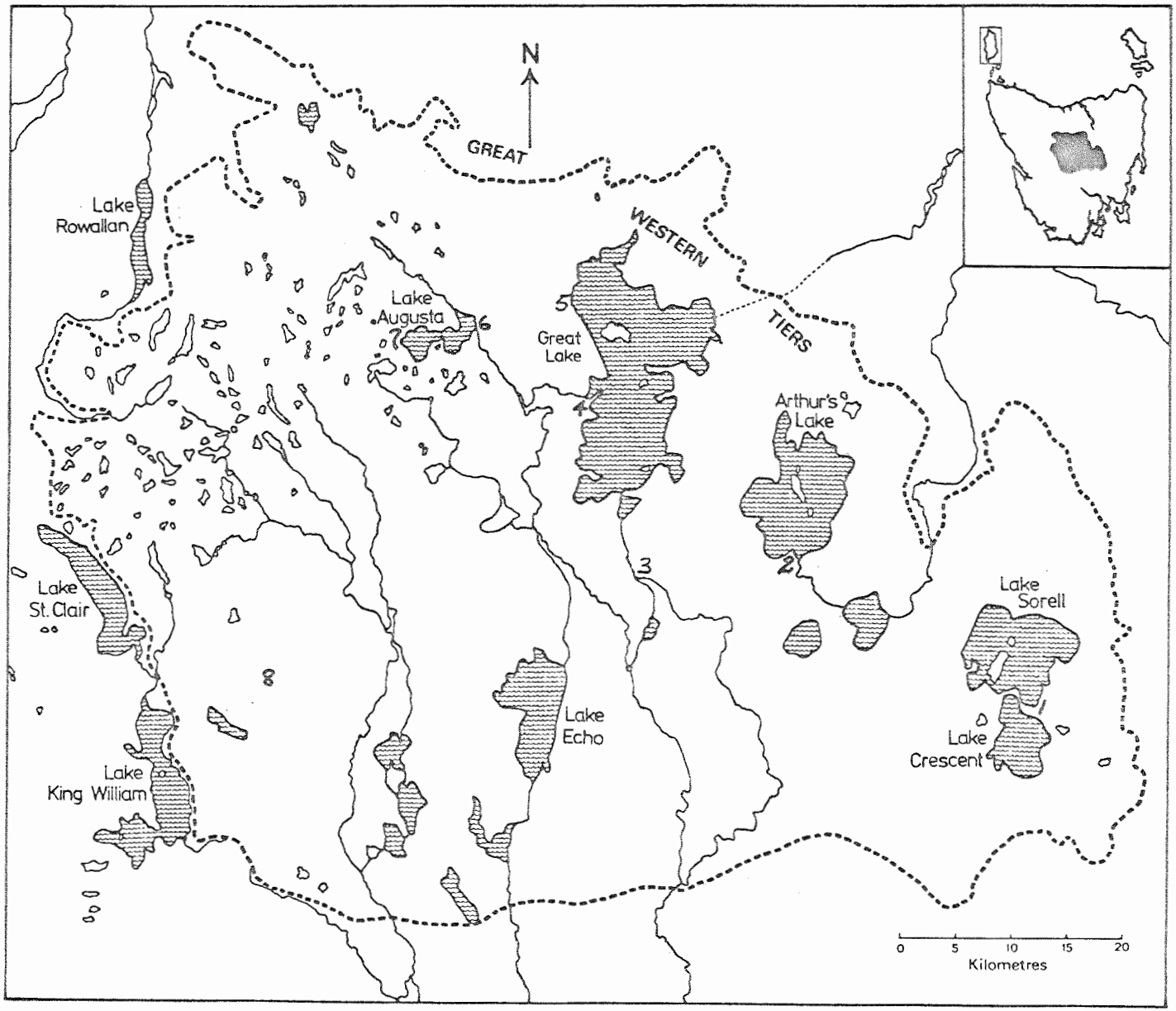
Introduction

Two species of *Plantago*, *P. paradoxa* and *P. glabrata*, occur separately and in admixture in the sub-alpine and alpine regions of Tasmania. The two species exhibit a high degree of variability in a number of characters (e.g. leaf-shape), both within and between populations. The species are perennial rosette herbs, and are readily recognised by their rootstocks and flowering scapes. However, the variability of leaf-shape exhibited by the species, even within apparently uniform habitats, makes them sometimes difficult to distinguish in the vegetative state.

The variation in leaf shape exhibited by the species is further complicated by seasonal variation. Observations over a number of years suggest that both species undergo seasonal alterations of shape, apparently converging during the winter months, but becoming divergent in summer. The co-occurrence of the taxa thus appears to provide a useful basis for a comparative study of their plastic and heritable responses when subjected to a similar range of macro- and micro-environmental changes.

The Study Area

The area chosen for study was the Central Plateau of Tasmania (Fig. 4.1), where the species occupy the grassland and herbfield associations bordering the many lakes and streams. The plateau is bounded approximately by latitudes 41.5°S and 42.4°S, and longitudes 146.0°E and 147.3°E.


N
GREAT
WESTERN
TIERS
Lake Rowallan
Lake Augusta
Great Lake
Arthur's Lake
Lake St. Clair
Lake King William
Lake Echo
Lake Sorell
Lake Crescent
1
2
3
4
5
6
0 5 10 15 20
Kilometres

Climate

Average annual rainfall varies from 700 mm to 2750 mm in a relatively short distance from east to west (Bur. Met. 1976). The rainfall is reliable, although droughts do occur. Snow and hail may occur in all months, but are most frequent in July-August and October-November respectively. Across the plateau, the average maximum monthly temperatures range from 4.3°C to 7.8°C in July and 15.2°C to 20.4°C in January. The corresponding minima are -1.8°C to 0.3°C in July and 4.7°C to 7.6°C in January. Frosts can occur in all months.

Geology

The plateau consists of two main nearly-horizontal layers of rock resting on a basement of older, steeply tilted rocks (Banks, 1972a). The surface layer is Jurassic dolerite, injected into sedimentary rocks about 165 million years ago. The basement consists of metamorphic, sedimentary and igneous rocks of varying age, intruded twice by granite, and folded into a mountain range about 370 million years ago.

Physiography

The plateau exhibits a marked NW-SE variation in altitude. The northern and western margins are generally above 1200 m, and the plateau grades to approximately 600 m in the south east. Three main surfaces are present (Banks, 1972). The St. Clair Surface lies between 750 and 825 m, the Lower Plateau Surface between 900 and 1050 m, with erosion residuals rising to 1200 m, and the Higher Plateau Surface, which rises from 1200 to 1420 m in the north west. The surfaces probably represent successive erosion levels separated in time by uplift phases.

The plateau is drained mainly to the south, but a few short rivers drain the western, northern and north-eastern rims.

Much of the western region was ice-covered and subject to glacial erosion during the last glaciation. The large number of lakes which dot the western half of the plateau derive from this period. The larger lakes to the east and south lie outside areas of known glaciation, and their origins are uncertain.

Soils

The plateau is characterised by alpine humus soils and moor peats (Nicolls, 1965). The alpine humus soils are associated specifically with periglacial solifluction deposits. These consist of dolerite boulders and fragments in an earthy matrix, which is coloured brown, and varies in texture from sand to clay, depending on the source. These soils typically show little development within a profile.

Large areas of the plateau show very little relief, and sites of impeded drainage which occur are characterised by the development of organic peats of varying depth.

Climatic Regimes of the Study Sites

The particular sites studied in the course of this work are shown in Fig. 4.1. The average annual rainfall is known for some of these sites, but there is no available temperature data. However, temperatures were available for eight other stations around the Central Plateau (Table 4.1), so these data were used to predict the temperatures of the study sites using a multiple regression technique. The altitude, and position of each station to the west and to the north of Interlaken (chosen arbitrarily as a base station), were known for all sites (Table4.2). These

values were used as independent variables and regressed against the temperature data. The regression equations obtained were as follows:

$$\text{Tmax (January)} = 25.805 - 0.009x_1 - 0.006x_2 - 0.003x_3,\ F(3,4) = 21.30^{**}$$

$$\text{Tmax (July)} = 12.843 - 0.008x_1 + 0.000x_2 - 0.011x_3,\ F(3,4) = 38.71^{**}$$

$$\text{Tmin (January)} = 9.607 - 0.004x_1 - 0.000x_2 - 0.001x_3,\ F(3,4) = 6.29$$

$$\text{Tmin (July)} = 1.693 - 0.003x_1 + 0.013x_2 - 0.011x_3,\ F(3,4) = 1.65$$

where x_1 = altitude (m), x_2 = km W of Interlaken, x_3 = km N of Interlaken

Only the first two of these regressions were significant,* so these equations were used to predict the average maximum temperatures in January and July at the study sites. Inspection of the temperature and rainfall data for these sites shows that there is a trend of increasing rainfall and decreasing temperatures in a westerly direction. However, the reduction in maximum temperatures appears to be associated most closely with altitude, whilst the increase in rainfall is associated more specifically with distance west of Interlaken. These relationships are consistent with my personal experience of the study area. The reasons for establishing the inter-relationships given here will become apparent in later sections of the study.

*The minimum temperatures cannot be predicted accurately in this way because of local effects of cold air drainage and "frost hollows".

Table 4.1. Physical and Climatic Data for Eight Stations on or Near the Central Plateau

Station	Altitude (m)	km W of Interlaken	km N of Interlaken	Ave. annual rainfall (mm)	Tmax Jan. (°C)	Tmax July (°C)	Tmin Jan. (°C)	Tmin July (°C)
Bronte Park	675.1	56.0	1.0	954	20.2	7.7	6.8	0.0
Cradle Valley	914.4	101.0	56.0	2774	17.2	4.6	6.3	0.1
Lake St. Clair	735.2	82.0	3.0	1514	18.3	7.6	7.1	1.1
Shannon	940.0	35.0	11.0	852	18.1	5.3	6.1	-1.1
Butler's Gorge	666.0	74.0	-14.0	1704	19.2	6.9	6.3	-0.2
Miena	1013.5	37.0	18.0	810	15.4	4.3	4.8	-1.9
Palmerston	176.8	14.7	41.5	838	23.8	11.0	8.5	0.3
Oatlands	432.2	-16.0	-17.5	567	22.2	9.4	8.5	1.3

Table 4.2. Physical and Climatic Data for the Eight Study Sites. (The temperature data have been predicted by multiple regression.)

Station	Altitude (m)	km W of Interlaken	km N of Interlaken	Ave. annual rainfall (mm)	Tmax Jan. (°C)	Tmax July (°C)
Interlaken	800	0.0	0.0	708	18.6	6.4
Arthurs Lake	951	20.5	12.5	775	17.1	5.1
Kannaleena	970	30.0	14.0	791	16.9	4.9
Canal Bay*	1020	38.2	27.5	c.900	16.3	4.4
Pine Creek	1034	41.0	35.5	988	16.1	4.3
Ouse River*	1151	50.0	33.0	c.1300	15.0	3.3
Lake Augusta*	1160	55.5	30.0	c.1400	14.9	3.2
Clarence Plains*	800	67.0	-1.5	c.1500	18.2	6.5

*Average annual rainfall at these sites has been interpolated from published rainfall contour maps (Bur. Met. 1976).

Habitats of the Species

The two species are widespread on the Central Plateau, and although they may occur together, some differences in their ecology are apparent.

P. paradoxa generally occupies wet and poorly drained sites, forming *P. paradoxa* closed herbfields, or occurring as a codominant species in *P. paradoxa - Ranunculus nanus - Velleia montana* closed herbfields. The species is also to be found as a colonizer in the early stages of degradation of bolster moor communities dominated by species such as *Abrotanella forsterioides* ("cushion plants").

P. glabrata usually occurs on higher ground, or sites less subject to flooding. It is found as a major intertussock herb in *Poa gunnii* or *Poa billardieri - Poa gunnii* grasslands, or forms part of the ground layer under predominantly Proteaceae - Epacridaceae shrubland communities. The species may also occur within sedgeland communities dominated by open growing sclerophyllous monocotyledons such as *Juncus pallidus*.

The two species occur in admixture on low lying grasslands near lake shores, forming *P. glabrata - P. paradoxa - Velleia montana* closed herbfields. Both are also common at lower altitudes on the plateau in areas sown with introduced pasture species for stock grazing.

At many of the lakeside areas on the Central Plateau, the herbfield, grassland and shrubland communities are contiguous. Thus in moving away from a lake shore, there is gradation from pure *P. paradoxa* through an admixed region to *P. glabrata* in the absence of *P. paradoxa*.

5. The Seasonal Variation in Leaf-Shape of *P. glabrata* and *P. paradoxa*

Introduction

Previous observations had indicated that the two species have very similar leaf-shapes in winter, although the form of their leaves is quite distinct in summer (Fig. 5.1). This effect might reasonably be supposed to be of adaptive advantage to the species. The plasticity might be determined directly by the environment, e.g. low temperatures could differentially limit cell enlargement or division in the two species, resulting in a convergence of shape. However, the effect might also be the resultant of indirect environmental control, such that metabolic pathways in the two species are switched on or off enabling them to overwinter in a (common) favourable state.

Before any assessment of these alternatives could be made, several problems had to be resolved:-

1. A suitable means of quantifying leaf-shape had to be found and the developmental pattern of leaf-shape variation in the species had to be ascertained.
2. It was necessary to demonstrate objectively the subjective impression of convergence in leaf-shape in the two species.
3. It was also necessary to evaluate the relative degree of plastic response of the two species and whether the response resulted from genotype-environment interactions.

It was decided to use the multivariate techniques of principal components analysis (PCA) and canonical variates analysis (CVA) (see pp.271-274). These techniques were used to quantify the various

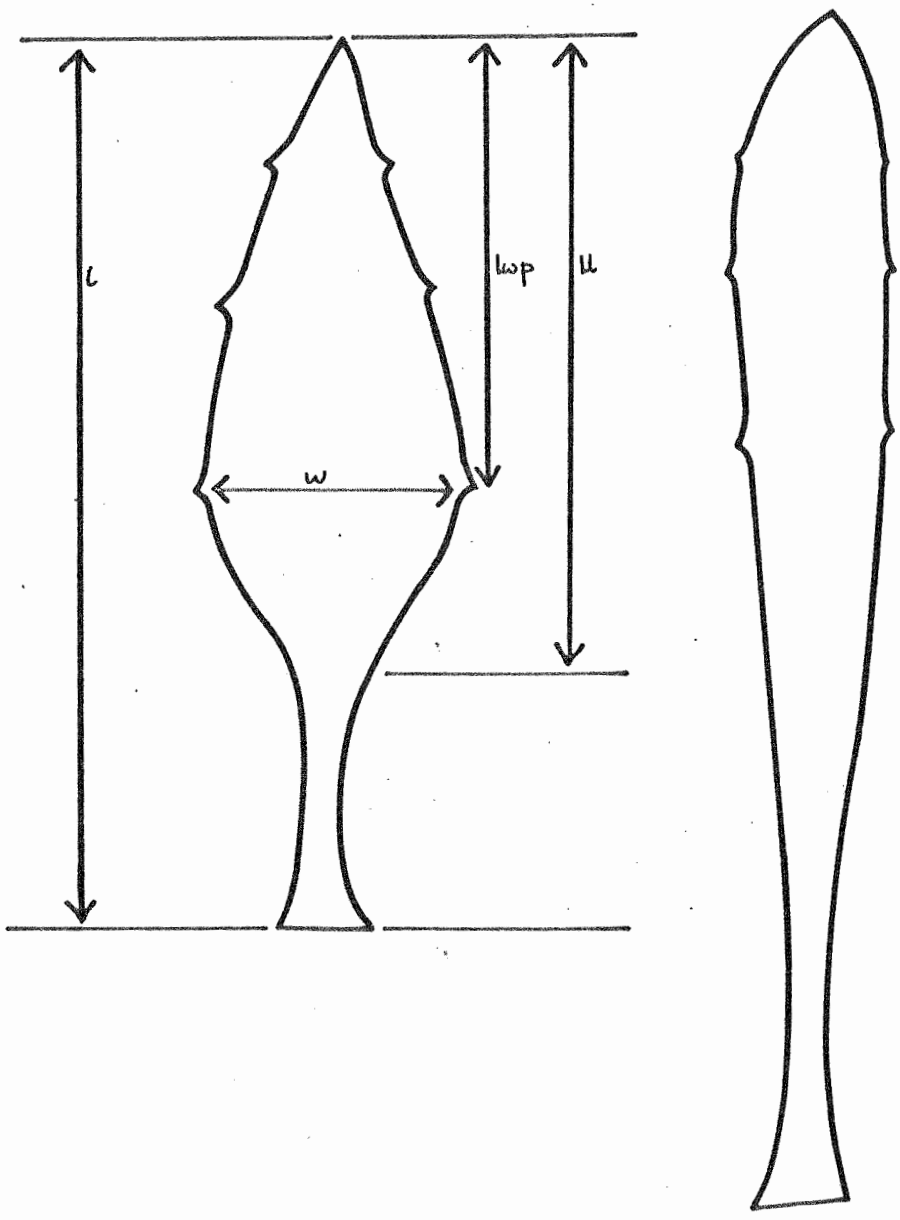


Fig. 5.1. Summer leaf shapes of *P. paradoxa* (left) and *P. glabrata* (right). The variables measured were length (*l*), length at widest part (*lwp*), lamina length (*ll*), and width (*w*).

aspects of leaf-shape so that they could be partitioned and assessed independently. The multivariate analytical method could then be utilized in transplant studies, controlled growth studies and heritability tests to disentangle the complex of correlated environmental and genetic factors expected to exert control over the observed variation.

Preliminary Studies

Variation in the Species During Leaf-Expansion

The aim of the study was to examine the effects of season on the leaf-shape of the two species. Such an analysis would be of limited value if the variability of leaf-shape within plants grown in a common environment outweighed that observed between plants. Some consideration must therefore be given to the developmental changes occurring in the growth patterns of the two species during leaf-expansion.

The present experiment was conducted to examine this facet of within plant variation, and at the same time to illustrate the efficiency with which the multivariate techniques of principal components and canonical variates discriminate leaf-shape in the species.

Materials and Methods

A total of 28 plants of each species were collected at random from a range of sites on the Central Plateau. The plants were transplanted into boxes and grown in the glasshouse (p. 45). After 8-10 weeks conditioning, a new crop of (10-29) leaves had formed on each plant. These new leaves were stripped sequentially from the oldest to the youngest (unexpanded) leaf, and each leaf

was scored for the four characters: leaf length (*l*), length from apex to widest part (*lwp*), length of lamina (*ll*) and maximum width (*w*).

These measurements were used to calculate the principal components for each of the 56 plants, based on the correlation matrices among the variables. The correlation matrices for 8 of the plants are given in Appendix C.1 , Table 1 (p. 287).

To illustrate the type of result obtained in these analyses, four plants of each species have been selected subjectively to represent the extremes of leaf-shape encountered within each species. The *P. glabrata* plants were classified as 'long-broad', 'short-broad', 'long-narrow' and'short-narrow' on a basis of the mature leaf-shape of each plant. The *P. paradoxa* plants were classified as either 'narrow' or 'broad' because the relative lengths of their leaves were not as variable as those of *P. glabrata*.

To illustrate the way in which multivariate techniques can be used to classify differences of leaf-shape, the data from the same eight plants were pooled and analysed by principal components (PCA) and by canonical variates (CVA). The PCA was based on the within 'groups' (i.e. plants) correlation matrix. The between (B) and within (W) plants sums of squares and products matrices (SSP) were calculated and used to derive canonical variates.

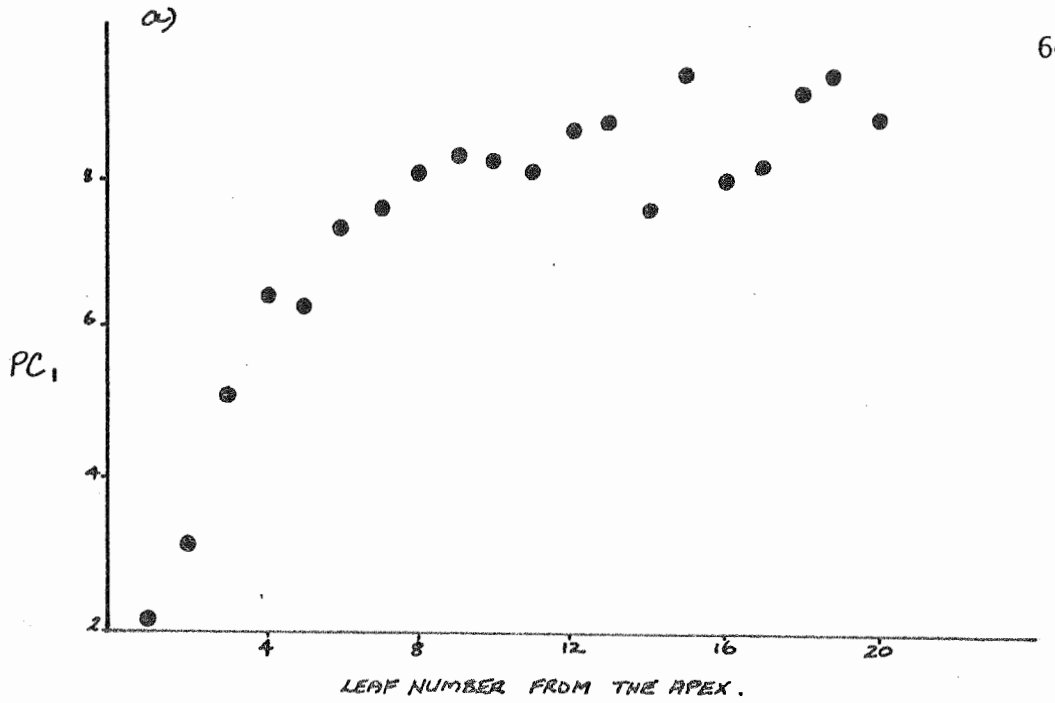
Results and Discussion

1. The Variation of Each Plant During Leaf-Expansion

The coefficients of the first principal component, and the percentage of the total variation taken up for each of the 56 plants are given in Table 2 of Appendix C.1 (p.288-289). In every case, the PCA within individuals resulted in the first principal component

taking up over 90% of the total variation. In *P. paradoxa*, the mean percentage of variation is 96.9% with a range of 90.7-99.2%. The corresponding values for *P. glabrata* are 96.9% and 92.1-99.3%. The hypothesis that this first component corresponds to equal relative growth among the measured dimensions (i.e. increase in 'leaf-size') would require a vector having elements equal to $1/\sqrt{p}$, where p is the number of variables (Joliceur, 1963). In the present case $p = 4$, so that the required vector is (0.5, 0.5, 0.5, 0.5). The elements of the first principal component obtained for every one of the 28 plants of the two species are very close to this value (pp.288-289). Thus the major developmental pattern occurring in all plants appears to be one of allometric increase in the size of the leaves.

Some examples of the way in which the scores of individual leaves along the first principal component change with leaf-number from the apex are presented in Figs. 5.2 and 5.3 . In each of these examples, there is a steady increase in the 'size' of the leaves of a plant as they expand, until they mature. Thereafter the response differs from plant to plant. The older leaves of three of the four *P. glabrata* plants fluctuate about an average value, whilst the oldest leaves of the fourth plant show a sequential reduction in size. The 'narrow' phenotypes of *P. paradoxa* show a steady increase in the first principal component to maturity, but insufficient leaves had grown to specify their subsequent response. The leaves of the two 'broad' phenotypes of *P. paradoxa* increase in size to maturity, and there is some evidence of a slight reduction in size thereafter.



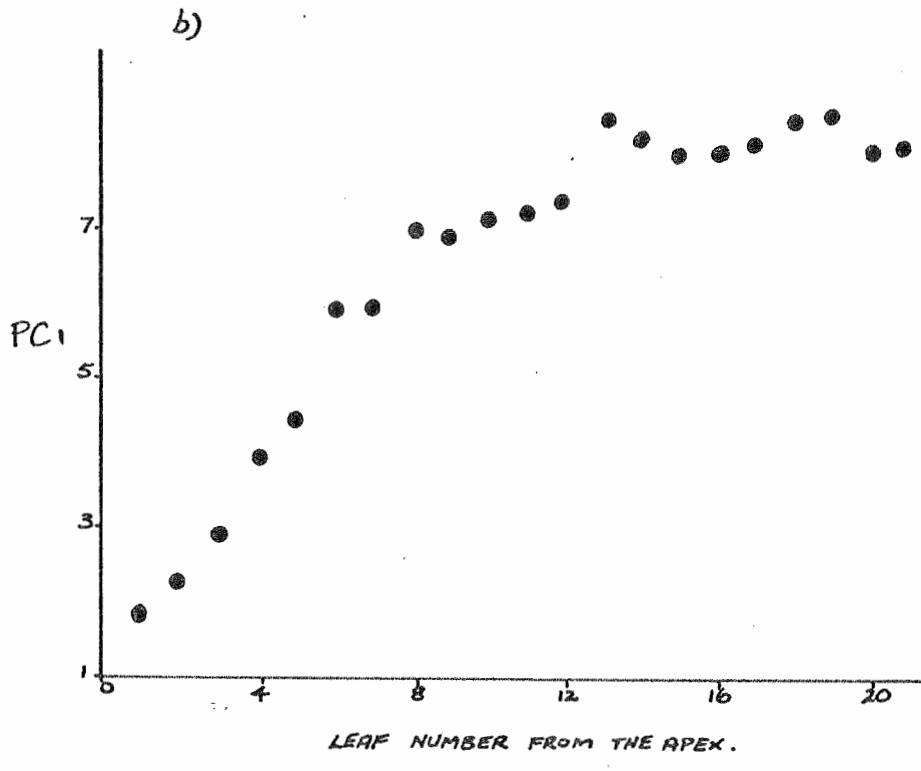


Fig. 5.2(a). The variation in leaf size with position of insertion in narrow leaf phenotypes of *P. glabrata*.
(a) Short, narrow leaf phenotype.
(b) Long, narrow leaf phenotype.

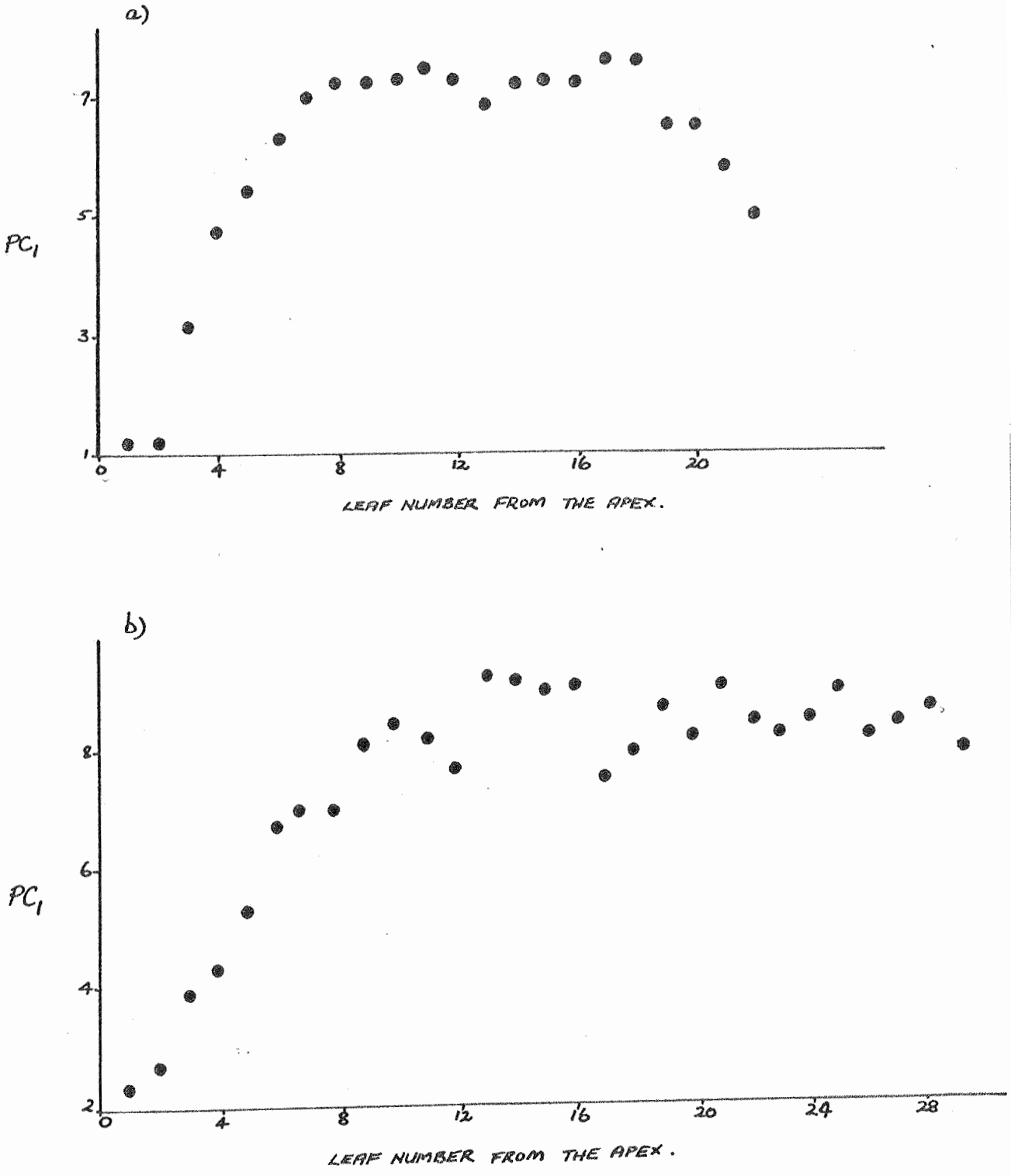


Fig. 5.2(b). The variation in leaf size with position of insertion in the broad leaf phenotypes of *P. glabrata*.
(a) Short, broad leaf type.
(b) Long, broad leaf type.

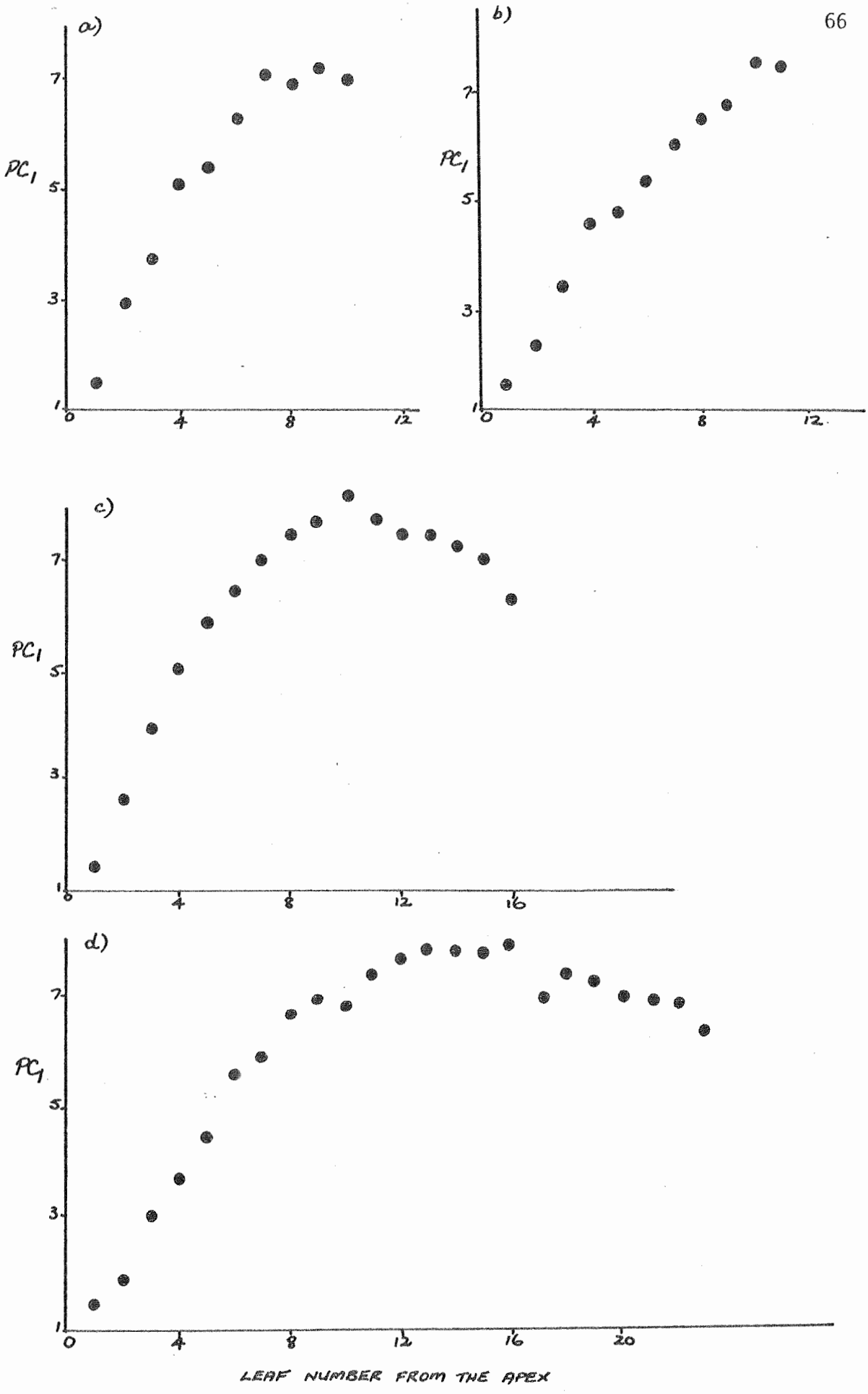


Fig. 5.3. The variation in leaf size with position of insertion in *P. paradoxa*.
(a) and (b) are narrow leaf phenotypes.
(c) and (d) are broad leaf phenotypes.

The remaining principal components were found to differ among the 56 plants and were largely specific for each individual(e.g.Table 3,pp.290-291). An examination was made of the plots of these components against leaf-number, but the variation they encompassed could not be ascribed readily to a factor such as position of leaf-insertion on the stem. These components might indicate possible genetically controlled canalization of growth, resulting in distinctive shape patterns of the leaves, which are specific for each genotype. However, they may also be attributable to 'developmental noise', i.e. more or less immediate response to difference in microenvironmental fluctuations, or to measurement error. The resolution of these alternatives would require the estimation of the heritability of the lesser components; this was beyond the scope of the present work.

2. Classification of the Eight Plants by PCA and CVA

(a) Principal Components

The PCA resulted in four axes (Appendix C.1, Table 4, p. 292) each of which has a fairly straightforward biological interpretation. The first component takes up 91% of the variation and is attributable to size, i.e. equal relative increase among the dimensions. The second vector resolves in a contrast of width with the other characters, i.e. differential growth along the length and breadth axes of the leaf. The third vector contrasts *lwp* with *l* and *ll*, i.e. represents differential growth along the long axis of the leaf. The fourth vector contrasts *l* and *ll*, and thus provides a measure of the relative petiole lengths of the leaves.

While no single axis clearly discriminates between the *P. glabrata* and *P. paradoxa* plants, the mean vectors of the eight plants are readily differentiated in the space defined

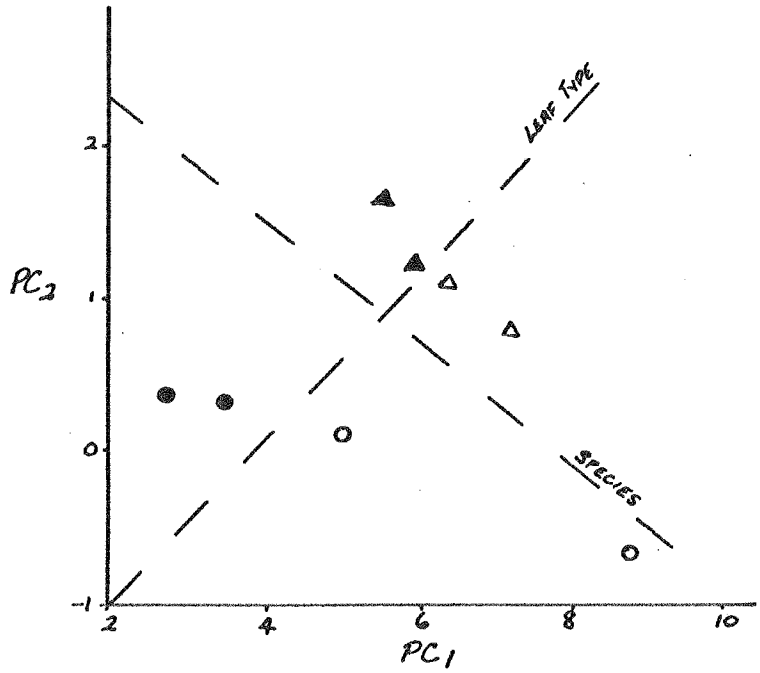


Fig. 5.4. PCA of the eight plants, based on the within group correlation matrix. Arbitrary axes have been superimposed. ○ *P. glabrata*, narrow leaf form; ● *P. paradoxa*, narrow. △ *P. glabrata*, broad leaf form; ▲ *P. paradoxa*, broad.

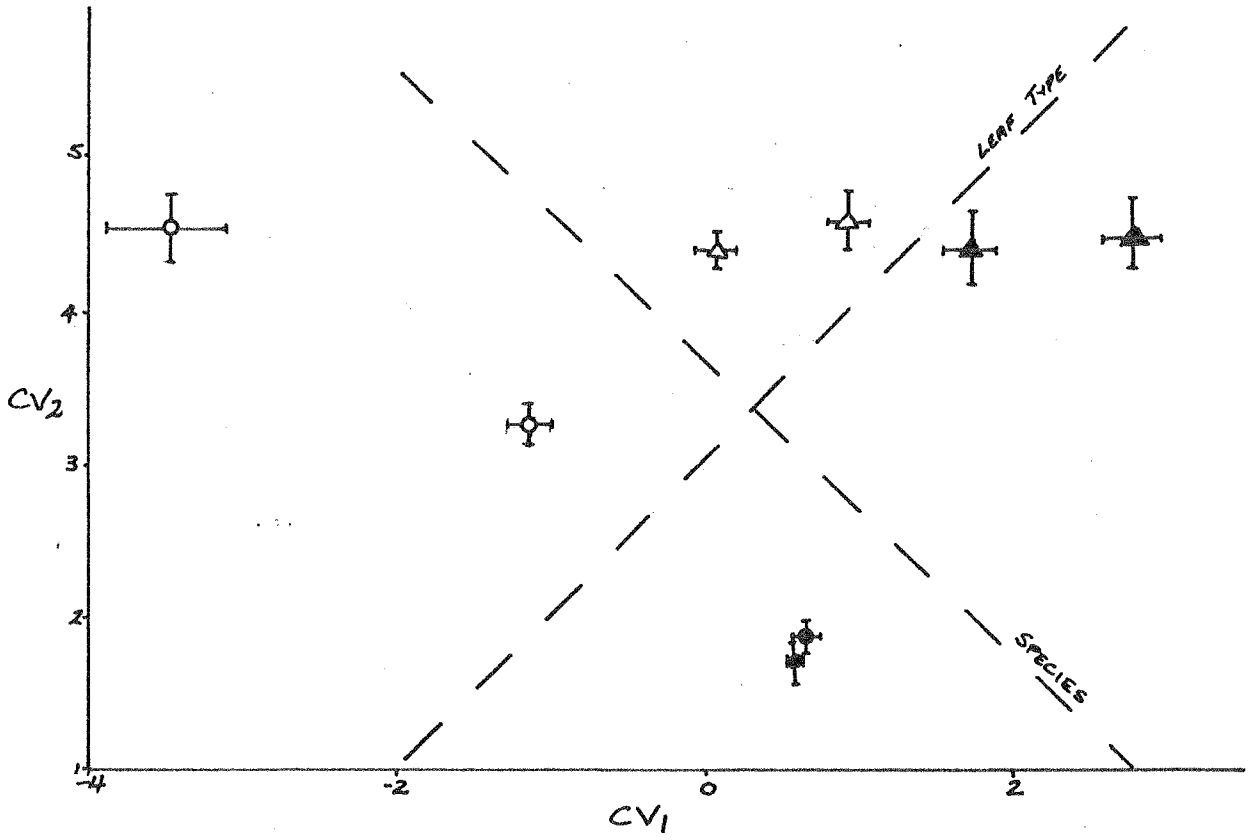


Fig. 5.5. CVA of the eight plants; the coding of phenotypes is as given for Fig. 5.4.

by any two of them (Fig. 5.4). Thus, if arbitrary axes are superimposed, the mean values are divided by species on one axis, and by broad and narrow leaf phenotypes on the second.

(b) Canonical Variates

A similar situation prevails after CVA (Appendix C.1, Table 5, p. 293). The space defined by the first two canonical axes clearly differentiates the two species and the narrow- and broad-leaf-types (Fig. 5.5). Furthermore, the analysis shows that, with the exception of the two narrow-leaf *P. paradoxa* plants, all the plants are phenotypically distinct. The first canonical variate is predominantly a contrast of *ll* and *w*, with a lesser contribution from *lwp*. The second variate contrasts *l* and *ll* and thus provides a measure of the relative petiole lengths of the plants. The contrast is much more marked in *P. paradoxa* than *P. glabrata*.

Conclusions

The results obtained from the 56 plants indicate that the major aspect of variation in the leaves of both species during expansion is related to a progressive enlargement of the leaves. There did not appear to be any change of shape associated with the expansion of leaves. In the experiments which follow, only mature, recently-expanded leaves were used, and it can be assumed that any minor differences of shape arising from measurement error or developmental noise are randomized within each treatment class.

The results of the multivariate analyses of the differences of leaf-shape between the eight plants have little biological significance because the sample size was so small. However, the

example shows that the multivariate methods provide an adequate means of assessing differences in the shape of leaves between plants. The examples also demonstrate in a simple manner, the way in which the vectors which result from PCA or CVA are ascribed biological meaning (i.e. 'reification' (Marriott, 1974)) in this thesis.

The Relationship Between Fresh and Pressed Leaves of *P. paradoxa* and *P. glabrata*

Introduction

In the studies which follow, it was not always possible to take the measurements immediately, so that a means of preserving the form of the individuals was required. This section reports on the degree to which changes in dimension occur between fresh and pressed leaves of *P. glabrata* and *P. paradoxa*.

Materials and Methods

A single leaf was collected from each of 15 plants of *P. paradoxa* and *P. glabrata*. The collections were made from plants growing in their usual habitats at Interlaken on the Central Plateau. The leaves were sealed in plastic bags with a small amount of water and transferred to the laboratory. Measurements of the four leaf dimensions, length (l), length to widest part (lwp), width at widest part (w) and lamina length (ll) were made on each leaf using 1 mm graph paper.

The leaves were numbered consecutively, laid flat on paper hand-towels between sheets of newspaper and pressed in a book-binders press. The leaves were re-measured after one week.

The data obtained were used in several ways:-

1. The two sets of measurements for each species were used to calculate paired t tests for each character.
2. Individual principal components analyses (PCA) of the four data sets were carried out to determine whether or not the relationships among leaf-forms are consistent with each species when pressed or fresh leaves are used.

3. A discriminant analysis was made of the differences of leaf-form which occur between species when fresh leaves are measured. A second analysis was made using pressed leaves. The multivariate distance (D^2) between fresh and pressed leaves within species was also calculated.

Results

Inspection of the mean values (Appendix C.1, Table 6, p. 295) indicates that some shrinkage occurs on pressing. The paired t tests show that in most cases the shrinkage is non-significant. However, the total lengths and lamina lengths of individual leaves of *P. paradoxa* are reduced significantly on pressing.

The correlation matrices and results of the PCA of leaf measurements made on fresh and pressed leaves of each species are given in Tables 7 and 8 of Appendix C.1 (pp. 295, 296). In each species, the four principal components have equivalent interpretations in both pressed and fresh leaves, and each component accounts for about the same amount of the total variation. For example, in both species the first principal component is a vector representing equi-dimensional proportions within both pressed and fresh leaves. In *P. glabrata* this first component accounts for about 77% and 79% of the total variation in fresh and pressed leaves respectively; in *P. paradoxa* the proportion is 80% in each case.

The second axis contrasts *lwp* with *w* in both sets of leaves from *P. glabrata*; in *P. paradoxa* the second axis is weighted mainly by *w* in both pressed and fresh leaves. Thus the interpretations of the major aspects of variation of form of leaves in the two species is the same, irrespective of whether measurements are made on fresh or pressed leaves.

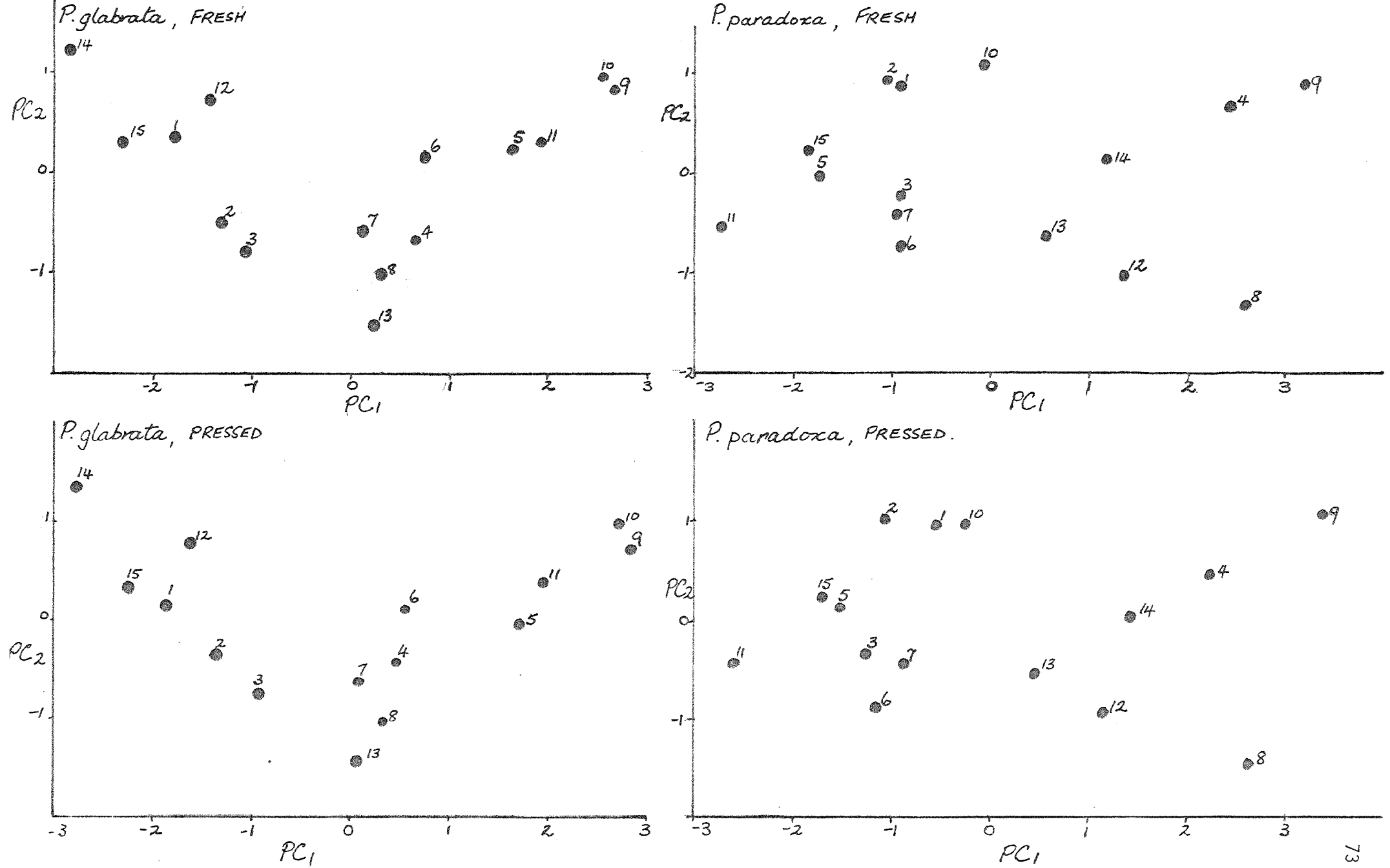


Fig. 5.6. Principal component ordinations of fresh and pressed leaves of *P. paradoxa* and *P. glabrata*.

The positions of each individual on the first and second principal component axes are plotted in Fig. 5.6. There are some minor variations within each species, but the relative dispositions of pressed leaves are very similar to those found within fresh leaves.

The discriminant analyses (Table 9 of Appendix C.1 , p. 297) show that there are significant differences of leaf-form between *P. paradoxa* and *P. glabrata* (D^2 fresh = 5.12***, D^2 pressed = 5.40***). However, there is no significant difference in the form of pressed and fresh leaves within each species (D^2 *P. paradoxa* = 0.34 n.s.; D^2 *P. glabrata* = 0.11 n.s.). The discriminant function obtained from the analysis of the data from fresh leaves has coefficients which are similar to those obtained from the measurements of pressed leaves. The joint regression of the discriminant scores obtained in each case is highly significant ($F_{1,28}$ = 999.54***), i.e. there is a high correlation (r = 0.986) between the discriminant scores of leaves of the two species when measured fresh and after pressing.

Discussion

The results indicate that some shrinkage of leaves does occur when leaves are pressed in the way described above. However, this shrinkage was discounted in further studies because:-

1. The overall leaf dimensions within each species are largely unaltered.
2. There is an overall similarity of form of individual leaves within each species when compared with its fellows.
3. The interspecific differences of form are maintained on pressing.

The method described above for the pressing of leaves was used whenever it was not possible to score leaves immediately. Within any one experiment all of the leaves were either fresh or pressed, i.e. each experiment was internally consistent.

5.2 Seasonal Convergence of Leaf Shape in a Common Environment

Introduction

At most locations there was considerable variation in leaf-shape in both *P. paradoxa* and *P. glabrata* and this variation appeared to be correlated with different microhabitats. The object of the present study was to examine the seasonal variation of leaf-shape in the species. Accordingly, a study site was selected in which the two species were intermixed, thereby enabling the seasonal aspects of the variation (both within and between the species) to be examined free of effects due to the microhabitat.

Materials and Methods

A quadrat, $1m^2$ in area, was marked out at Arthur's Lake (Altitude 951 m) on a site where the species were growing in admixture (Table 5.2., p. 84). Twenty plants of each species in the quadrat were selected for measurement. The same plants were located at each sampling time by assigned coordinates on the quadrat. The plants were sampled once each season during 1972. At each sampling the youngest fully expanded leaf was removed and pressed for later measurement. The leaf characters measured were length *l*, length of widest part *lwp*, and width *w*. The overall morphological separation between the species at the different periods of time were estimated by the calculation of the Mahalanobis Distance, D^2 (Rao, 1952). An analysis by principal components (PCA) was undertaken to obtain uncorrelated sets of variables with which to examine the seasonal changes of leaf-shape in the two species. Since the variances of the original characters differed widely (Appendix C.2, Table 1 , p. 298),the PCA was performed on the pooled covariance matrix constructed from the

logs of the original characters.

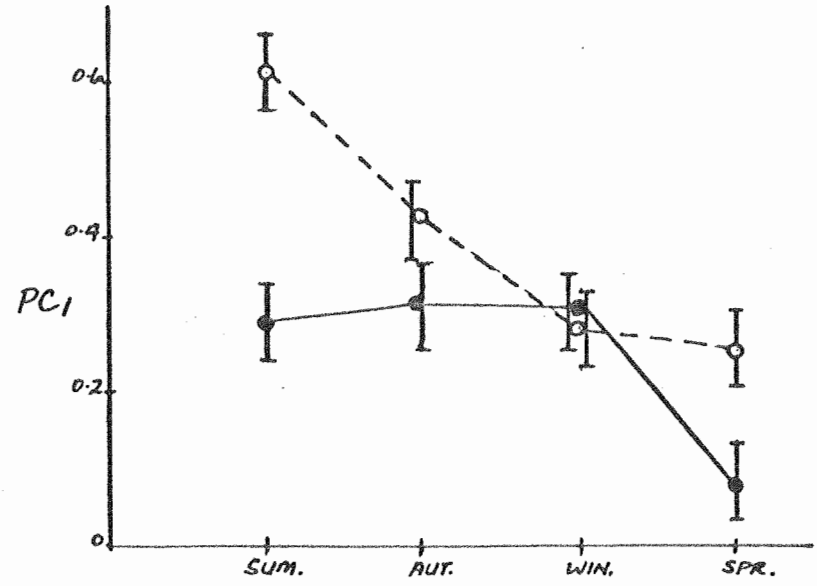
Results

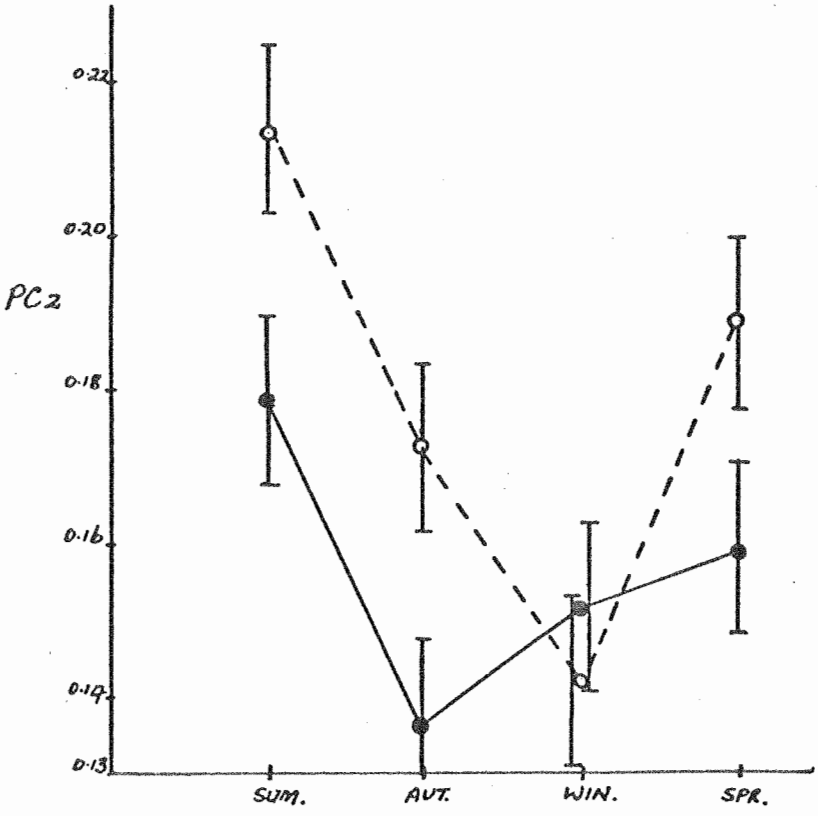
The means and matrices of pooled covariance between species pairs for the three leaf characters at each sampling are given in Table 2, Appendix C.2. The matrices were assessed for equivalence, and found to be homogeneous ($X_{18}^2 = 18.73$, $0.5 > P > 0.3$). Table 5.1 gives the D^2 values and corresponding variance ratios obtained for each season. It is apparent that the D^2 values are significant in summer and spring, but are non-significant in winter.

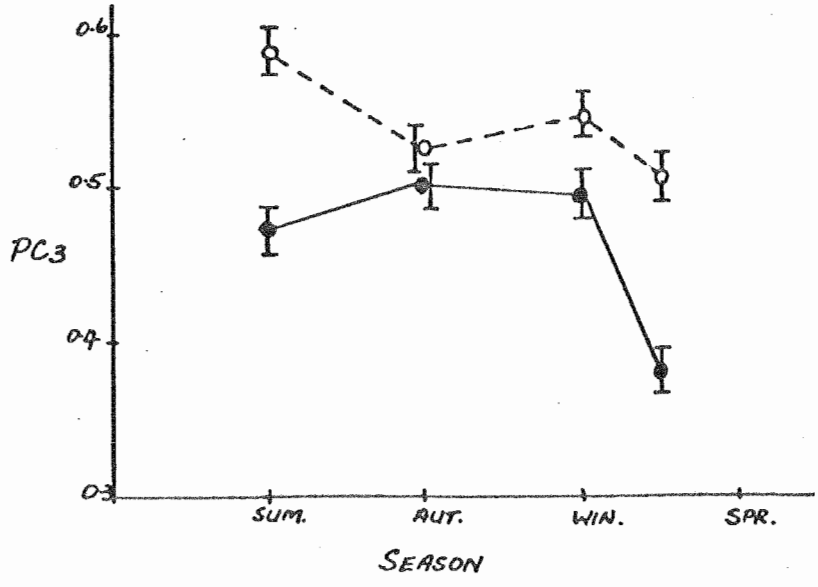
Table 5.1. D^2 values obtained between the two species growing in admixture at Arthur's Lake.

Season	D^2	Variance Ratio	Significance Level
Summer	1.95	6.24	0.01
Autumn	0.57	1.18	NS
Winter	0.51	1.64	NS
Spring	2.21	7.07	0.001

The PCA resulted in three eigenvalues, each of which is distinct. Inspection of the associated eigenvectors reveals that the three components have relatively simple structures. The first component contains eigenvectors which are all positive, and of equivalent magnitude, suggesting that it corresponds to an equi-dimensional increase of the original variables. This is interpreted as a measure of size. The second principal component contrasts *lwp* with *l* and *w*, whilst the third is a contrast of *l* and *w*, the coefficient for *lwp* being relatively small. Plots of the transformed mean vectors of the samples are presented in Fig. 5.7. These graphs demonstrate


0·4
0·2
0
PC1
SUM.
AUT.
WIN.
SPR.


0·22
0·20
0·18
0·16
0·14
0·13
PC2
SUM.
AUT.
WIN.
SPR.


0·6
0·5
0·4
0·3
PC3
SUM.
AUT.
WIN.
SPR.
SEASON

the marked convergence in both size and the two components of leaf-shape for the two species during the winter months.

Discussion

The two multivariate methods indicate that in winter there is a convergence of leaf-form in the two species. Inspection of Fig. 5.7 shows that the convergence results from seasonal changes in the size and shape of leaves of both species. However, the plastic responses in the two species become manifest at different times. Thus *P. paradoxa* leaves are stable in size (PC1) and the width-length contrast (PC3) from summer to winter, but have changed by spring. In *P. glabrata* the predominant changes in these characters occur between summer and autumn. Both species exhibit the greatest change in the *lwp* component of shape (PC2) during summer to autumn; *P. paradoxa* remains stable thereafter. The trend observed for *P. glabrata* characterises the subjective impression of the cyclic nature of the seasonal shifts in leaf-shape.

The convergence of leaf-shape in the species involves seasonal changes in both the relative position of *lwp* and of *w* concomitant with a reduction in the total leaf length. Thus *lwp* must move "up" the leaf in *P. paradoxa* and "down" the leaf in *P. glabrata*, and both species become relatively narrower, i.e. the ovate leaves of *P. paradoxa*, and the oblanceolate-obovate leaves of *P. glabrata*, which are present on the plants in summer, are replaced by linear-elliptic leaves in winter. The value of the multivariate approach, and in particular the PCA of the data, lies in the ability of the technique to give uncorrelated sets of variables for further examination.

Only relatively simple contrasts of shape were involved in the present study. If the effects of season on the overall size

of leaves are ignored, then an analysis using the ratios *w/l* and *lwp/l* would have yielded essentially the same results as those obtained from the PCA. The equivalence of the two ways of analysing the data is implicit in the independence of the two components of shape extracted by the PCA. The two components are of the form:

$x_1 \log l - x_2 \log lwp + x_3 \log w$, where x_3 is small

and $-y_1 \log l + y_2 \log lwp + y_3 \log w$, where y_2 is small

The first of these components contrasts *l* with *lwp* (i.e. $\simeq$ *lwp/l*), the second component contrasts *l* with *w* (i.e. $\simeq$ *w/l*) and the two components are independent.

However, the PCA has several advantages over a series of univariate analyses. Firstly, the PCA more accurately describes the inter-relationships among the variables. Also, the PCA has removed the correlations from among the variables, and so demonstrated that more complex changes of shape are not involved. For example, there is no *a priori* reason why the leaves of the species could not have shown a coordinated change in both *lwp* and *w* relative to leaf-length in response to seasonal change.

In the present study, the two characteristics have been shown to be independent. Thus,in both species,significant changes in the value of each character occur in different seasons. This fact suggests three alternatives: the two characteristics are responding to different environmental variables, the responses are elicited by different levels of a particular environmental factor, or both of these mechanisms are operating. Whilst the experiment does not distinguish between these alternatives, the PCA has provided a useful means of generating hypotheses about the nature of the seasonal convergence and has freed the investigation from the

problems of covariation among the original variables.

One further point has arisen from this study, and from the preliminary studies (pp. 61-75). In every case when the raw data or their logarithms are used as variables in the analyses, the major proportion of the resulting variation can be ascribed to differences in the size of leaves. Furthermore, inspection of the (independent) minor components shows that they usually describe aspects of shape which involve a contrast of the largest dimension (*l*) with the lesser dimensions. Thus size effects can be precluded by using the ratios of the lesser dimensions to *l* as characters for analysis. This procedure is adopted in the remaining studies of the seasonal response of leaf-shape in the two species. For ease of comparison, the ratios are converted to percentages. Frequency distributions have been plotted for many of the within treatment groups, and in every case are near-normal. Therefore it is not necessary to make angular transformations of the data.

The aim of the following experiments is to discriminate between grouped sets of data, so that analysis by canonical variates (CVA) is preferable to PCA. However, unless differences of size of leaves are removed from the analysis, the technique of CVA will confound aspects of size and shape in achieving a maximum discrimination.

5.3 The Seasonal Variation in Leaf-Shape of *P. paradoxa* and *P. glabrata* from Different Habitats

Introduction

The convergence of leaf-shape of the two species in winter is pronounced in situations where they grow in admixture. However, the convergence may occur only in the particular type of habitat studied or it may be a more general phenomenon. The response could be due solely to a local environmental fluctuation such as temporary flooding, or it may be a more general response of both species to harsh winter conditions, as might be expected from, for example, a temperature-induced reduction in metabolic rates. The response might also involve pre-adaptive mechanisms triggered by some other cyclic event such as photoperiod.

The convergence could result from the interaction of particular genotypes with the environment. The regions of admixture are ecotonal between the typical 'wet' communities containing *P. paradoxa*, and the drier sites occupied by *P. glabrata*, and in the ecotone both species may be occurring at the boundaries of their respective ecological tolerances. The observed plasticity could therefore reflect the general adaptive strategies adopted by the species in extending their range. However, the admixed plants also might be genetically different from the main body of the population, so that the observed response is restricted to only those genotypes capable of colonizing the marginal areas.

In the previous experiment (pp. 76-82), the probability of genotype reduplication in the sense of Harberd (1957) is very high since such a small area was sampled. This applies especially to *P. paradoxa*, in which the possibilities for out-crossing are more limited. The aim of the present experiment was to conduct a

Table 5.2. Site Descriptions of *Plantago* Plots.

Plot No.	Species	Locality	Vegetative Association* and Site Description
Admixed plot	*P. paradoxa* + *P. glabrata*	Arthur's Lake (A.L.)	*P. glabrata-P. paradoxa-Velleia montana* closed herbfield; plants well drained, unshaded, with ground cover 100%.
1	*P. paradoxa*	A.L.	*P. paradoxa* closed herbfield; plants unshaded in low lying depression, frequently flooded.
2	*P. paradoxa*	Kannaleena (Kan.)	*P. paradoxa-Ranunculus nanus-Velleia montana* closed herbfield; plants unshaded, in depression.
3	*P. paradoxa*	Pine Creek (P.C.)	*P. paradoxa* closed herbfield; plants on relatively high ground but periodically inundated by creek.
4	*P. glabrata*	A.L.	*Juncus pallidus* sedgeland; plants shaded and subject to periodic flooding.
5	*P. glabrata*	A.L.	*Poa gunnii* closed grassland; plants unshaded and well drained.
6	*P. glabrata*	A.L.	*Olearia algida* low open shrubland; cover 10%, plants on bare ground with little other herbaceous ground cover.
7	*P. glabrata*	Kan.	*Poa billardieri-P. gunnii* closed grassland; plants well drained, partially shaded by tussocks.
8	*P. glabrata*	Kan.	*Poa gunnii* closed grassland; plants well drained and unshaded.
9	*P. glabrata*	P.C.	*Poa billardieri-P. gunnii* grassland; plants occur on bare ground as intertussock herbs, unshaded, drainage free.
10	*P. glabrata*	P.C.	*Richea acerosa-Olearia algida* low shrubland, plants shaded, well drained.

*The vegetation associations given are after Jackson (1972, 1974).

comparative study of the seasonal variation of leaf-shape in the species when they occupy different habitats, and at the same time to examine whether there are phenotypic differences to be observed in the shapes of leaves within each species at different locations.

Materials and Methods

1. Field Data

Permanent plots containing each species were marked out at three locations: Arthur's Lake, Kannaleena and Pine Creek (Fig. 4.1, p. 53). Arthur's Lake and Kannaleena have fairly similar climates (Table 4.2, p. 57), but Pine Creek is colder and receives more rainfall.

The habitats occupied by *P. paradoxa* at each of the locations were very uniform, so that only one plot containing this species was located within each area. The habitats of *P. glabrata* were more heterogeneous (Table 5.2). Three plots containing this species were located at Arthur's Lake, and two at each of Kannaleena and Pine Creek, to give seven plots of *P. glabrata* in all.

The sampling procedure was carried out during 1972 in the same way as described for the admixed plot (p. 76), except that 15 plants per quadrat were measured, and a fourth variable, lamina length *ll* was measured on each leaf. The experiment was discontinued after one year because of damage to the plots by vandals.

2. Data Analysis

(a) Intraspecific variation in leaf-shape

Due to the unbalanced nature of the experiment (7 plots of *P. glabrata* and only 3 of *P. paradoxa*) it was not possible to break down the analysis into species, site, habitat within site, season and the various interactions. In order to examine the

intraspecific variation in leaf-shape, univariate analyses of variance (ANOVA) were conducted on the characters *lwp/l*, *w/l* and *ll/l* for each species separately. The frequency distributions of these ratios were approximately normal, so it was not considered necessary to make angular transformations of the data. The ANOVA's were straight-forward for *P. paradoxa*, resulting in a simple two-way partition by site and season. In *P. glabrata*, the heterogeneity between the plots made it unreasonable to group habitats across sites. The analyses were therefore conducted as in *P. paradoxa*, partitioning by plot, season and their interaction, rather than using a three-way partition of plot, site and season.

In both species, the model used in the analyses of variance was model I (Snedecor and Cochran, 1967). Thus both seasons and sites are considered to be fixed. The plots were chosen subjectively to represent a number of different habitats, so that it was not reasonable to assume that they represented a random sampling of the total range of habitats available to the species. The results of the analyses therefore are applicable only to the particular habitats studied.

(b) Interspecific variation in leaf-shape

The data from all 10 plots (i.e. with species confounded), were then re-analysed in a two-way analysis of variance to examine the variation in leaf-shape of plot with season. The multivariate response of the species to season was examined by canonical variates. The matrix of D^2 values obtained on analysis was used as the basis for the construction of a minimum spanning tree (Gower and Ross, 1969) among the plots in each season.

(c) Missing values

The leaves of *P. glabrata* are grazed by herbivores, and at any one sampling time it was not always possible to collect 15 undamaged leaves within every plot. However, a minimum of 13 leaves was available for each plot/season combination. In all, there were 9 missing values. The ANOVA program of GENSTAT (p. 49) interpolates missing values, but one degree of freedom is lost in each case. This is shown in the ANOVA tables (Appendix C.3, Tables 4 and 5), by '(9)' next to the error degrees of freedom.

Results

Full tables of the results are given in Appendix C.3 , pp.300-307) where the mean values of each variable for each species/site/season are given in Table 1.

1. Leaf-Shape Variation in *P. paradoxa*

The analyses of variance for the three characters show that there are significant seasonal differences in leaf-shape in *P. paradoxa* (Appendix C.3, Table 3). However, the species shows no differentiation of leaf-shape between sites. The *lwp/l* ratio of the leaves differ between winter and spring, and both are different from the summer and autumn results. The summer and autumn leaf-shapes are similar (Table 2a of Appendix C.3, p.301, Fig. 5.8). The leaves exhibit a progressive increase in *ll/l* from summer through to spring. The leaves are much broader relative to their length in spring, but have similar forms from summer to winter.

2. Leaf-Shape Variation in *P. glabrata*

The *P. glabrata* plots are more variable. In each of the three analyses of variance, significant results were obtained for sites,

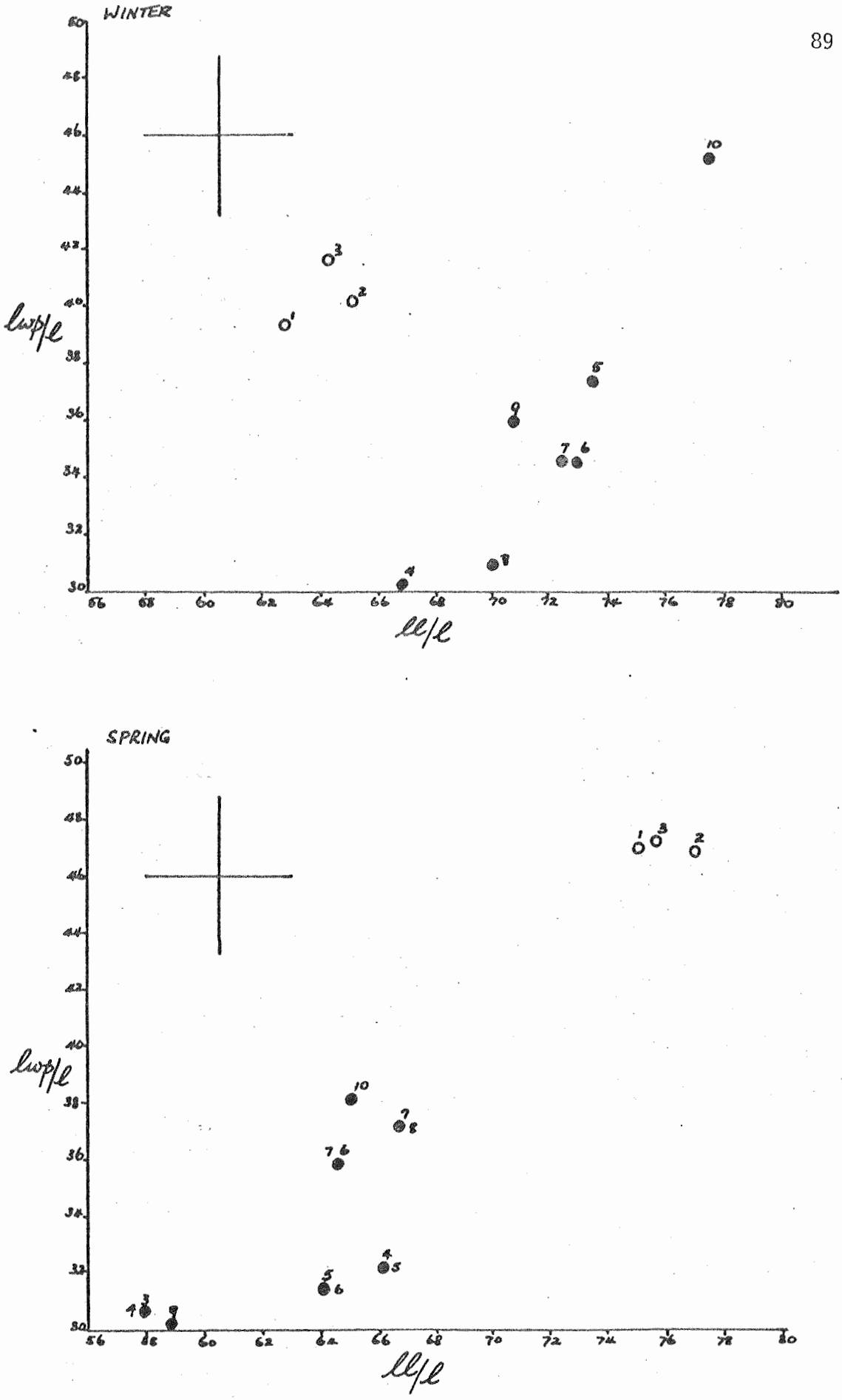
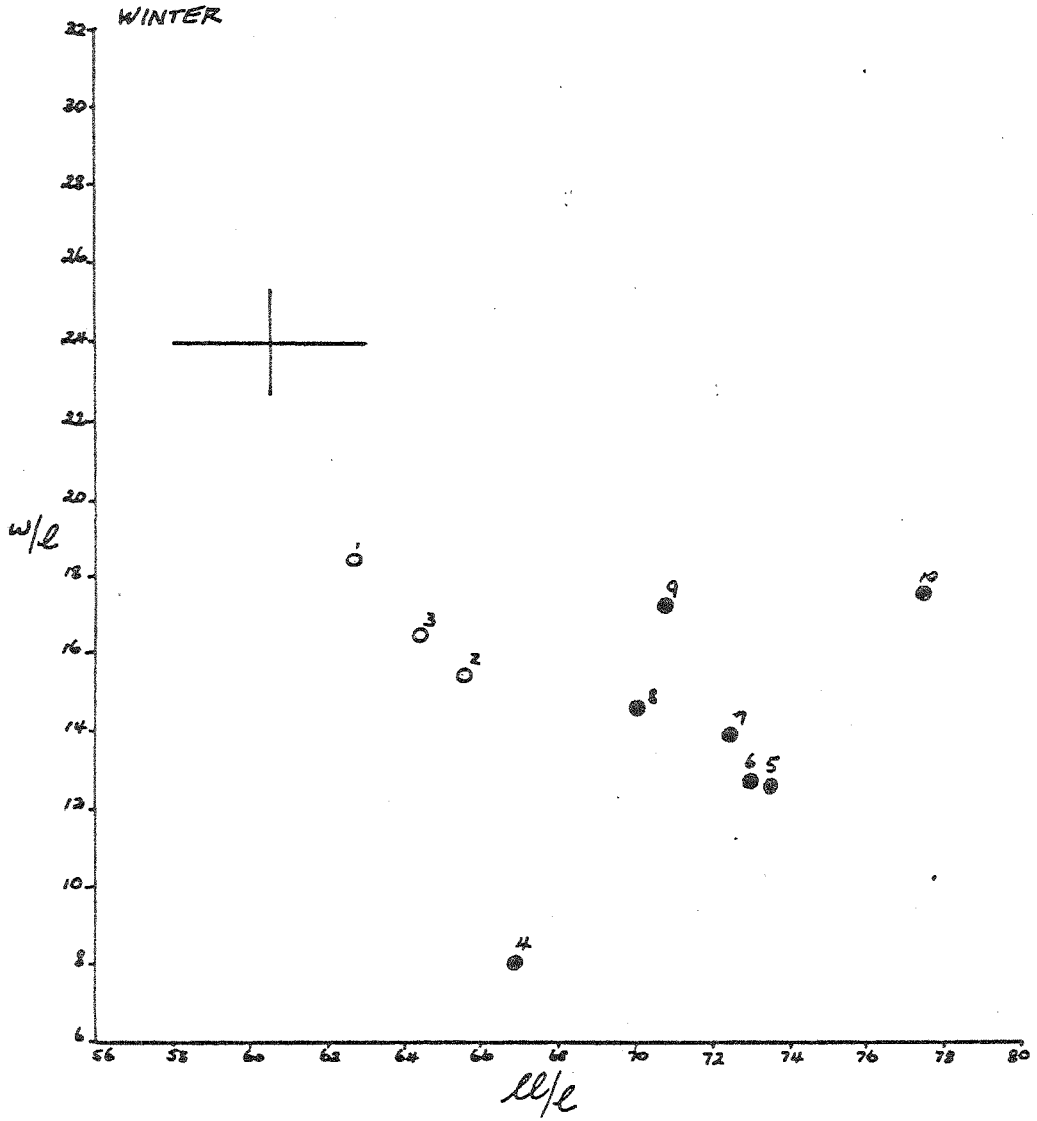
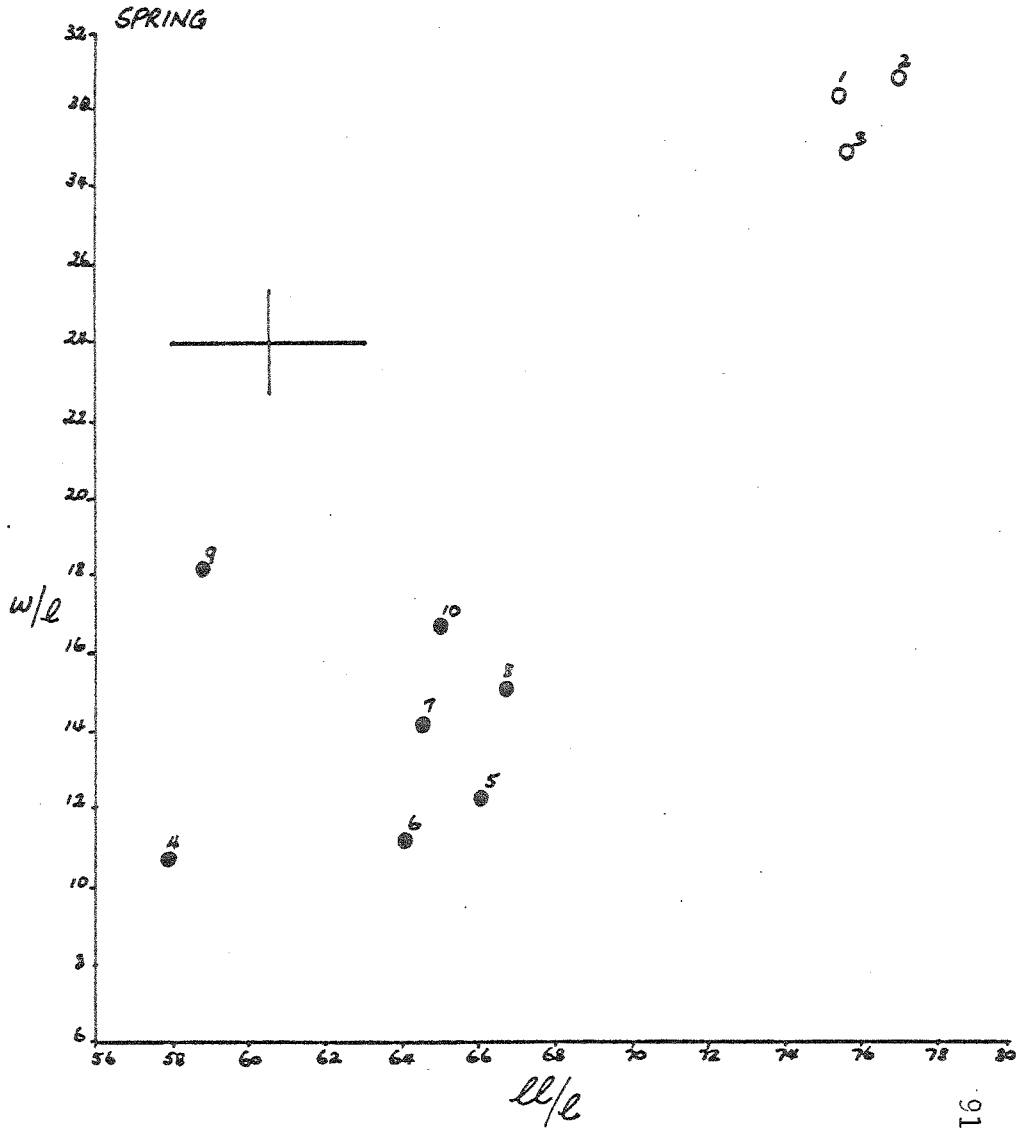


Fig. 5.8. The seasonal changes in mean values of *lwp/l* and *ll/l* for *P. paradoxa* and *P. glabrata* from the ten plots. Numbering of the plots follows Table 1. 95% confidence limits are shown.


WINTER
w/l
ll/l
32
30
28
26
24
22
20
18
16
14
12
10
8
6
56
58
60
62
64
66
68
70
72
74
76
78
80
1
2
3
4
5
6
7
8
9
10


SPRING
w/l
ll/l
32
30
28
26
24
22
20
18
16
14
12
10
8
6
56
58
60
62
64
66
68
70
72
74
76
78
80
1
2
3
4
5
6
7
8
9
10

seasons and their interaction (Appendix C.3 , Table 4 , p. 303). Thus not only does the species differ in leaf-shape between plots, but the plots also exhibit differential responses to seasonal change. The effects of these interactions are best considered in relationship to the multivariate response of plots with season.

Considered together, the *P. glabrata* plots show a reduction in *lwp/l* ratio from summer to autumn, and remain more or less stable thereafter (Appendix C.3, Table 2.6, Fig. 5.8). This contrasts with the response observed in *P. paradoxa*. The *ll/l* response of *P. glabrata* is similar to that of *P. paradoxa*, but in the opposite direction. The *w/l* ratios differ only marginally between seasons.

Analysis of the site differences (Appendix C.3,Table2.c,p.301)shows plot 4 from Arthur's Lake differs in all respects from the remaining plots. These plots have similar values for *lwp/l* and *ll/l* ratios, but there is evidence of intrapopulation variation at Pine Creek (plots 9 and 10). The *w/l* ratios are site specific; the leaves of the plants become progressively broader with increasing altitude and westerly influence (Fig. 5.9).

3. The Multivariate Response of the Two Species with Season

(a) Interpretation of axes

The CVA resulted in four significant canonical variates, the first two of which accounted for 79% of the total variation (Appendix C.3, Table 6 , p. 305). Inspection of the standardised eigenvectors associated with each eigenvalue reveals that the first is predominantly a contrast of *ll* and *w*, i.e. provides a score for the relative leaf-widths of the plants. The second variate contrasts *l* and *ll*, with a lesser contribution from *w*, and thus contrasts narrow-petiolate and broad sub-sessile leaves. The third variate contrasts *l* and *ll*, i.e. is a measure

of relative petiole length, whilst the fourth measures the position of the widest point. The mean values of the canonical variates for the species in each season are given in Table 7, of Appendix C.3 (p. 306). These variates are independent, which is surprising, since the second variate approximates a compound of the first and third. However, inspection of the graphs obtained (Figs. 5.10, 5.11), or of the mean values(p. 306), shows that there are independent aspects to the variation which are partitioned by the variates in much the same way as the univariate analyses (Appendix C. 3 , Table 5 , p. 304). Thus the first variate grades the plots on relative leaf width in a similar fashion to the univariate anlaysis of *w/l*. The major variation along the second axis is the contrast between *P. glabrata* and *P. paradoxa* in spring. This variate also contrasts the spring leaf-shapes of each species with those of the other seasons, comparable with the results obtained in the univariate analyses of *w/l* and *ll/l*. However, the multivariate contrast suggests that seasonally the *ll/l* and *w/l* ratios are covariates.

(b) Intraspecific variation

The canonical analysis clearly demonstrates the overall similarity of leaf-shape among populations of *P. paradoxa* although there is some evidence of differential plastic response between them in winter on the second and third canonical axes. This contrasts with the situation in *P. glabrata* which over the same range of localities in every season is differentiated in leaf-shape between populations. Furthermore, there is evidence of intrapopulation variation at two of the localities: at Arthur's Lake plot 4 is different from plots 5 and 6, and at Pine Creek plot 9 differs from plot 10.

(c) The Seasonal Convergence of Shape in the Species

The multivariate analysis shows that although there is some convergence between the species when situated in different habitats, in general they maintain their differences of leaf-shape. This is clarified by an analysis of the multivariate distances between the plots. The results of two such analyses are given in Tables 5.3 and 5.4.

Table 5.3. Minimum values of D^2 between *P. paradoxa* and *P. glabrata* in each season.

Season	D^2	Variance Ratio
Summer	2.68	4.48**
Autumn	1.71	2.86*
Winter	1.47	2.45
Spring	3.15	5.28**

Table 5.4. Averaged distances between *P. paradoxa* and *P. glabrata* in each season.

Season	D^2	Variance Ratio
Summer	3.12	5.22**
Autumn	2.94	4.93**
Winter	2.56	4.29**
Spring	4.10	6.86***

In Table 5.3 the D^2 values are the minimum distances between *P. paradoxa* and *P. glabrata* (AppendixC.3,Table 8, p.307), and shows the convergence of the plots in the winter months. This is equivalent to a single linkage cluster analysis between the species. In Table 5.4 an averaged D^2 value is obtained between *P. paradoxa* and *P. glabrata* (plot 4 excluded). This is an approximation to an average linkage cluster analysis between the species. On this basis

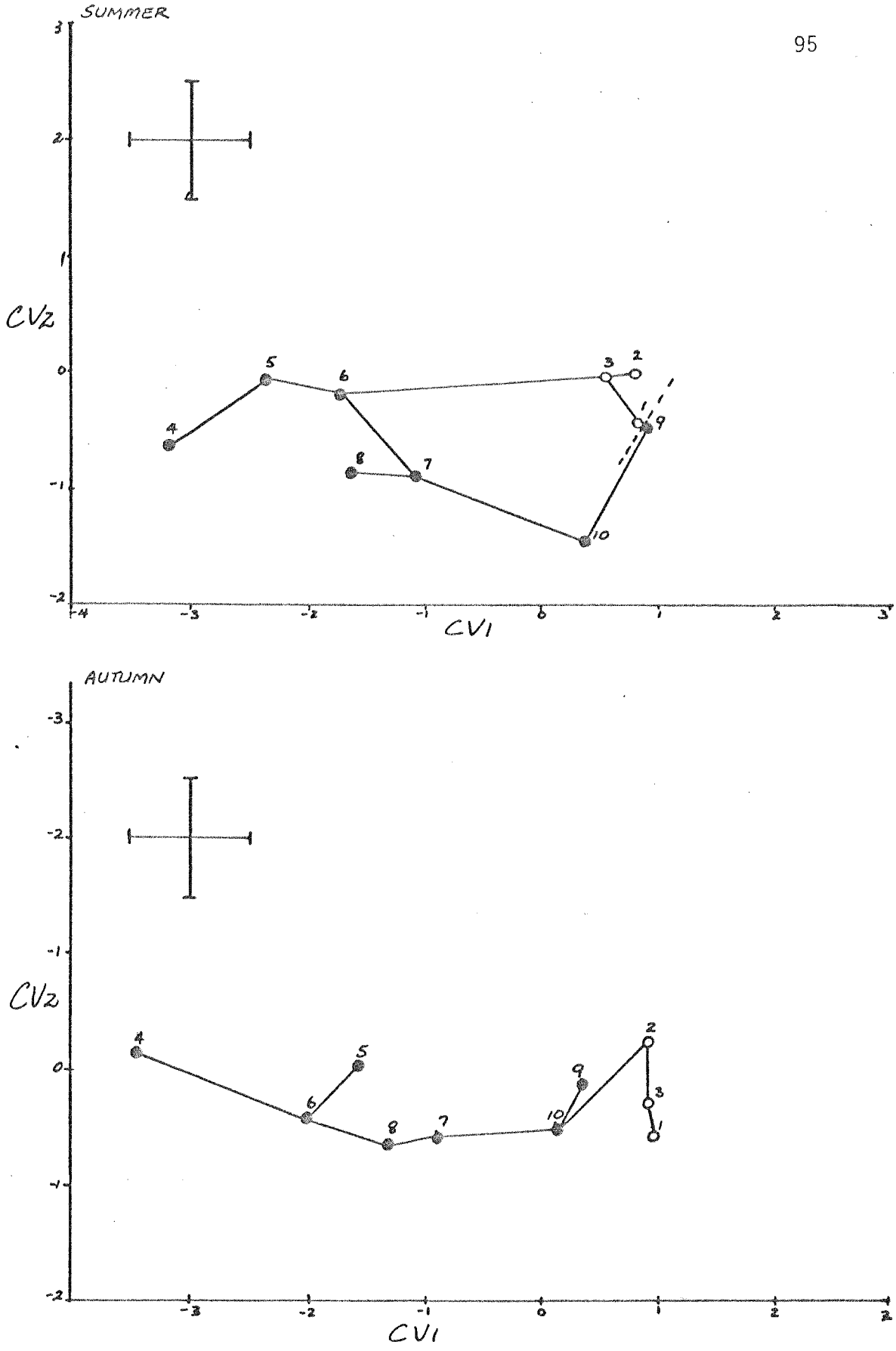


Fig. 5.10. The seasonal changes in mean values of the first two canonical variates for *P. paradoxa* and *P. glabrata* from the ten plots. The plots are joined by the minimum spanning tree. Numbering of the plots follows Table 1. 95% confidence limits are shown.

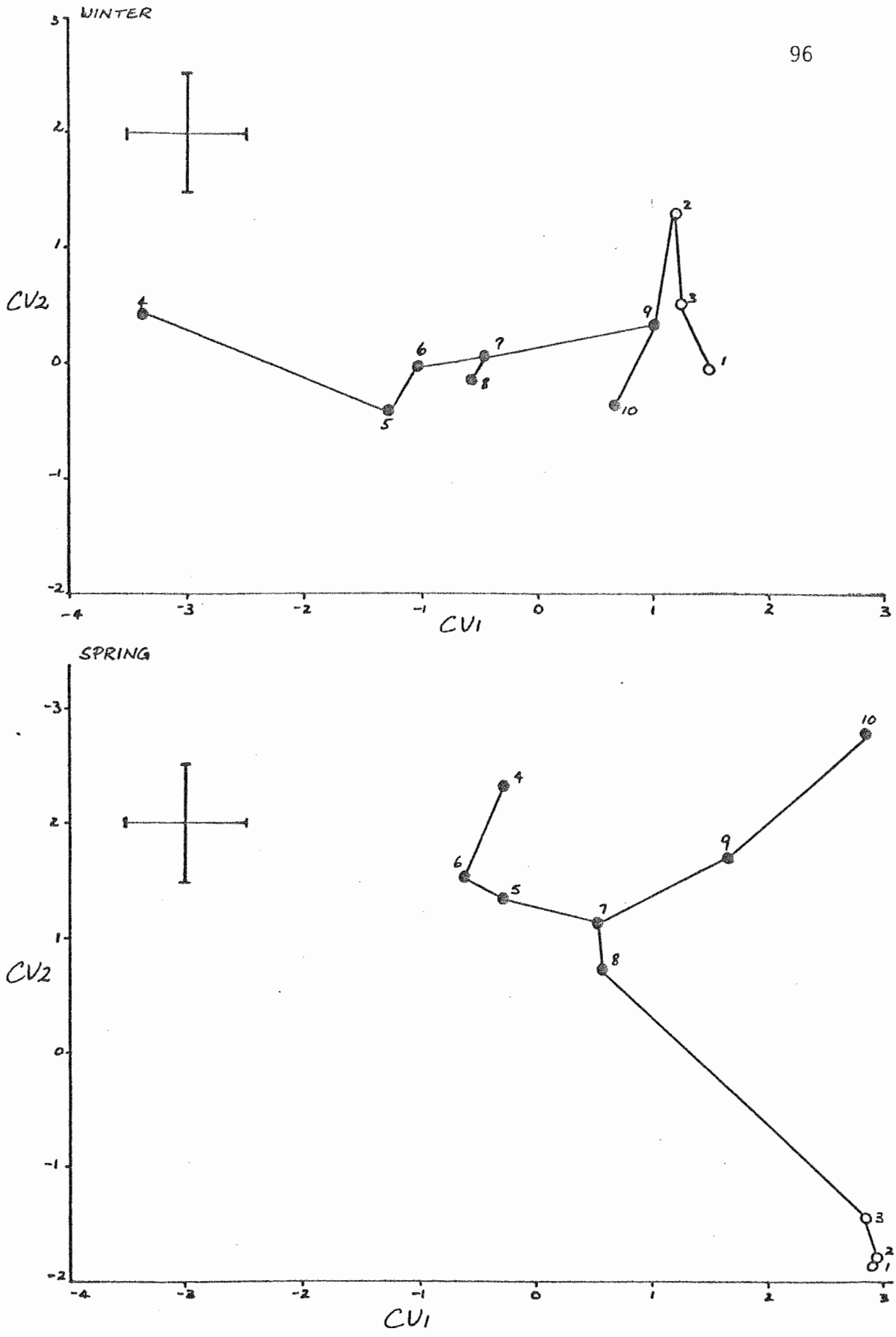


Fig. 5.10. The seasonal changes in mean values of the first two canonical variates for *P. paradoxa* and *P. glabrata* from the ten plots. The plots are joined by the minimum spanning tree. Numbering of the plots follows Table 1. 95% confidence limits are shown.

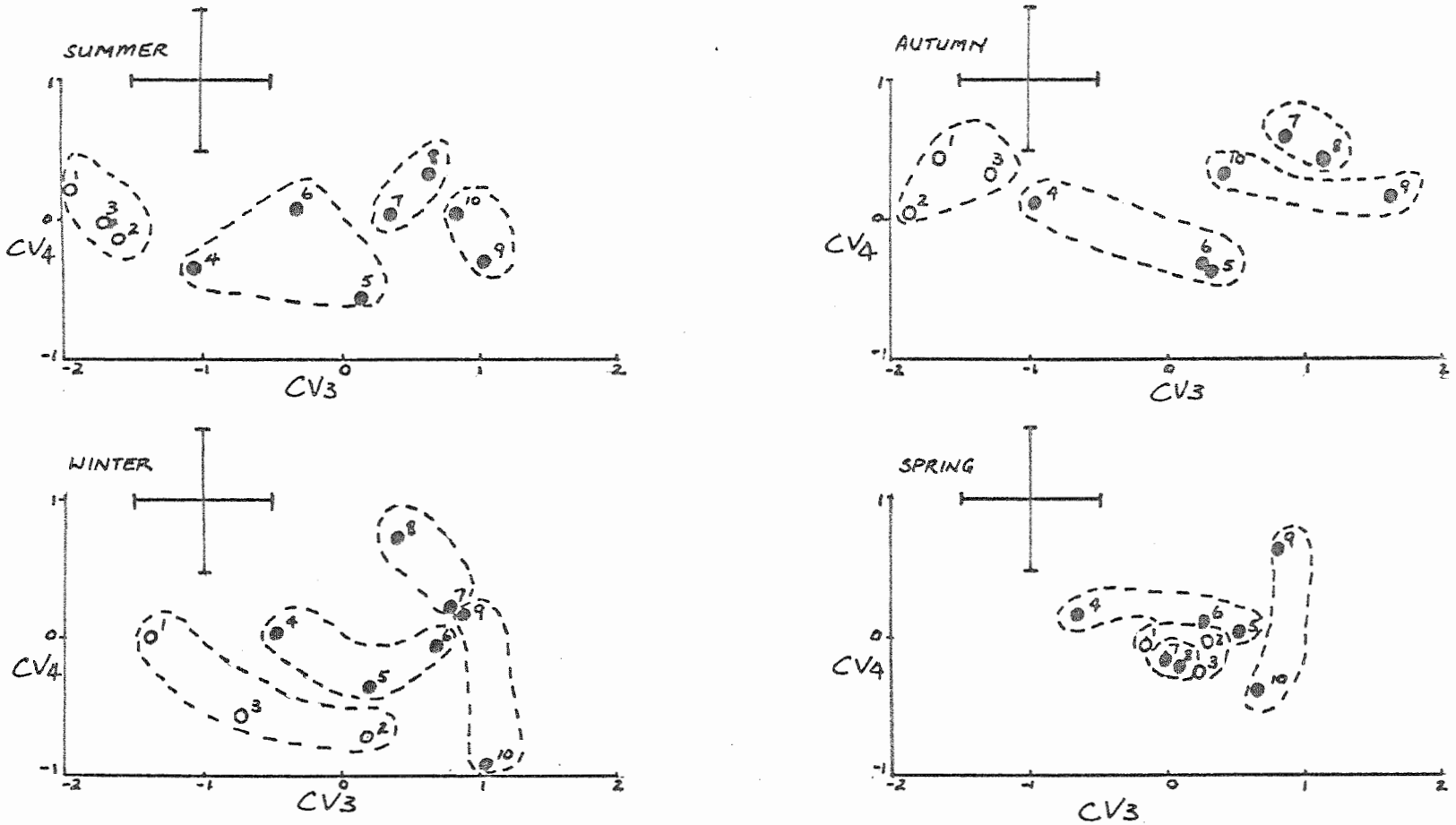


Fig. 5.11. The seasonal changes in mean values of the third and fourth canonical variates for *P. paradoxa* and *P. glabrata* from the ten plots. The dashed lines indicate species-site combinations. Numbering of the plots follows Table 1. 95% confidence limits are shown.

the species are distinct in all seasons, although a trend to lower values of D^2 occurs in winter. The application of the variance ratio approximation (Rao, 1952) may be unjustified statistically. However, it serves to illustrate the trend in each case.

Discussion

The multivariate analyses have demonstrated that both species exhibited changes in their leaf-shape in response to season, in all of the habitats which were studied. By and large, the interspecific differences were maintained. The seasonal change in the relative position of *lwp* in the leaves of both species was similar to that observed when the two were growing in admixture. This suggests that the response is not site specific, but is under the control of a more general environmental factor.

The differential response of the species in *ll/l* ratio (and the second canonical variate) can be ascribed to a combination of habitat factors and flowering strategies. Petiole length in both species apparently increases in wet habitats. Since the sites occupied by *P. paradoxa* are wetter than those occupied by *P. glabrata* (see p. 58), the leaves of *P. paradoxa* have longer petioles for most of the year. However, the relative petiole length of the two species is reversed in spring with the onset of flowering. At this time *P. paradoxa* forms very compact rosettes of shortly petiolate leaves, with sub-sessile inflorescences. By the fruiting stage, the flowering scapes have elongated and the rosettes again become spreading with petiolate leaves. *P. glabrata* tends to maintain compact rosettes with shortly petiolate leaves in winter, but in spring the plants 'bolt' so that the leaves become erect with longer petioles.

The multivariate studies showed that leaf-shape in *P. paradoxa* was similar for each of the habitats studied, although some evidence of differential plastic response to season was found. This may be the result of genetically determined differences in the expression of the response, but more probably reflects differences in the degree of environmental stress to which the plants were subject.

The results provided evidence of differentiation in leaf-shape among the populations of *P.glabrata*. There is a progressive increase in the *w/l* ratio of the leaves of plants from Arthur's Lake, Kannaleena and Pine Creek respectively. This ordering corresponds with the increasing elevation and distance west of Interlaken of the locations, and is associated with increasing rainfall and a reduction of temperatures (Table4.2, p.57). Thus the variation in the *w/l* ratio may be ecoclinal. However, only three locations were studied and the changes in the various climatic characteristics are correlated over this range, so that it is not possible from these data to associate the variation in leaf-shape with a particular environmental characteristic. A more detailed investigation into this aspect of variation in *P. glabrata* is given in a later chapter (p. 167). Also, the experiment is incapable of determining whether the variation is genetically based or whether it is a product of the particular environmental regime of each habitat.

The experiment indicated intrapopulation differences in leaf-shape of *P. glabrata*. At Arthur's Lake the leaf-shapes of plants in plot 4 were generally different from those in plots 5 and 6. This plot differs from plots 5 and 6 being swampy and having a partial cover of *Juncus pallidus*. The wet conditions and shading produces longer and more linear-oblanceolate leaves in summer.

However, the plants of all three plots are covered in snow for long periods during June-August, and the new leaves which are produced in spring are elliptic and much reduced in length. Thus the difference probably represents a differential plastic response to microhabitat, since the variation is only manifest seasonally. On the basis of these results, it is not possible to say whether the differential plastic response is itself genetically determined, i.e. has resulted from past selection.

Differences were also found to occur between the leaf-shapes of plants in plots 9 and 10 at Pine Creek. At this site there was a marked differentiation of leaf-shape with habitat, and this was maintained in all seasons. The differences observed here were also apparent at other sites on the Central Plateau, suggesting that a further evaluation was warranted.

The use of multivariate statistics has helped to clarify the interactions of leaf-shape with season in the species. The traditional use of these techniques has been to maximise the distances between pre-designated groups and/or to reduce the number of dimensions required for their study. In this investigation all of the canonical variates were significant, so that the technique did not produce a reduction in dimension. However, the technique was able to partition different aspects of variation which could be either confounded, overlooked or exaggerated by a series of univariate analyses, especially since the original variables were inter-correlated. In general the analysis has shown that the simple ratios of the other characters to length provide useful contrasts of form if considered in isolation, but that overall differences in leaf-shape are best studied by the multivariate techniques. This was adopted as standard procedure for the remaining

experiments. The univariate analyses of variance are given in the appendices, whilst discussion in the body of the thesis centres on the multivariate contrasts.

5.4 Experimental Garden Studies

Introduction

The results of the two previous experiments indicate that the seasonal convergence of leaf-shape in the two species is largely a result of microhabitat. However, the potential for the response may be conditioned directly by the environment, or it could be genetically based, i.e. there may have been past selection for the ability to exhibit the response. A first approach to distinguishing between these alternatives is to transplant some plants from a range of habitats into a common environment and to observe their (averaged) response. If interspecific convergence results, then it can be assumed (with some qualification) that the response is conditioned directly by the habitat, irrespective of genotype. However, there may still have been past selection for the plastic response in all of the genotypes tested. If the plants are cloned prior to transplanting, then the cloned material would provide a means of estimating errors in the subsequent analysis, and the response of individual genotypes can be ascertained. If the degree of the response is found to differ among genotypes, progeny testing would be needed to establish the heritability of the response.

A problem with transplant material is that an observed response may have been conditioned during the previous development of the plants, i.e. testing of progeny is needed also to test for pre-conditioning. Another way of estimating the degree to which previous conditioning affects the response of individuals is to use reciprocal transplants of the cloned genotypes from two (or more) widely disparate locations. Unless the environmental regimes of the locations are similar, the pre-conditioning experienced by the two

sets of plants is likely to be different. The determination of the plastic response exhibited by the genotypes across sites may then provide an estimate of the effects of previous environments on genotype. At the same time, the inclusion of plants from different locations is one way of testing whether the previously observed ecotypic differentiation of leaf-shape in *P. glabrata* (p. 99) has a genetic basis.

The experimental garden study reported in this section was designed to answer some of these questions. However, there were a number of problems which limited the information available for subsequent analysis.

Materials and Methods

1. The Sites

Two sites on the Central Plateau were selected for the establishment of the gardens. These were at Interlaken and Lake Augusta (Fig. 4.1). The sites were selected for a number of reasons:-

(a) They represented the most readily accessible climatic extremes on the Central Plateau (p. 57).

(b) The leaf-shapes of *P. glabrata* at the two sites were very different. At Lake Augusta the summer leaves of the plants ranged from lanceolate to broadly ovate-elliptic-obovate, whilst at Interlaken the leaves were linear to oblanceolate-elliptic.

(c) At each site there was a fairly uniform region in which the two species were naturally admixed. Thus transplantation should not result in any adverse effects arising from the habitat differentially favouring one or other species.

A summary of the vegetation and physical characteristics of each site is given in Table 5.5.

Table 5.5: A Summary of the Characteristics of the Experimental Garden Sites at Lake Augusta and Interlaken.

Characteristic	Lake Augusta	Interlaken
Altitude (m)	1160	800
Annual rainfall (mm)	c. 1400	708
% relative variability of rainfall	14	20
Estimated average temperature - Max (January)	14.9	18.6
Min (July)	-1.3	-0.8
Parent material	dolerite	dolerite
Soil type	clay loam with some humic development in upper 30 cm	loam
Evidence of solifluction	present	absent
Drainage	free, but subject to occasional flooding	free, but subject to occasional flooding
Predominant vegetation	sub-alpine meadow-herbfield	pasture grasses intermixed with native herbs
% ground cover	100	100
Grazing	rabbits, some summer sheep grazing	rabbits, cattle in all seasons
Fertilizer application	none	probably periodically topdressed

2. The Gardens

The positions of the gardens were chosen subjectively within the admixed region of each site. The gardens were fenced with rabbit-proof fencing to prevent grazing. The fences formed squares of side-length 11 m, with one side set parallel to the lake edge.

The gardens were subdivided into 484 (22 x 22) 0.5 x 0.5 m units and the presence or absence of each species in each unit was determined. The number of occurrences of each species in each column of 22 units parallel to the lake was used to determine their frequency of occurrence. Species presence or absence in the 484 quadrats was also used to test the degree of association between species at each site (Grieg-Smith, 1964).

3. The Transplants

At each site 30 plants of each species were selected by a stratified random technique. A grid of 100 m square was located at each site. This included habitats in which each species occurred as 'pure stands' and areas in which they were co-occurring. In order to minimise the possibility of over-sampling a single genotype, the plants were selected at random subject to their restriction to 4 m^2 quadrats within the grid.

The plants were cloned in the glasshouse to give four replicates of each genotype. These were transplanted into plastic pots in a vermiculite-soil mixture and replaced in the glasshouse for 6 weeks. After this time the plants were hardened outside the glasshouse for a further eight weeks. This material formed the basis for the selection of 16 x 4 plants of each species-site combination. These plants were transferred to the gardens and were planted out in duplicate in the early spring of 1974 to give two randomized blocks of 64 plants in each garden. The plants were planted at a 30 cm spacing with each block.

4. Sampling

The plots were sampled once during winter and again in the summer of 1975. The garden at Interlaken had to be abandoned. At the first sampling it was found that the fence supports had been removed and the area had been heavily grazed and trodden by cattle, so that the plants were unrecognizable, and could not be used in the subsequent analyses. However, the degree of association between the species in their native habitats (i.e. prior to establishment of the gardens) has been calculated for both sites.

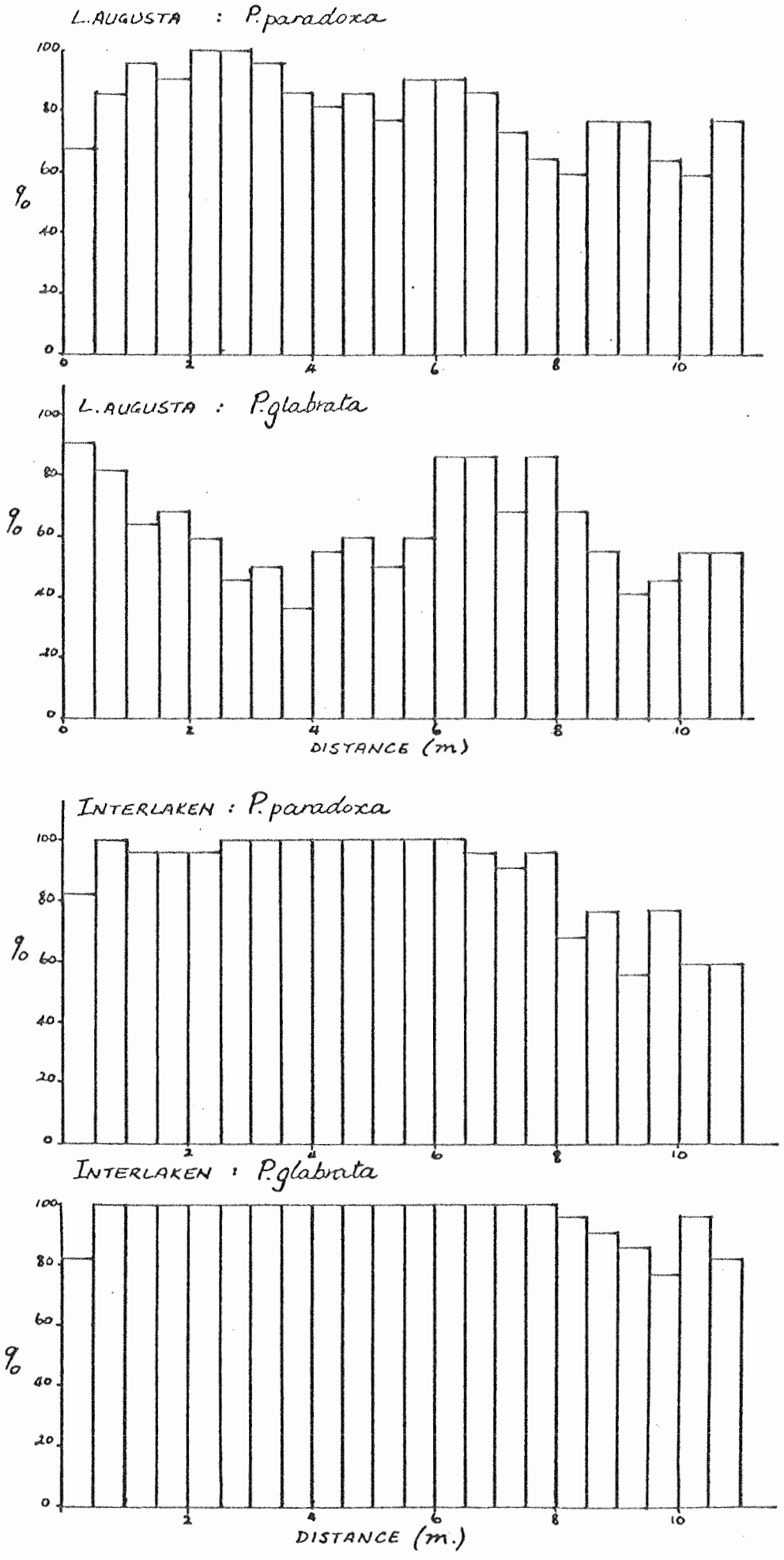
A further problem arose because many of the plants from all species/sites combinations did not establish in one of the randomised blocks at Lake Augusta. The number that remained was considered inadequate for statistical manipulation. Thus the whole experiment needs to be repeated using more plants and less accessible locations. Despite these problems, some useful results were obtained from the remaining block of 64 (2 x 2 x 16) plants at Lake Augusta. Fifty-seven (15 + 15 + 15 + 12) of these plants survived, and data from these were used in the analyses.

Analysis of Data

A three-way, univariate analysis of variance was conducted for each of the three ratios w/l, lwp/l and ll/l, which were measured on the plants from one randomised block at Lake Augusta. The analyses were partitioned by species, site and season and their various interactions. Model I (Snedecor and Cochran, 1967) was assumed because all three effects are fixed.

The same data were incorporated in a CVA to determine the multivariate response of leaf-shape to season.

Fig. 5.12. Frequency of occurrence of *P. paradoxa* and *P. glabrata* in the experimental garden sites at Lake Augusta and Interlaken.



Results

1. Internal Homogeneity of the Garden Sites

Frequency histograms of the occurrence of the two species at Lake Augusta and at Interlaken are given in Fig.5.12. These show that the species occur with reasonable frequency across the gardens at both sites. The co-occurrence of the species within the area was confirmed by the X^2 analysis. At both sites *P. paradoxa* and *P. glabrata* were found to be positively associated ($X_1^2 = 3.85^*$ at Lake Augusta, $X_1^2 = 6.93^{**}$ at Interlaken), indicating that the gardens could be assumed homogeneous for the growth of both species.

2. Leaf-Shape Analysis

The univariate ANOVA's show that there are highly significant seasonal differences in all three ratios (AppendixC.4 , Table 2 , p.308), but only the *w/l* ratio shows a significant species/season interaction ($F_{1,49} = 5.1^*$). The *w/l* ratio differs between sites ($F_{1,49} = 31.58^{***}$), but there are no significant differences for this factor in respect of the *ll/l* or *lwp/l* ratios.

CVA of the leaf characters of the plants from both sites grown at Lake Augusta resulted in three significant canonical variates (Appendix C.4, Table 3). The first distinguishes between seasonal differences of leaf-shape for the four species/site combinations (Fig. 5.13). The summer leaves of each species are distinguished by site on the second axis, but the four species/site groups become convergent in winter. A seasonal convergence of shape is also elicited on the third axis between the leaves of the plants of *P. glabrata* and *P. paradoxa* which originated at Interlaken.

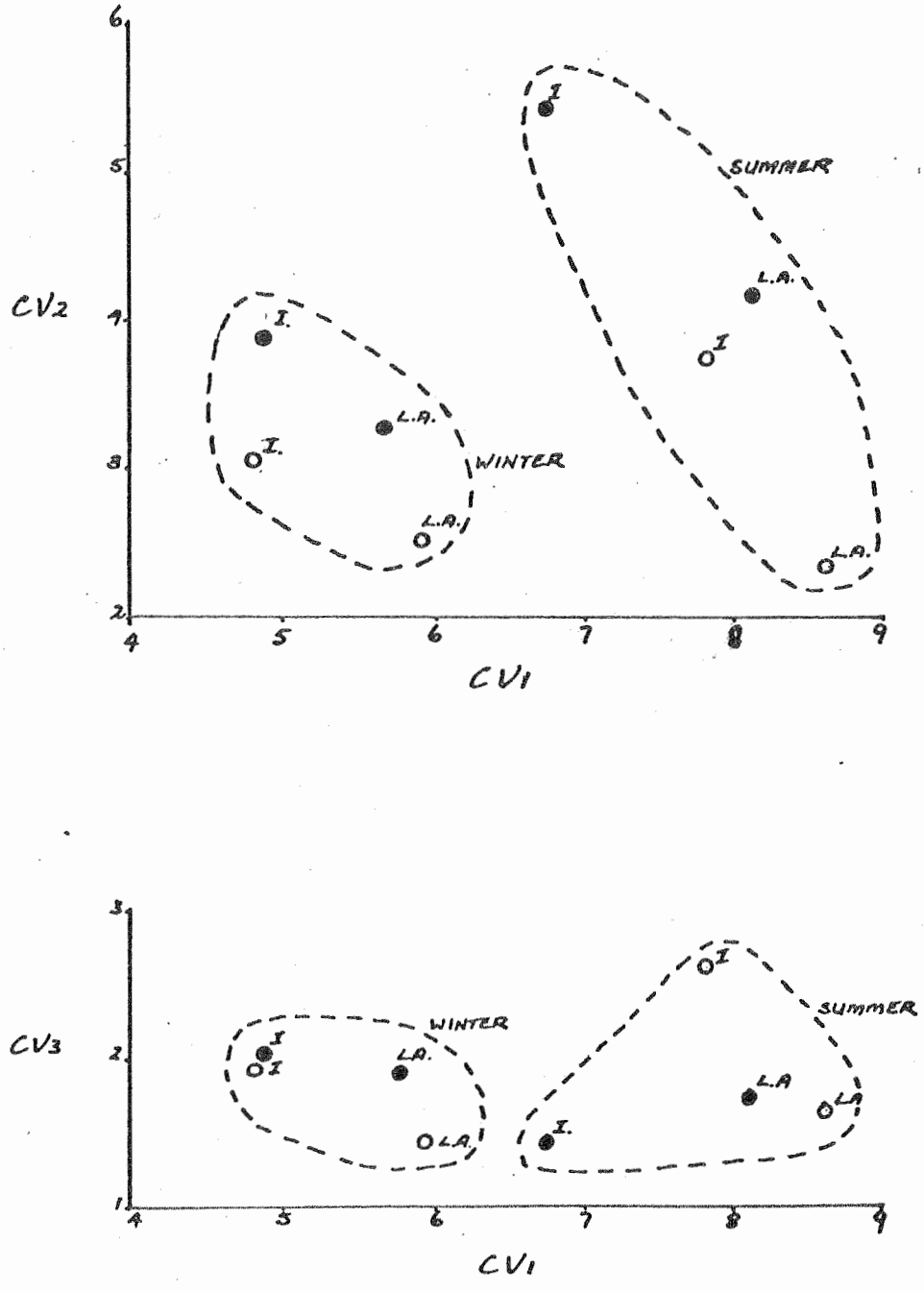


Fig. 5.13. CVA of the seasonal variation in leaf-shape of *P. paradoxa* and *P. glabrata* plants grown in the experimental garden at Lake Augusta.
L.A. Lake Augusta ● *P. glabrata*
I Interlaken ○ *P. paradoxa*

However, the plants which originated from Lake Augusta show little seasonal variation in this character.

Inspection of the standardized eigenvectors (Appendix C.4, Table 3,p.310),and comparing these with the mean values of the original variables (Appendix C.4, Table 1, p. 308),shows that the variation along the first canonical axis can largely be ascribed to a concomitant seasonal change in relative petiole length of the leaves, and a reduction in their relative widths. The summer leaf-shapes of the species-sites are graded in the same sense. Thus the summer leaves of both species on plants from Lake Augusta are relatively broad and shortly petiolate compared with those from Interlaken, when both are grown at Lake Augusta.

The second axis reflects the contrast between the small changes in *lwp/l* and *w/l* relative to *ll/l* of *P. glabrata* from Interlaken compared with the larger shifts in these characters in plants from the other species-sites combinations. The third axis illustrates the marked convergence in *lwp/l* ratio in winter of the two species from Interlaken when grown at Lake Augusta. The two species have similar values for this character in summer, and remain similar in winter in the case of the plants which originated at Lake Augusta.

Discussion

It is unfortunate that the results from the garden at Interlaken are unavailable, because any interpretation of the results from the garden at Lake Augusta must be made with the proviso that (unlike the transplants which originated from Interlaken), the transplants from Lake Augusta are 'at home'.

Nevertheless, a number of interesting facets of leaf-shape variation in the species have emerged from this experiment:

1. The leaf-shapes of the species at each site are clearly differentiated on the first two canonical axes. Since the plants were grown in a randomized design, microenvironmental effects will also be randomized, so that it can be concluded that the leaf-forms of each species from each site are genetically distinct. This confirms the results obtained earlier for *P. glabrata*. However, there was no previous indication of leaf-shape differentiation among sites for *P. paradoxa*. The plants used in this experiment originated from sites (Interlaken and Lake Augusta) which have much greater differences in their climatic regimes than those which occur at the sites studied earlier (Arthurs Lake, Kannaleena, Pine Creek) (see Table 4.2, p. 57). Superficially, it would appear that the difference in climate between the sites may be accompanied by ecotypic differentiation between the extreme populations, but not between the intermediate populations. However, it must be remembered that, unlike the extreme populations, plants from the three intermediate populations have yet to be tested in a common environment.

2. There is a more or less equivalent seasonal response in both species from both sites when grown in a common environment. The characters affected are the relative leaf-widths and the petiole lengths of the two species. Whilst some evidence of seasonal change in leaf-width was obtained when the species were growing in different habitats, it appears that this response may be stimulated largely by local environmental factors.

3. There is evidence of differential response to seasonal change in the *lwp/l* ratio between the populations of the two species. The *P. paradoxa* and *P. glabrata* plants which originated at Interlaken are widely divergent in this character in summer, but become convergent in winter. However, whilst plants of the two species from Lake Augusta exhibit a trend in the same direction, their leaves do not differ greatly in this character in summer or winter.

4. In the short term, the seasonal differences which prevail between Lake Augusta and Interlaken do not appear to be agents of selection between ecotypes in mature plants grown at Lake Augusta. The results from the garden at Interlaken would be needed to verify whether the converse is true. However, the limited evidence of this experiment suggests that seasonal plasticity of leaf-shape enables the plants to mask those genetic differences which are to be observed in more equable conditions.

6. Glasshouse Studies on Factors Affecting Leaf-Shape in *P. paradoxa* and *P. glabrata*

Introduction

The field studies have demonstrated that both species exhibit changes in leaf-shape in response to altered environmental conditions. The multivariate analyses have shown that the visual impression of winter convergence in the leaf-shape of the species is the result of independent aspects of variation in the different leaf-characters.

The onset of winter brings associated changes in a number of environmental variables, any of which might affect the changes of leaf-form. Obvious seasonal factors which could influence leaf-shape include altered soil moisture levels, temperature, light intensity and light duration. This chapter reports the results of some investigations into the response of the species to changes in the levels of these factors under glasshouse conditions.

Materials and Methods

The techniques of cultivation which were used in these experiments and the growing conditions available in the glasshouse, phytotron and growth cabinets, have been outlined in the General Materials and Methods (p. 45). Unless specifically stated otherwise, these conditions apply to the experiments reported in this section.

The Inundation Experiment

Twelve plants each of *P. paradoxa* and *P. glabrata* were cloned to give matched pairs of plants from stock gathered at

Arthur's Lake. The 48 plants were grown individually in plastic pots containing a vermiculite-gravel mixture. The plastic pots were put into cans and placed in a randomised block. The plants were grown, either under a normal (daily) watering regime, or sitting in water so that their apices were just covered (Fig. 6.1).

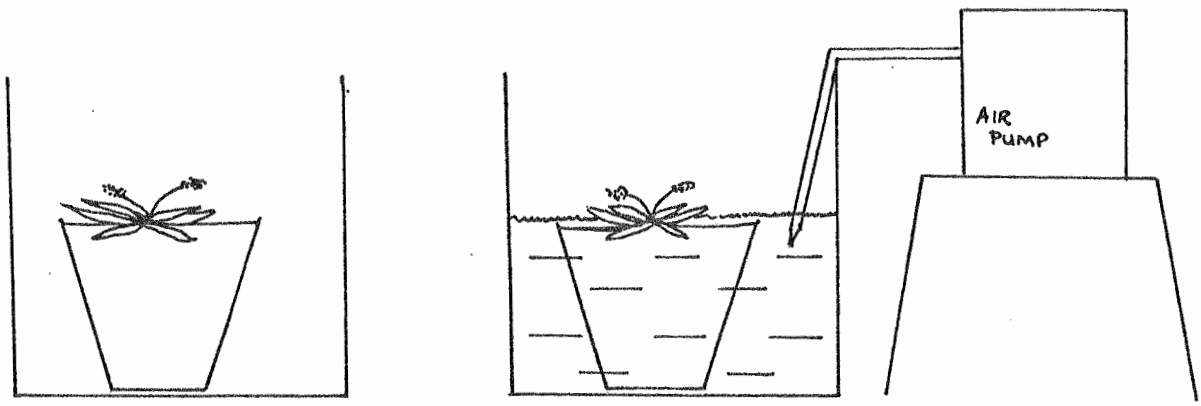


Fig. 6.1. Experimental design used in the inundation experiment.

The submerged plants were kept aerated by air pumps connected via rubber hoses to Pasteur pipettes placed in the water. The differences in light intensity perceived at the apices of treated and control plants were assumed to be negligible, although the possibility of altered light quality remained. Under these conditions it was not possible to achieve accurate control of temperature. Temperature differences were monitored periodically and were found to average $5^{o}C$, ranging from 18-$20^{o}C$ water temperature to 23-$25^{o}C$ air temperature in the vicinity of the untreated plants.The water in the cans was changed twice weekly, and the plants were left for 8 weeks prior to harvesting. The 3 most

recently expanded leaves were excised from each plant and pressed for scoring.

Temperature Experiments

Two experiments were conducted to examine the effects of temperature on leaf-shape of the species using stock material from Arthur's Lake. In the first of these, one cloned sample of each of 24 plants of the two species was grown in growth cabinets under a 12 hour photoperiod at 10, 15, 20 and 25°C. Leaves from these plants were harvested and scored after 12 weeks. In the second experiment, 10 plants each of *P. glabrata* and *P. paradoxa* were cloned to give 3 replicates per genotype. These were grown in growth cabinets at 6, 9 and 12°C under a 24 hour photoperiod. The plants were removed for scoring after 11 weeks. The light intensity was set at 21,000 lux (= 210 microeinsteins $m^{-2}sec^{-1}$) in both temperature experiments.

The Effect of Light Intensity

Twelve plants each of *P. glabrata* and *P. paradoxa* were cloned to give replicate genotypes for each shading treatment using stock material from Arthur's Lake. The plants were grown one to a can, placed in two adjacent rows for each treatment, alternating the species in each row. The plants were grown under frames fitted with 'Saylon' shade cloth to give 28, 52, 72 and 81% reduction in ambient light intensity. The shade cloths are purported not to alter differentially the spectral quality of the incident light. A fifth set of control plants were set up with no shade cloth. The positions of the treatments were allocated to minimise row effects along the glasshouse. Three recently

matured leaves per plant were harvested after 12 weeks and pressed.

The Effect of Photoperiod

The response of the two species to 24 hour and 8 hour photoperiods was studied in the phytotron. Seed of the two species was collected from three locations:- Arthur's Lake, Kannaleena and Pine Creek. Seedlings were planted out into plastic tote boxes, 24 plants per box, and four boxes per species-site combination. Two boxes of each were grown under long days or short days for a period of 26 weeks. Five leaves per plant were removed and pressed for later measurement. In addition to the leaf-measurements, the plants were scored weekly for the presence of flower buds in the leaf-axils. The effects of photoperiod on flowering are reported elsewhere (Chapter 9). The mean values of the leaf-characters were calculated over all the plants in each of the tote boxes to give two replicates of the averaged response of each species-site-daylength combination.

Interaction of Temperature and Light Regimes

The combined effects of light and temperature were studied on plants grown from seed. The seed was collected in bulk from each species at Interlaken and Lake Augusta. Twenty plants per treatment were used. At the time of the experiment, only limited cabinet facilities were available, and it was not possible to achieve a fully factorial design of light intensity, daylength and temperature. Accordingly, two temperatures ($10^{o}C$ and $17.5^{o}C$) and three light regimes, were selected (long days, low

light intensity; short days, high light intensity; and short days, low light intensity). The light levels were adjusted so that the total incident light under short days and high intensity was the same as that under long days and low intensity. The photoperiods used were 8 and 24 hours, and the light intensities were set at 17,000 and 51,000 lux using an EEL light meter. The incident light energies were checked subsequently with a Li-Cor quantum sensor and found to be 170 and 510 microeinsteins m^{-2} sec^{-1}.

The plants were grown on the apron of the glasshouse for 6 weeks prior to the commencement of treatment, and grown in the cabinets for 12 weeks prior to measurement.

The Measurement of Leaf-Shape

Leaves of the plants were scored for the characters length *l*, position of widest part *lwp*, length of lamina *ll*, and width *w*. Initially, these characters were used either untransformed or as logarithms in multivariate analyses of all experiments. It was found that these analyses usually resulted in simple contrasts of form, e.g. between *l* and *w*, *l* and *ll*, etc., and the results were usually confounded with 'size' effects. It was decided to use the ratios of the characters *lwp*, *ll* and *w* to *l* as the variates in all analyses. In this way the effect of size was removed, and the vectors resulting from the multivariate analyses were relatively simple to interpret. Univariate analyses of variance were carried out for each character in each experiment. Model I (Snedecor and Cochran,1967) is assumed in each case.

Results and Discussion

Detailed tabulations of results are presented in Appendix D.

The Effect of Inundation on Leaf-Shape in the Species

Plants of the two species were grown either under a normal watering schedule, or with their apices submerged in water. The mean values and univariate ANOVA's of each character are given in Appendix D, Tables 1(a) and 1(b), respectively. A CVA of the changes in leaf-characters resulted in two highly significant canonical variates (Appendix D, Table 1(c), p. 311). The first variate contains 92% of the total variation and on this axis there is a clear convergence of leaf-shape between the species when growing in water (Fig. 6.2). The two species exhibit a response in the same direction on CV_1 although by far the greatest change occurs in the leaf-shape of *P. paradoxa*. Inspection of the standardized canonical variate shows that CV_1 contrasts leaves which are broadly ovate, i.e. of relatively large width and widest point near the base of the lamina, with leaves which are more linear-elliptic. This is a reasonable approximation to the respective leaf-shapes of *P. paradoxa* when grown in non-submerged and submerged conditions. It also discriminates between the non-submerged leaf-shapes of the two species. The second variate contrasts the *lwp/l* ratio with *ll/l* ratio. The response on this axis is the same for the leaves of both species, and reflects their increase in relative petiole lengths when grown in water.

The field experiments showed that the seasonal convergence of leaf-shape of the species is most pronounced when they occupy a common environment. This suggests that local changes in

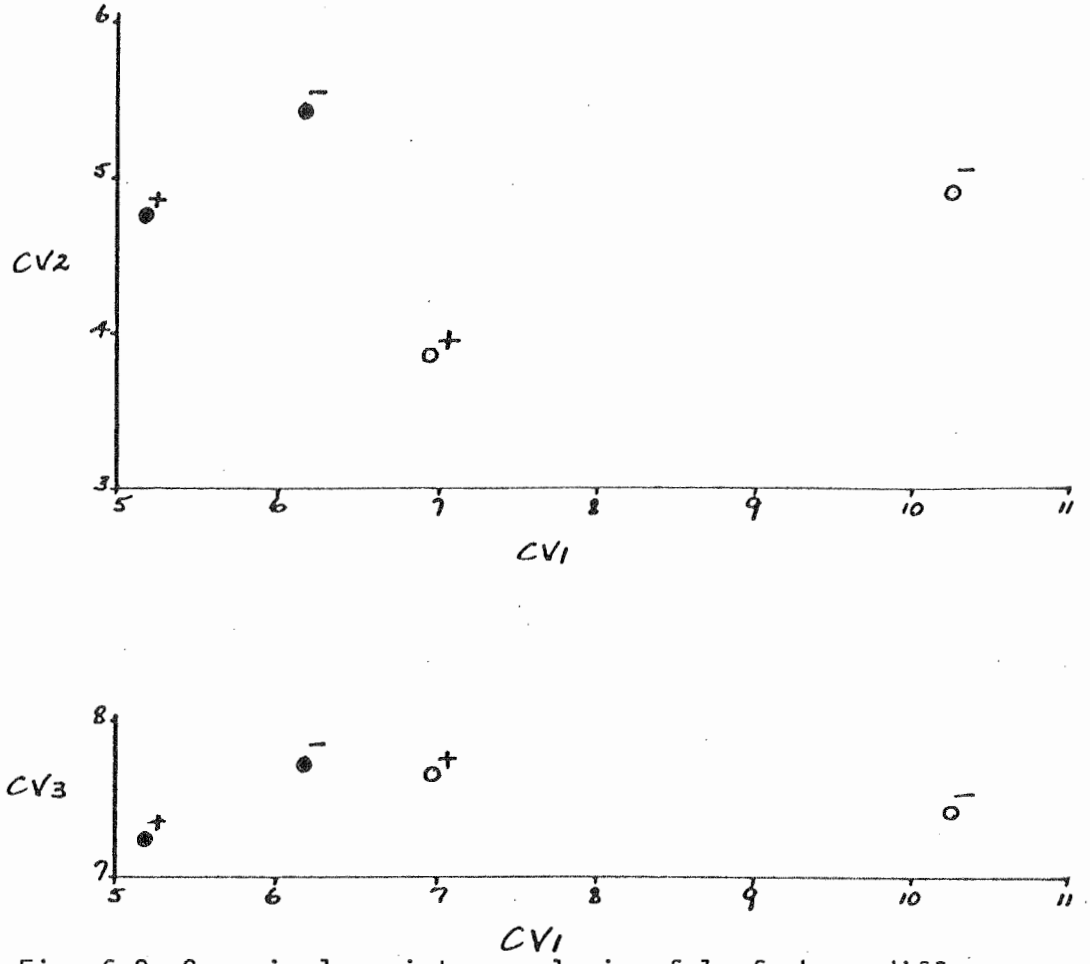


Fig. 6.2. Canonical variates analysis of leaf shape differences between *P. glabrata* ● and *P. paradoxa* o when grown in submerged (+) and non-submerged (-) conditions.

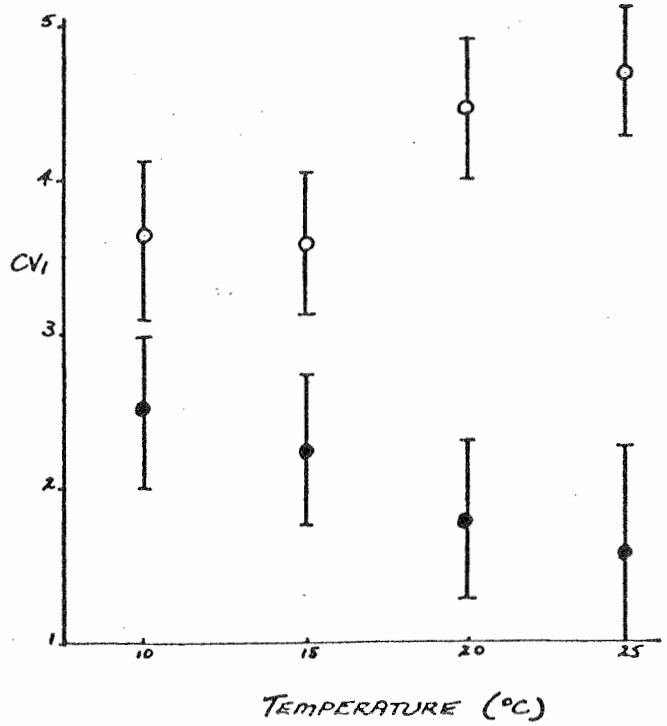


Fig. 6.3. The variation on the first canonical axis of leaves of *P. glabrata* ● and *P. paradoxa* o when grown under 4 different temperatures.

microhabitats provide major determinants of the similarity in leaf-shape, rather than changes in environmental factors which take place over wider ranges. The most obvious factor which exhibits local differences between summer and winter is the amount of surface water lying on the ground. In winter, the admixed sites are frequently covered by water, whilst the bulk of the *P. glabrata* populations are on higher or better drained ground. Under the conditions of the present experiment, it was found that submergence *per se* is sufficient to bring about a convergence of leaf-shape in the species.

The Effect of Temperature on Leaf-Shape in the Species

(a) Plants grown under a 12-hour photoperiod at 10, 15, 20 and 25°C

The mean values for each treatment class are given in Appendix D, Table 2a(i) (p. 313).

The univariate analyses of variance show that there are significant differences in the *ll/l* and *w/l* ratios between species, and the characters respond to changes in temperature (Appendix D, Table 2(a)(ii), p. 313). The *lwp/l* ratio apparently differs between species, but is unaffected by temperature. The CVA resulted in one highly significant eigenvalue which accounted for 88% of the total variation (Appendix D, Table 2(c)(iii), p. 313). If the mean values of the species on the first canonical variate are plotted against temperature (Fig. 6.3), then there is a definite trend towards convergence of leaf-shape in the two species with reduced temperature, although the species remain distinct at the lowest temperature. The lowering of temperature appears to cause a co-ordinated change of leaf-shape in the species and this shift is opposite in direction.

Inspection of the standardized coefficients of the first canonical variate reveals that it consists of a contrast of *lwp/l*

and *w/l* with *ll/l*, i.e. it contrasts the broadly ovate leaves of *P. paradoxa* with the attenuate, oblanceolate-obovate leaves of *P. glabrata* at higher temperatures. The contrast is reduced at lower temperatures, when the leaf-shape of both species becomes more linear elliptic, the relative leaf-width being reduced in *P. paradoxa* and increased in *P. glabrata*.

(b) Plants grown under a 24 hour photoperiod at 6, 9 and 12°C.

The results of this experiment are set out in Table 2(b) of Appendix D (p.314).

The univariate analyses indicate that the *lwp/l* ratio of leaves of the two species remain distinct at these temperatures. There is a marginally significant interaction between species and temperature in both *ll/l* and *w/l*. The CVA resulted in a single significant eigenvector, which reflects the differences in the *lwp/l* ratios of the two species. Although non-significant, the second canonical variate contrasts the *w/l* and *ll/l* ratios, and the species produce a response which is opposite in direction on this axis.

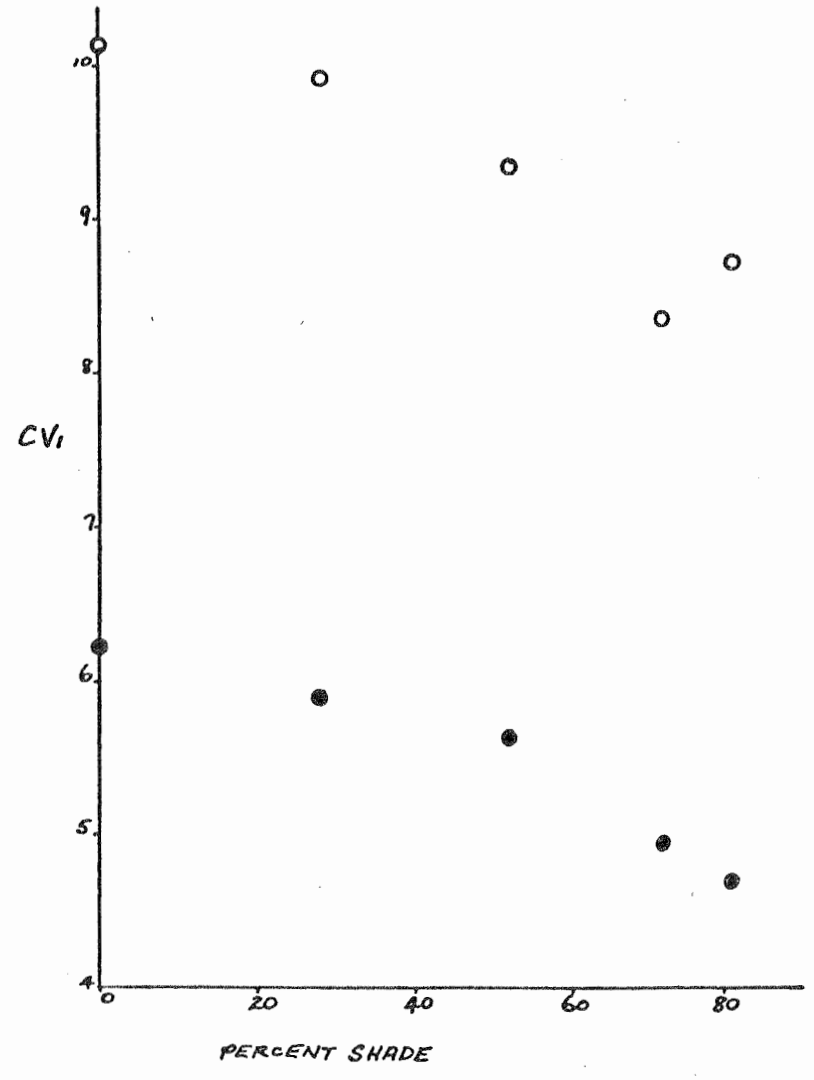
From these experiments it appears that the response of leaf-shape to temperature may be limited to some extent by photoperiod in both species. Under a shorter photoperiod, the leaf-shape of the two species is convergent concomitant with a reduction in temperature. Under long days, a trend towards convergence at low temperatures was also elicited, but the leaf-shapes adopted by the species remained distinct by virtue of the photoperiodic control of the *lwp/l* ratio.

The Effect of Light Intensity of Leaf-Shape in the Species

(Appendix D, Tables 3(a)-(e), p. 316).

The univariate analyses of variance show that there is a significant response to altered light intensity in all three characters in both species. Canonical analysis resulted in two highly significant eigenvectors. Graphs of the mean values of these canonical variates for each species against the degree of shade are presented in Fig. 6.4. The first variate effectively discriminates between species, and shows that the differences are maintained at all light intensities. The second variate shows a linear response in both species to decreased light intensity. The analysis illustrates some of the problems inherent in reification of the canonical axes (Marriot, 1974). From a consideration of Fig. 6.4, it is clear that within each species the first and second canonical variates are highly correlated. The reason the two axes are independent is the interspecific differences. This can be seen in Fig. 6.5. Thus the actual change in leaf-shape due to shading in each species is a compound of the variation measured by both axes.

If the species are analysed independently, then in both species only the first canonical variate is significant, and plots of the mean values of this character show a linear decrease with decreasing light intensity (Fig. 6.6). In *P. glabrata* the vector is heavily weighted by the *ll/l* ratio. In *P. paradoxa*, the *ll/l* ratio is strongly weighted, but there is also a lesser contribution from *w/l*. Thus, in *P. glabrata* the primary response to reduced light intensity is the production of petiolate leaves and the significant differences in *lwp/l* and *w/l* obtained in the



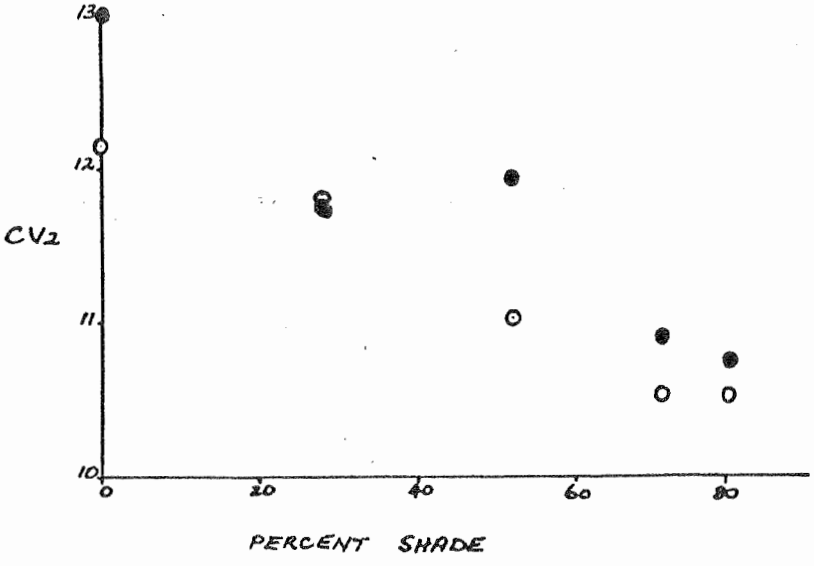


Fig. 6.4. The effect of shading on leaf shape in *P. glabrata* ● and *P. paradoxa* ○ , as measured by the two significant canonical variates.

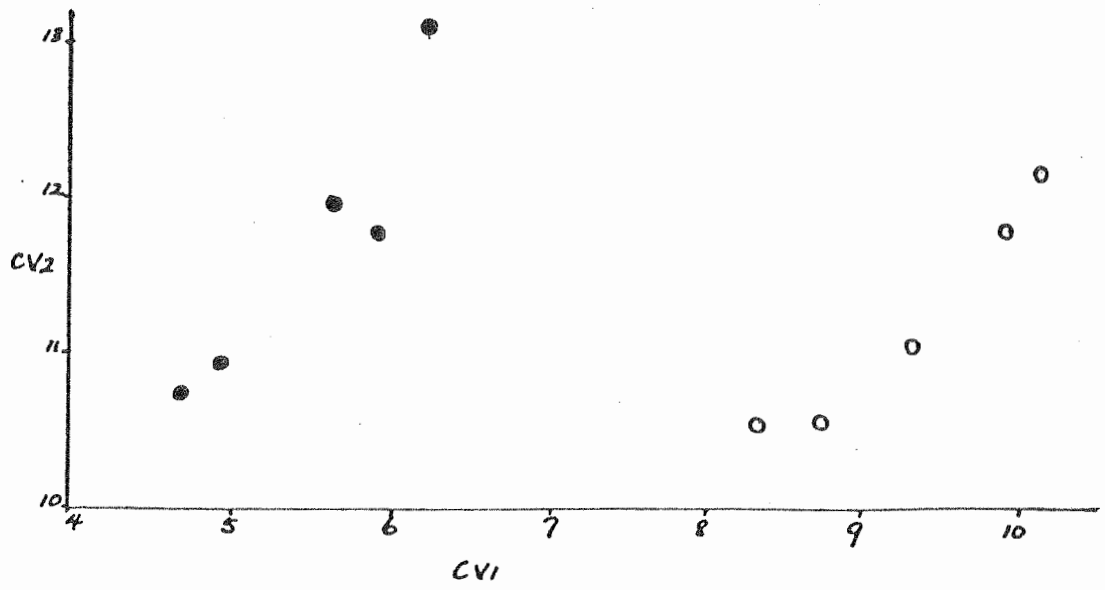


Fig. 6.5. The mean values at 5 different light intensities of *P. glabrata* ● and *P. paradoxa* ○ on the first two canonical variates.

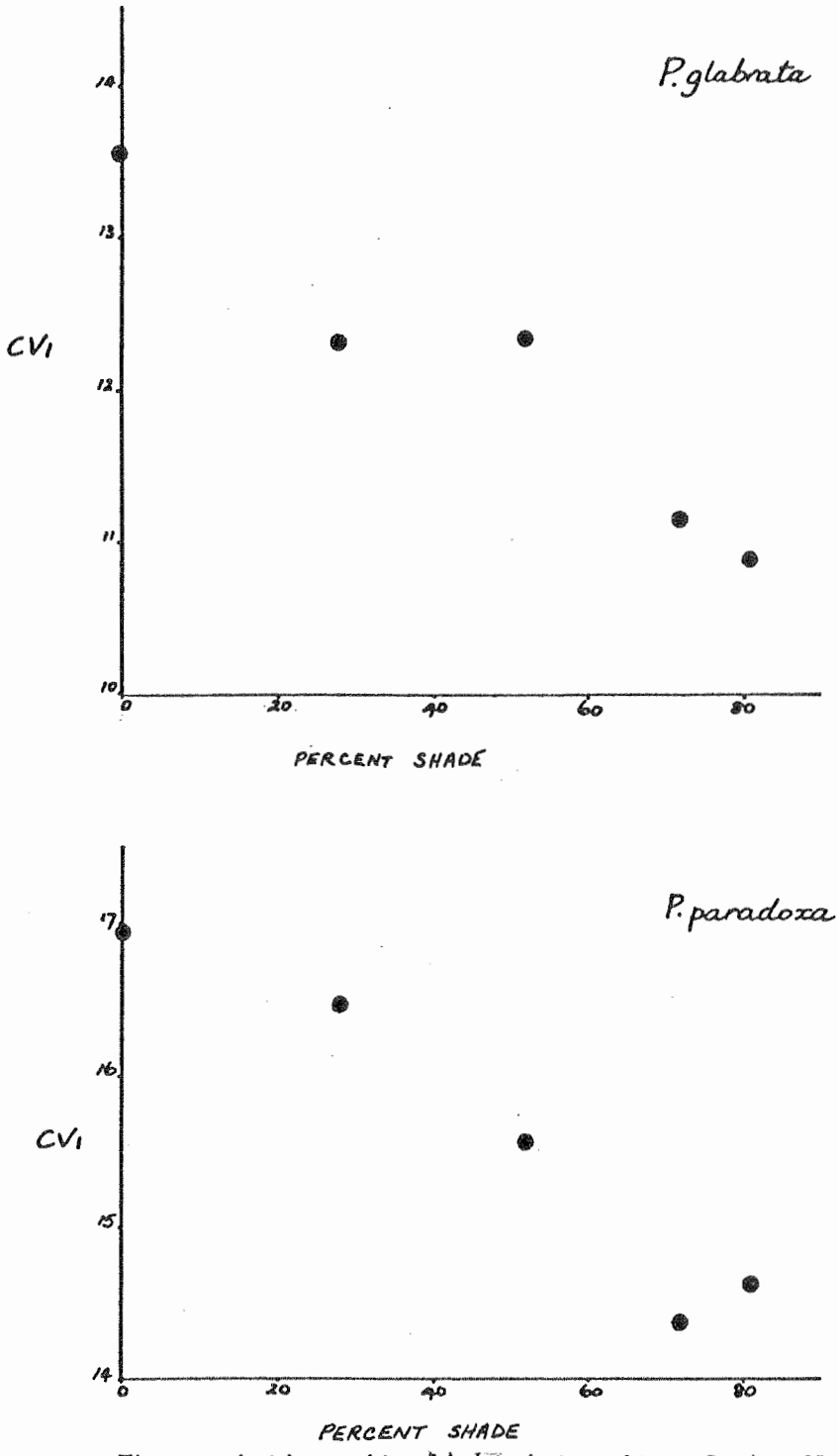


Fig. 6.6. The variation with light intensity of the first canonical variate when *P. glabrata* and *P. paradoxa* are analysed independently.

univariate analyses can be ascribed to the covariation of these characters with the *ll/l* ratio. The leaves of *P. paradoxa* also become more petiolate under reduced light intensities, and there is a concomitant reduction in their relative widths.

The Effect of Photoperiod on Leaf-Shape in the Species

(a) The response of the species to photoperiod

The mean values of the *lwp/l*, *ll/l* and *w/l* ratios for each species-site-daylength combination are given in Appendix D, Table 4(a) (p. 320). The univariate analyses of variance demonstrate a significant species-photoperiod interaction for both the *lwp/l* and *ll/l* ratios (Appendix D, Table 4(b), p. 321). There are significant differences between species and daylength in the *w/l* ratio. The canonical analysis resulted in three significant eigenvectors (Table 4(c) of Appendix D, p. 322). Graphs of the first and second, and first and third canonical variates are presented in Fig. 6.7. The first and second canonical variates distinguish between the species under both photoperiods, although the differences in leaf-shape are reduced under short days. The response exhibited by the species is opposite in direction between photoperiods, and is most pronounced in *P. glabrata*. The first variate resolves in a contrast between the *lwp/l* and *w/l* ratios with the *ll/l* ratio, whilst the second contrasts the *ll/l* and *w/l* ratios. An inspection of the mean values of the original characters (Appendix D, Table 4) indicates that there is an increase in both the relative width, and relative position of maximum breadth in leaves of *P. glabrata* in short days, whereas the opposite is true for *P. paradoxa*.

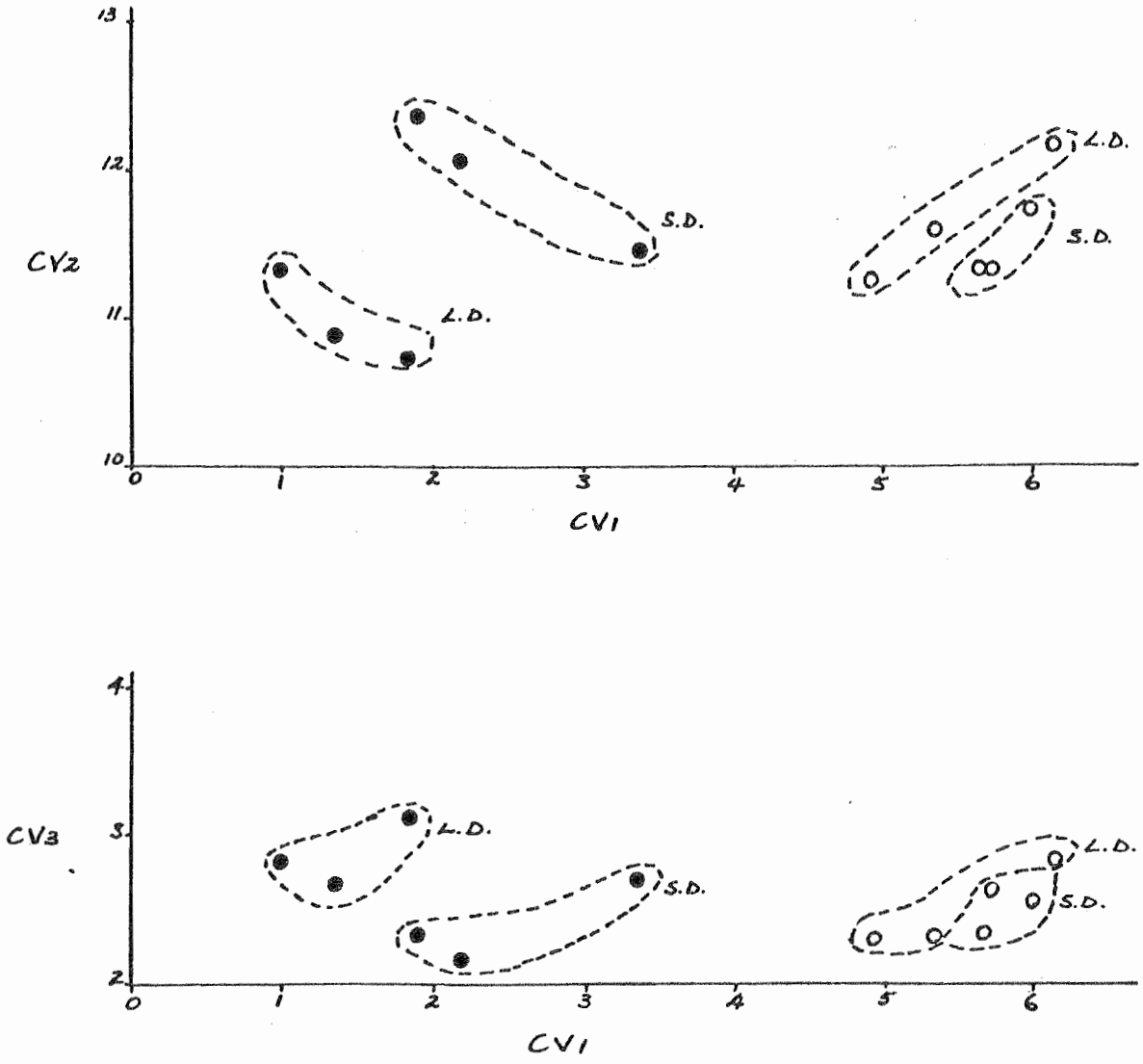


Fig. 6.7. Canonical variates analysis of the response of *P. glabrata* ● and *P. paradoxa* o to long days (LD) and short days (SD).

(b) Intraspecific differences of leaf-shape

The canonical analysis indicates that the differences of leaf-shape in *P. glabrata* observed in the field studies are maintained under glasshouse conditions. The effect is more pronounced if the *w/l* ratio of leaves from each of the three populations are considered (Fig. 6.8). The leaves of plants from Pine Creek are much broader than those from Kannaleena and Arthur's Lake, and the difference becomes more pronounced under short days.

In *P. paradoxa* the leaf-shapes of the three populations are distinct under long days, but the distinction is removed under short days. Possibly the distinction is an artefact resulting from the growth of plants in an artificial environment (continuous light) which is never experienced by plants in the wild. However, the high consistency of performance within plants from each location (as evidenced by the standard errors in Fig. 6.8) suggests that the tolerance limits of the plants probably have not been exceeded.

It is interesting that *P. paradoxa* exhibits site-specific differentiation of leaf-shape under glasshouse conditions when no evidence for differences of leaf-shape was found among the parent populations in the field. It appears that the restrictions imposed on *P. paradoxa* by the particular microhabitats in the field have led to a canalization of leaf-form which is recurrent at each site. This canalization limits the phenotypic expression of the underlying differences of leaf-form which occur between locations and which are expressed under non-limiting conditions.

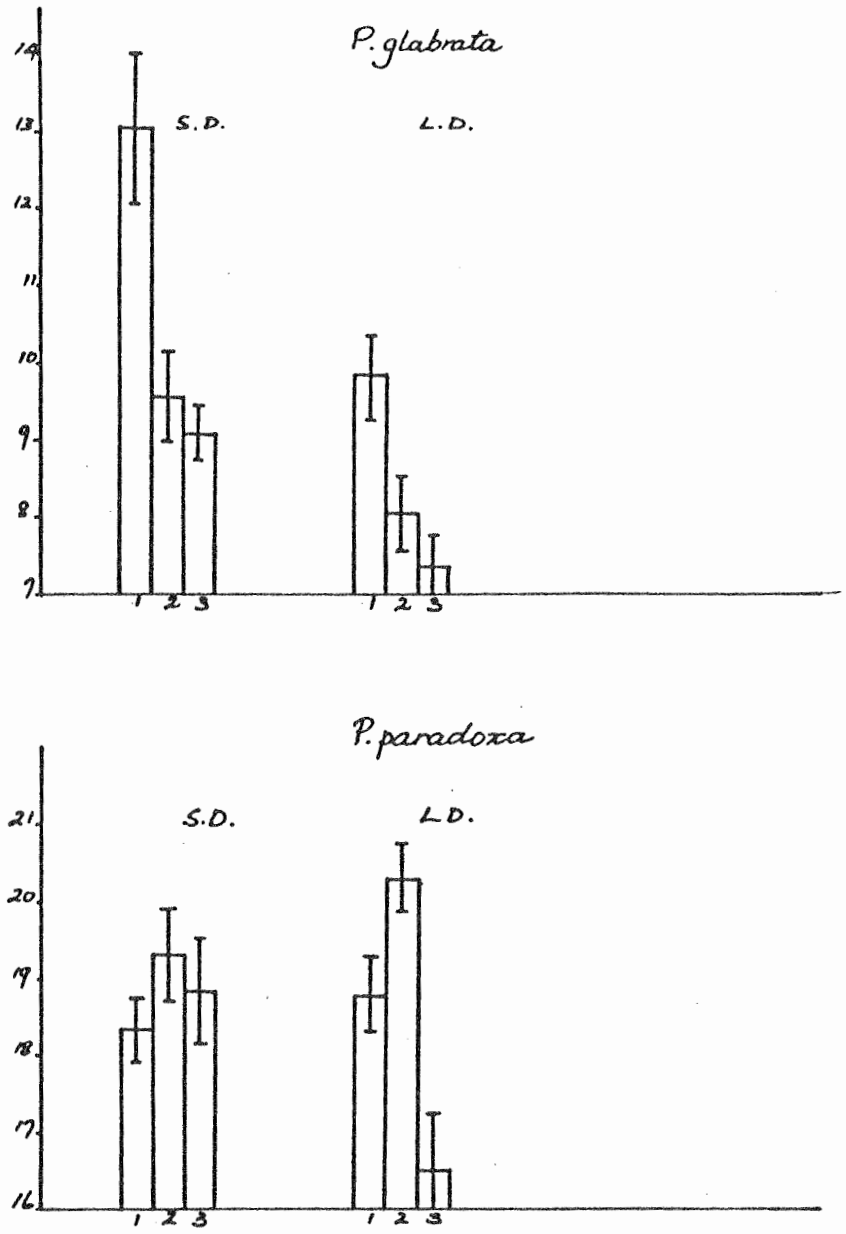


Fig. 6.8. Mean values of the *w/l* ratios of *P. glabrata* *P. paradoxa* under long days (LD) and short days (SD). The population samples are from Pine Creek (1), Kannaleena (2), and Arthur's Lake (3).

There is a difference in the response of the two species with respect to inter-population differentiation of leaf-shape. Both species exhibit differences of leaf-shape between populations in glasshouse conditions, but only *P. glabrata* exhibits these differences in the field. Possibly these results reflect alternative adaptive strategies adopted by the species in response to the different habitats occupied by each at the different locations. Equally, the results may reflect direct phenotypic modification imposed on *P. paradoxa* (but not *P. glabrata*) by the generally high water-table which occurs in the habitats occupied by this species. In the winter months, the leaf-shapes of both species converge, especially when they co-occur in habitats which are wet. However, *P. glabrata* occupies a range of habitats at any one of the three locations (p. 84), whereas *P. paradoxa* uniformly occupies habitats which have a high water table, even in summer.

The Effect of Light-Temperature Interactions on Leaf-Shape in *P. glabrata* and *P. paradoxa*

The mean values of the three leaf characters (*lwp/l, ll/l* and *w/l*) for each species-site under the two temperature and three light regimes, are presented in Appendix D, Table 5(a)(p.323). The four-way analyses of variance for each character resulted in highly significant interactions between species, site, temperature and light (Appendix D, Table 5(b), p.325). These results indicate that there is a complex response to leaf-shape to the altered environmental levels by both species from both sites. This is confirmed by the CVA (Appendix D, Table 5(c), p. 326). The

canonical analysis resulted in three highly significant roots, and these are very nearly unit vectors which respectively represent the original characters *w/l*, *lwp/l* and *ll/l* in decreasing order of magnitude of variances. The canonical analysis is therefore of no value in determining the relative contributions of the various environmental levels to changes in leaf-shape at each species-site. In this case there were two other possible approaches. The first was to partition the SSP matrices by species, season, temperature, light and their various interactions, and calculate the discriminant functions appropriate to each. The second approach, and the one adopted here, was to treat each species-site combination separately, and use the canonical correlation method to obtain sets of coefficients describing the environmental factors, and the concomitant descriptions of leaf-shape. This involved the establishment of a dummy variable (-1,1) to describe the two temperature levels, and two other dummy variables to describe the three light levels. The correlation matrix obtained between the dummy variables and the leaf-shape characters was then partitioned between environments and shape characters, to obtain internally correlated sets of coefficients which were uncorrelated between sets. The whole procedure is fundamentally similar to that of multiple regression in the univariate case, where it is required to relate the response of a (dependent) character to a number of independent variables.

The correlation matrices and results of the analysis are presented in Appendix D, Table 5(d) (p.327). In each case only two significant sets of canonical correlations were obtained. The

analysis of each species-site combination will be considered in turn:-

(i) *P. glabrata*, Lake Augusta

The first set of canonical coefficients shows that the *lwp/l* ratio is associated predominantly with changes in photoperiod, although both temperature and light intensity also affect this character. The second set of coefficients relate the *w/l* ratio to temperature, and there is some evidence that the three shape ratios are affected differentially by the three environmental variables.

(ii) *P. glabrata*, Interlaken

The first canonical coefficients are heavily weighted by the *lwp/l* ratio, but in this case the character is most strongly associated with light intensity rather than daylength, and is also affected by temperature. The second (independent) set of coefficients again relate the *w/l* ratio with temperature, and to some extent light intensity.

(iii) *P. paradoxa*, Lake Augusta

The simplest interpretation of the first set of canonical coefficients is that temperature and daylength interact to produce contrasting effects on the *lwp/l* and *w/l* ratios. The second significant correlation shows a positive association between daylength and light intensity and a negative relationship between these variables and the *w/l* ratio.

(iv) *P. paradoxa*, Interlaken

The first coefficients are weighted predominantly by daylength, and this is associated positively with the *w/l* ratio and negatively with the *lwp/l* ratio. The second set of coefficients shows a positive relationship of temperature with the *w/l* ratio.

In a general way, the study of the effects of the interaction of temperature and light confirm the results obtained when these factors were examined independently. Thus shifts in the *w/l* ratios of leaves appear to be largely temperature controlled in both species, although there is a secondary effect due to photoperiod. Conversely, the *lwp/l* ratio is primarily under photoperiodic control, but is also influenced by temperature. The experiment goes some way towards disentangling the effects of light duration and total incident light energy. These factors would be confounded in the photoperiod experiment(p.126) where it was assumed that light intensity was non-limiting under both photoperiods (8 and 24 hour). The multivariate analysis did not show a significant association between light intensity and the petiole length as measured by the *ll/l* ratio. This is probably due to the relatively low light energies used in this experiment in comparison with the shading experiment (p.122).

The light-temperature interaction experiment is significant since it establishes that the response of the different characters to each environmental factor is to some extent specific to each species-site combination. The specificity occurs in two ways. Firstly, there is a difference between species and between sites in the degree of plasticity exhibited by a particular character in response to the altered level of the environmental factor under consideration. Secondly, within each species, the phenotypic expression of the leaf-shape characters is controlled to a different extent by the various environmental factors.

Concluding Remarks

The leaf-characters of each species were affected to some extent by all of the environmental factors which were studied under glasshouse conditions. The changes induced by the various factors in relation to the observed summer and winter leaf-shapes of the species are summarised in Fig. 6.9.

Not surprisingly, the response to a particular factor was found to be dependent on the provenance, especially for *P. glabrata*. However, it is instructive to consider the implications of the glasshouse studies in relation to the habitats and seasonal effects observed in the field. The comparison can only be tentative, because the results strictly are applicable only to the particular plants and environments in which they were studied. For this reason, the discussion here will be limited to the populations at Arthur's Lake, from which the plants used in the photoperiod, temperature, light intensity and submergence experiments were derived.

At Arthur's Lake, *P. paradoxa* occurs on low-lying wet areas, *P. glabrata* occurs on higher ground, and there are regions of admixture where the two species grow on sites subject to periodic flooding. The habitats occupied by *P. glabrata* can be subdivided further into those which are shaded, and those which are unshaded.

On the basis of the glasshouse results, it is possible to predict the phenotypic modifications of form which may be evoked in the leaves of plants in response to the different habitats and to season. For this purpose, the summer leaves of the species which occur in admixture at an unshaded, non-submerged site, will be considered as the base-line from which phenotypic modification

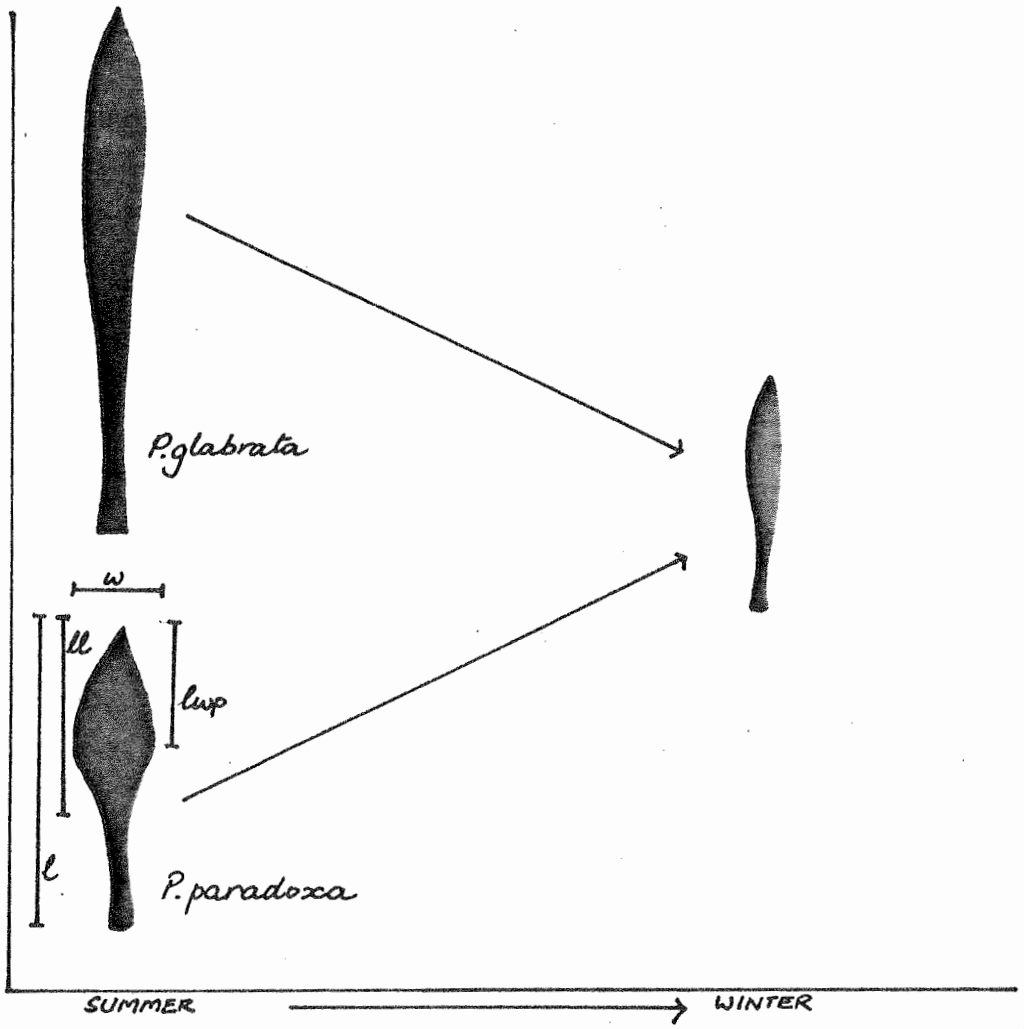


Fig. 6.9. The seasonal response of leaf shape in *P. paradoxa* and *P. glabrata*. The table below summarises the effects of environmental factors on individual leaf characters in the species.

leaf/character	species	lower temperature	shorter photoperiod	lower light intensity	increasing inundation
relative length *l*	*P. paradoxa*	→	→	←	←
	P. glabrata	→	→	←	←
relative lamina length *ll*	*P. paradoxa*	-	-	→	→
	P. glabrata	-	-	→	→
relative position of widest part *lwp*	*P. paradoxa*	→	→	-	-
	P. glabrata	→	→	-	-
relative width *w*	*P. paradoxa*	→	→	←	→
	P. glabrata	→	→	←	→

takes place. Under these conditions, the leaves of *P. paradoxa* are broadly-ovate and more or less petiolate compared with *P. glabrata* leaves which are longer, oblanceolate and not markedly petiolate (Fig. 6.9).

If the site is flooded, then the leaves of both species become petiolate, and those of *P. paradoxa* become more elongate, with some reduction in their relative width. Thus there is a partial convergence of leaf-form, but the leaves of the species remain distinct because their *lwp/l* and *w/l* ratios differ. If the plants are shaded, the leaves of both species become more petiolate, but the species will differ again in their *lwp/l* and *w/l* ratio. The effect of lower temperatures on the species (e.g. an unseasonable cold spell) is to produce a convergence of the *w/l* ratios of the two species, but the *lwp/l* ratios remain distinct.

However, with the onset of winter, temperatures are reduced (and the *w/l* ratios are convergent), the photoperiod is short(and the *lwp/l* ratios converge) and there is an increased duration and degree of flooding of the admixed habitat (and the *ll/l* ratios converge) so that the convergence of leaf-form between the species is absolute with respect to the measured characters. A trend towards convergence is also elicited in winter even when the plants are growing in disjunct habitats, but in this case the convergence is not absolute, because of the different microtopographical conditions. The *P. paradoxa* plants are immersed in water for considerable periods, whilst those of *P. antarctica* occur on freely draining sites.

It appears from this discussion that the ultimate leaf-form adopted by a particular plant of either species will depend on the magnitude and direction of the response of individual aspects of leaf-shape to the environmental change. For example, temperature produced a differential response in the *w/l* ratio of leaves in the

species. However, this character is also affected to some extent by light intensity, daylength and immersion in water. The degree to which the *w/l* ratio is affected by these other factors depends on the particular species and on the particular provenance of each species. Conversely, it appears that the different leaf-characters may be differentially affected by single environmental factors. Thus alteration of photoperiod resulted in changes in both the *w/l* and *lwp/l* ratios of *P. paradoxa*, whereas, in *P. glabrata*, the major effect of this factor was to produce changes in the *lwp/l* ratio.

The changes of leaf-form induced in the glasshouse studies were generally in the same direction as those observed at all sites in the field between summer and winter. The field studies showed that the *w/l* and *lwp/l* ratios vary independently in each species, and this effect was also observed in the controlled growth studies. The response of each species to a reduction in temperature and daylength was opposite in direction and these factors at least partly exert an independent control of the *w/l* and *lwp/l* ratios respectively. In the field, there was a trend towards winter convergence of leaf-shape, and this occurred irrespective of the habitats occupied by the species. This effect appears to correspond to the overall seasonal reduction in both temperature and daylength which accompanies the onset of winter.

The seasonal convergence was most marked in situations where the two species grew in admixture and this is the only situation in which *P. glabrata* is normally subject to immersion in water. The convergence therefore appears to be the result of changes in a number of local and widespread

environmental variables. The onset of winter brings associated changes in each of these variables, and their additive effect on the leaf-shape of the two species is one of convergence.

The controlled environment studies, and the heritability tests (Chapter 7), show that within each species there are differences of leaf-shape which appear to be maintained genetically. These differences occur between populations, and between individuals of a single population of each species. In *P. glabrata* the different leaf-shapes are maintained in the field, although plastic modification of leaf-form is also apparent. In *P. paradoxa* the genetic differences are only made manifest when the plants are grown in non-limiting environments. This suggests that in both species there exists some environmental canalization of leaf-shape, which to some extent masks the underlying genetic variability. This effect may be induced by the particular habitats in which the plants grow.

The fact that the plasticity of leaf-shape is able to buffer genetic variability in the sense of Gregor and Watson (1961), implies that the plasticity is of adaptive importance. The importance might merely be that the response allows the individual to over-winter in a favourable dormant state. In this regard, the control exerted by the environment may result in either an active or passive response by the plant to produce a different leaf-form. For example, reduced temperatures could be expected both to limit the supply of carbohydrate to the apex, and to decrease the rate of general metabolic activity, resulting in a reduction of the number of cell divisions. For the same reason, low temperatures may also result in a reduction of the degree of cell expansion.

Either mechanism would lead to an essentially passive response by the species to low temperatures. However, such a response could still be considered as adaptive in one sense. If the plant is able to survive the stress of winter in a dormant state, and is able to produce new leaves in spring, it will have minimised the metabolic costs entailed in the maintenance of the summer leaf-form.

The seasonal convergence of leaf-form in the species could be likened to the absolute convergence produced by winter deciduous species. However, in most cases the deciduous habit is tied to the environmental control of photoperiod, which allows the plants to pre-adapt to winter conditions (Bradshaw, 1965). Thus the deciduous habit is not passive in the above sense.

The convergence of leaf-shape in the *Plantago* spp. may also be active, since it cannot be ascribed solely to a temperature induced reduction of metabolic activity. The effect of temperature was to produce alterations of leaf-shape which were opposite in direction in each species. This implies that there is some degree of active response to temperature by at least one of the species. Kimball and Salisbury (1974) have demonstrated that plant species growing under snow may still exhibit active development but at a reduced level.

The changes in the *lwp/l* ratio were found to be predominantly under the control of photoperiod. It is difficult to visualize the way in which this change could correspond directly to altered physiological function in the plant.

Subjective observation suggested that the leaf-thickness of both species increased in winter. The measurement of this character was impractical, due to the interference of major veins along the leaf-lamina. It is possible that the changes in

the relative position of the widest part of the leaf may be necessarily associated with the change in leaf-thickness, in order to confer a relatively greater mechanical stability to the leaf.

When grown in water, the plants of both species produce leaves which have similar forms, but which are very different from the leaves produced by non-submerged plants. It is probable that the changes in leaf-shape associated with submergence reflect altered physiological processes in the plant. It was found in *P. paradoxa* that the structural changes accompanying submergence are also associated with differences in stomatal number (Chapter 8). The plants growing in the submerged environment would have transpirational requirements which are very different from those of plants growing in a freely drained situation. Similarly, both the rate of exchange, and the external balance between carbon dioxide and oxygen, would differ between an aqueous and an aerial environment.

7. The Correlation of Leaf-Shape between Parent and Progeny Plants of the Two Species, When Grown in a Common Environment

Introduction

The previous experiments have shown that leaf-shape in both species is plastic in response to changing environmental conditions. However, within any one environment, differences of leaf-shape between the plants of each species were still apparent. The aim of this study was to examine the relationship between the leaf-shapes of parent plants and their progeny when grown in a common environment.

Materials and Methods

Ten plants of each species from each of the three sites (Arthur's Lake, Kannaleena and Pine Creek) were selected at random from among the plants grown in long days in the photoperiod experiment. The remaining plants were discarded. To normalise the plastic response the thirty plants selected for each species were re-potted and grown in the glasshouse in a randomised block design for a further three months.

These plants formed the basis of the progeny testing. In the case of *P. glabrata*, all of the plants were open-pollinated, and it is likely that both self- and cross-fertilization occurred in every plant. This situation is superficially similar to a poly-cross (Falconer, 1970), but self-fertilization is precluded in a poly-cross where the aim is to examine the combining ability of the different 'lines'. It can be assumed that each plant of *P. paradoxa* was self-pollinated, because they were grown individually in cans, and the flowering scapes in this species

are sessile at anthesis.

It was not possible to grow on the progeny from all 60 plants, because of limitations on glasshouse space, instead, the following procedure was used to reduce the number of plants grown while covering the range of variability present.

Five mature leaves per plant were excised and measured for the leaf characters *l*, *lwp*, *ll* and *w*. These measurements were incorporated in a preliminary CVA of the thirty plants for each species. Within each species, the relative positions of their mean vectors along the first two canonical axes, were used as the criteria for the selection of twelve parent plants for progeny testing (*cf*. Riggs, 1973). Seed was collected from these plants, and progeny plants (*ca*. 30) were grown under the same conditions as the parents (Appendix E, Table 2, p.330).

A single mature leaf was excised from each progeny plant and measured for the same four characters. The leaves were picked after the onset of flowering, to minimize ontogenic effects. The progeny measurements were utilized in a PCA to determine the inter-relationships among the variables in each species.

As a result of this analysis the means of progeny values of the leaf-shape descriptors *lwp/l*, *ll/l*, and *w/l* for each species were regressed on the mean parental value as described by Falconer (1970).

To test the independence of these three shape descriptors, the mean values obtained for each from the parents and progeny were incorporated in a canonical correlation analysis (Marriot, 1974) to examine the multivariate heritability of shape of leaves in the two species. The mathematical basis of canonical

correlation is given in Appendix A.2. The partitioning of the correlation matrix in the present experiment takes the form:-

$$\left[\begin{array}{c|c} Rxx & Rxy \\ \hline Ryx & Ryy \end{array}\right] = \left[\begin{array}{ccc|ccc} 1 & Rab & Rac & Rad & Rae & Raf \\ & 1 & Rbc & Rbd & Rbe & Rbf \\ & & 1 & Rcd & Rce & Rcf \\ \hline & & & 1 & Rde & Rdf \\ & & & & 1 & Ref \\ & & & & & 1 \end{array}\right]$$

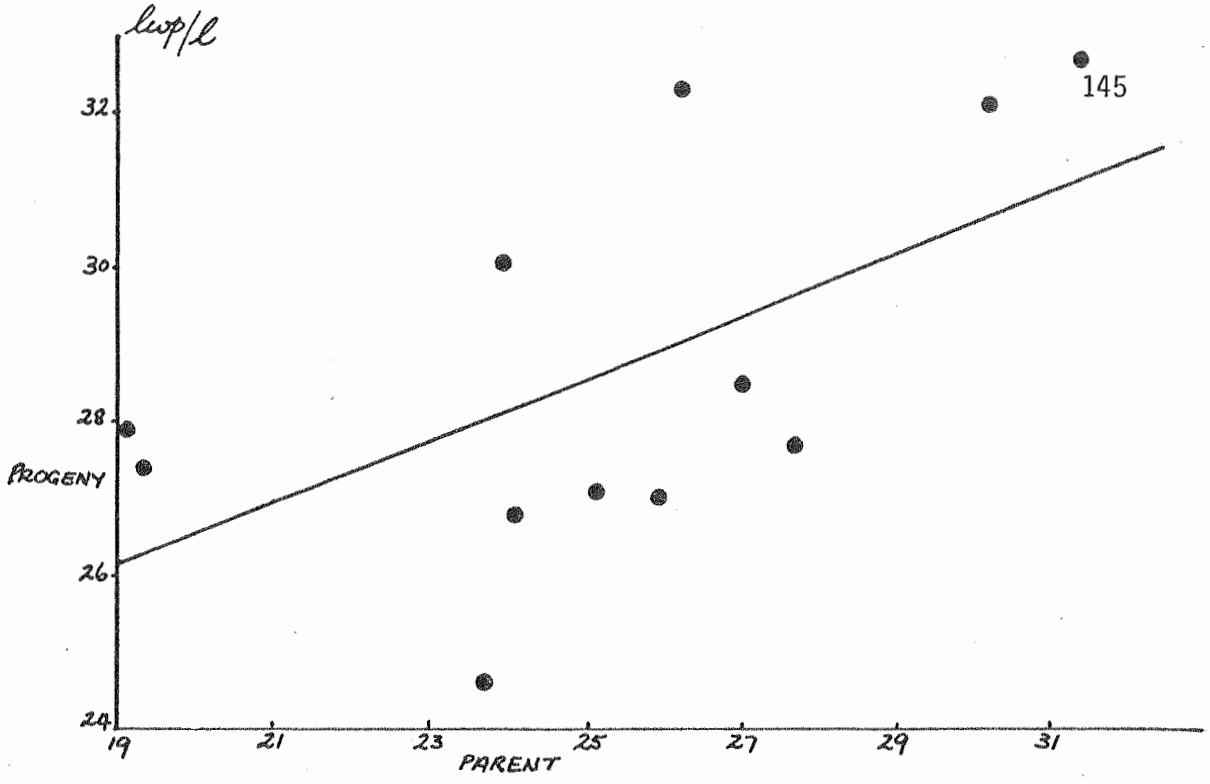
where a-c and d-f are the parent and progeny values of *lwp/l*, *ll/l* and *w/l* respectively, and R is the correlation coefficient.

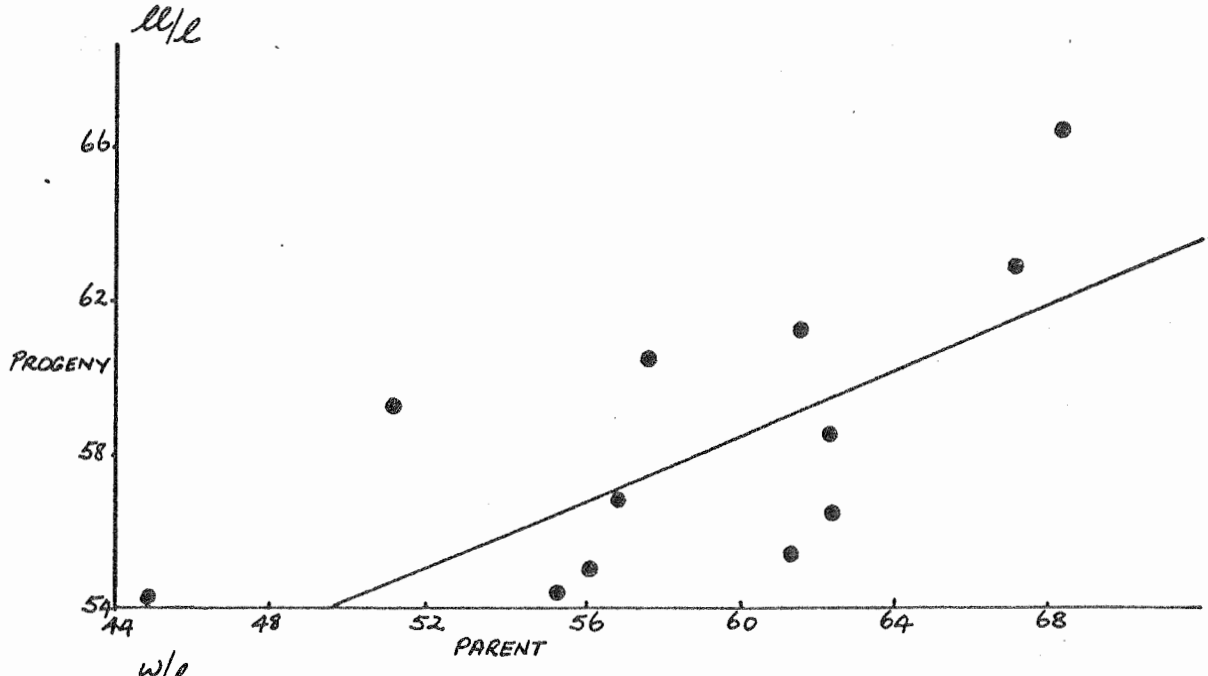
Results

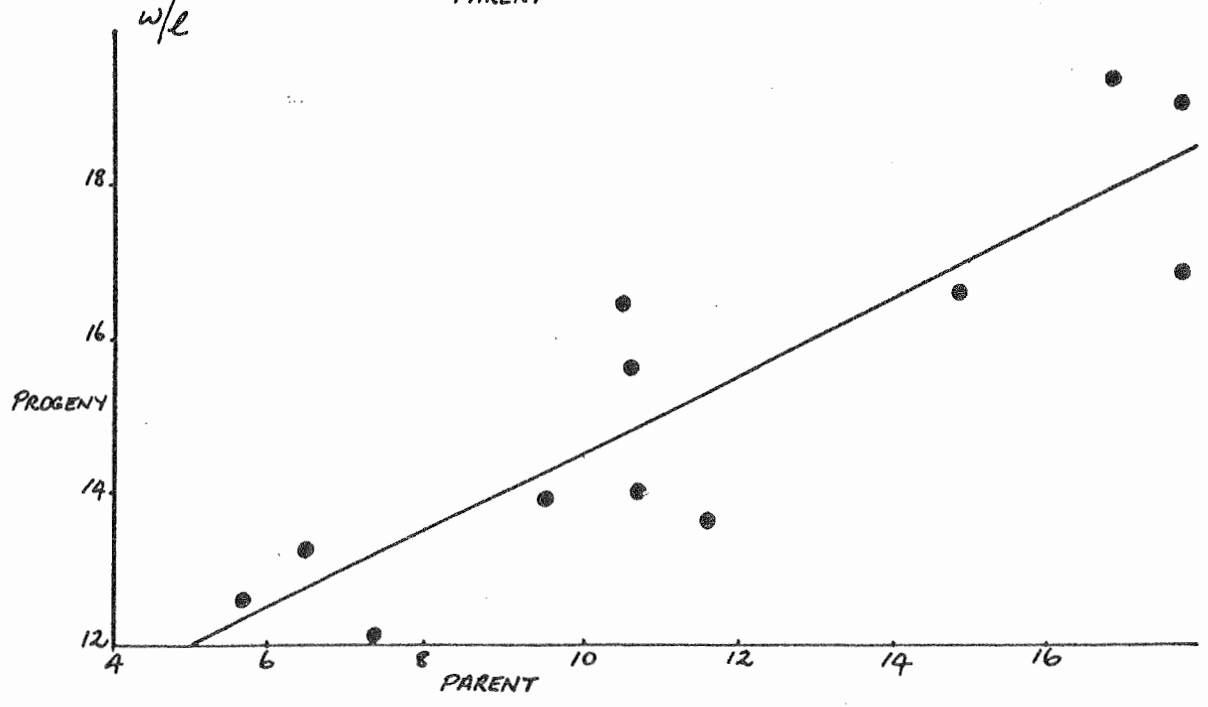
(a) The Progeny Test Using the Univariate Indices of Shape

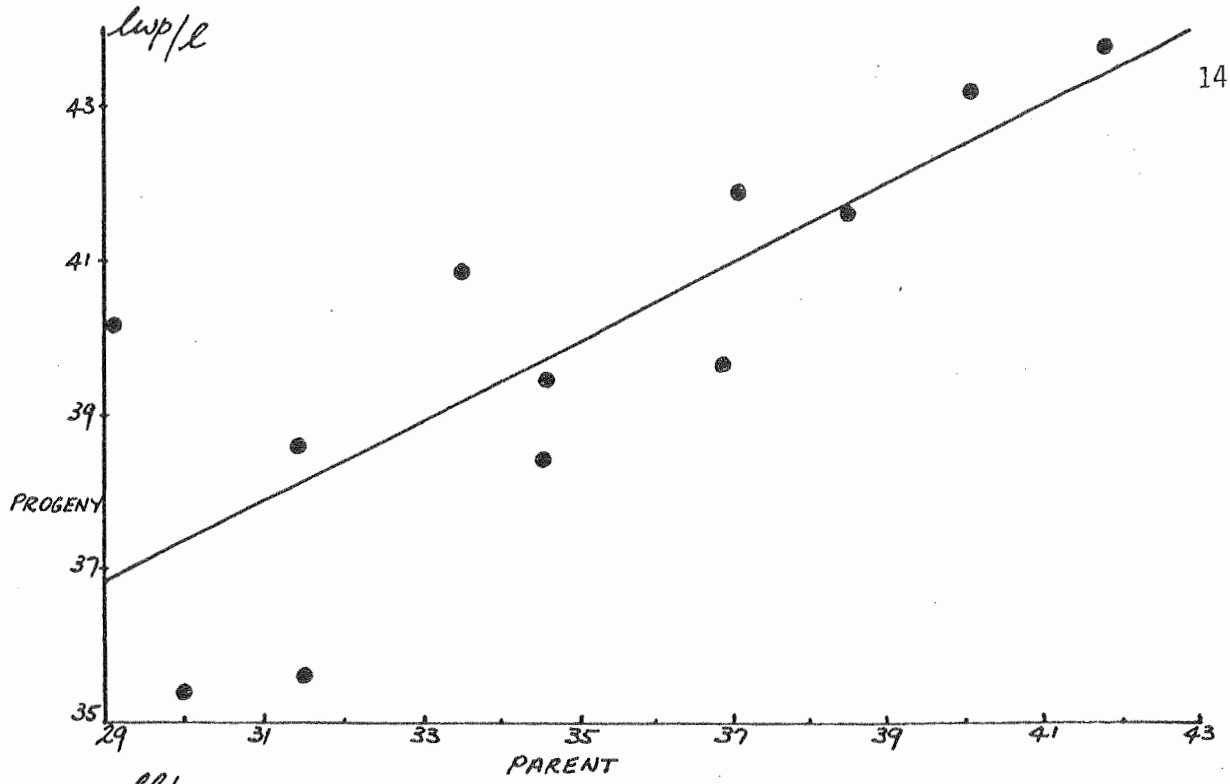
Detailed tabulations of the results are given in Appendix E, Tables 1-3 (p. 329). The PCA indicates that size effects comprise the largest part of the variation among progeny plants in the two species (82% in *P. glabrata* and 92% in *P. paradoxa*). In both species, the remaining vectors consist of contrasts of *w* to *l*, *lwp* to *l* and *ll* to *l* in order of decreasing magnitude of variance. Since these contrasts obtain along orthogonal principal axes, the analysis suggests that the simple ratios *w/l*, *lwp/l* and *ll/l* are suitable descriptors of leaf-shape for the testing of parent-progeny relationships.

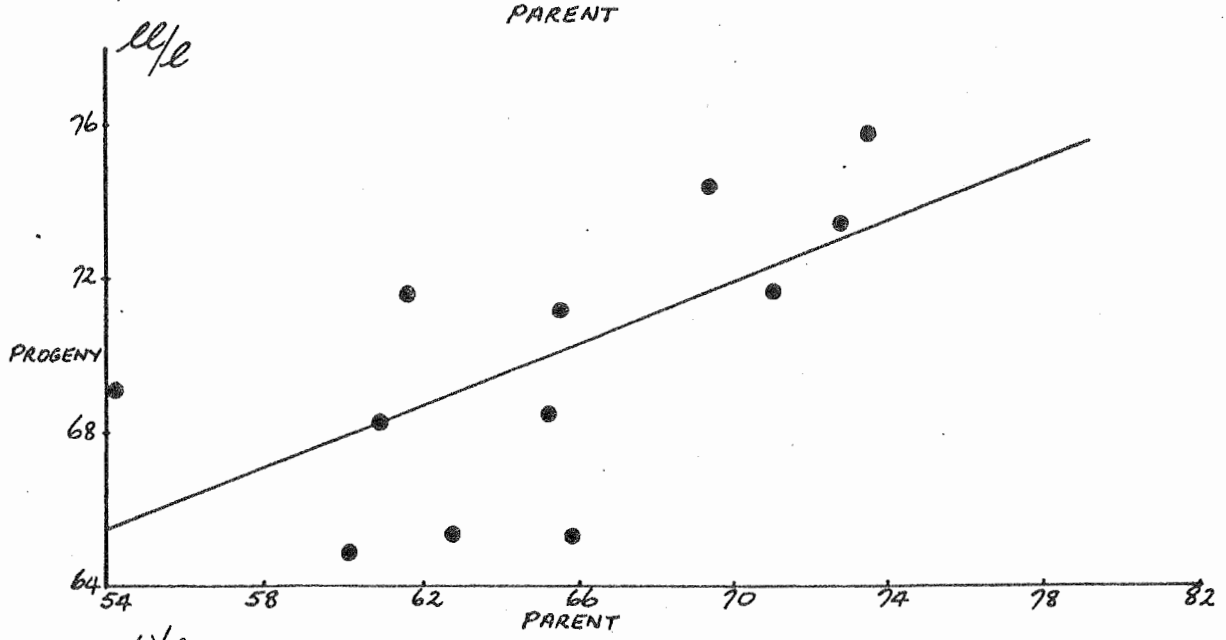
The regressions for these characters of progeny on parents in each species are presented in Figs. 7.1. and 7.2. The

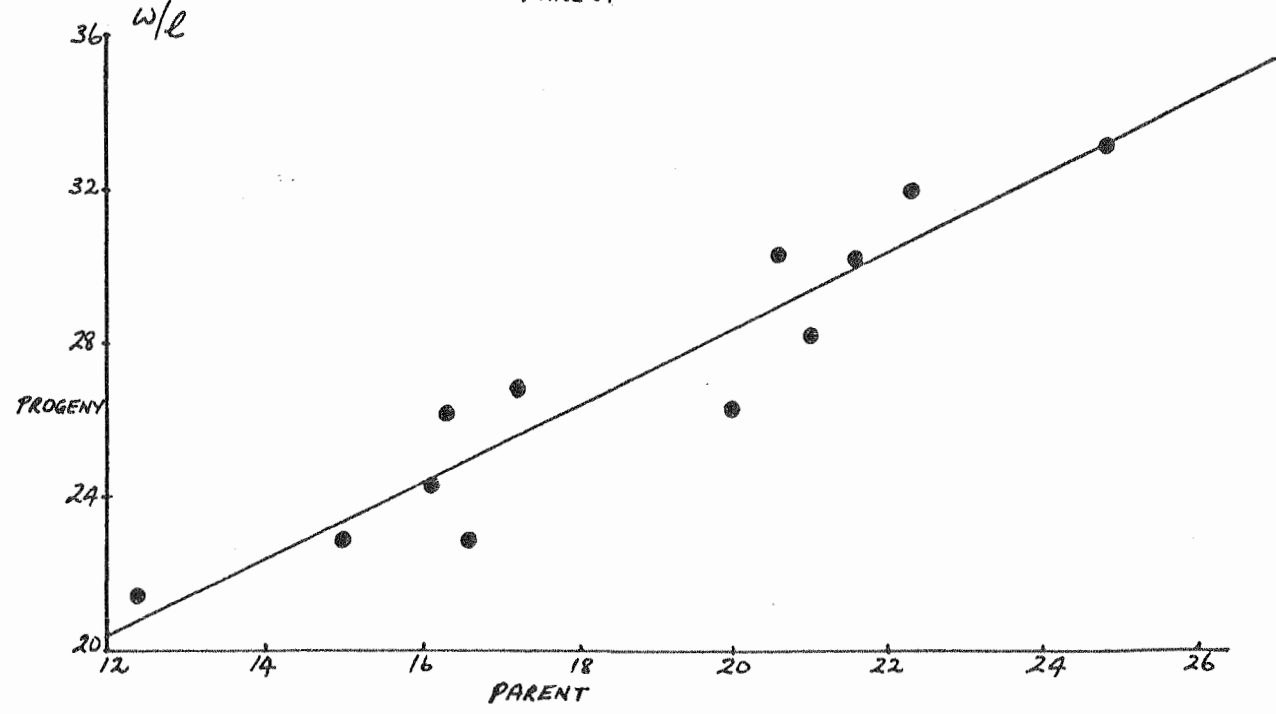

lwp/l
32
30
28
PROGENY
26
24
19
21
23
25
27
29
31
PARENT


ll/l
66
62
PROGENY
58
54
44
48
52
56
60
64
68
PARENT


w/l
18
16
PROGENY
14
12
4
6
8
10
12
14
16
PARENT


lwp/l
43
41
39
PROGENY
37
35
29
31
33
35
37
39
41
43
PARENT


ll/l
76
72
PROGENY
68
64
54
58
62
66
70
74
78
82
PARENT


w/l
36
32
28
PROGENY
24
20
12
14
16
18
20
22
24
26
PARENT

regression coefficients (b), intercepts (a), correlation coefficients (r), and F ratios for significance in each case are given in Table 7.1.

Table 7.1. The regression of progeny on parents for three leaf-shape descriptors in *P. glabrata* and *P. paradoxa*.

Species	Descriptor	b	a	r	F(1,10)
P. glabrata	*w/l*	0.507	9.38	0.891	38.34***
	lwp/l	0.399	18.58	0.581	5.08*
	ll/l	0.387	35.64	0.669	8.11*
P. paradoxa	*w/l*	1.003	8.31	0.943	80.06***
	lwp/l	0.516	21.87	0.790	16.58**
	ll/l	0.407	43.46	0.639	6.88*

The regressions of *w/l* are highly significant in both species, indicating that there is a significant genetic component in the expression of this character under the prevailing environmental conditions. The regression of *lwp/l* of parent/progeny is highly significant in *P. paradoxa*, but just attains the 5% level of significance in *P. glabrata*. The *ll/l* regressions also reach the 5% significance level in the two species.

(b) Canonical Correlation Analysis

The first two sets of canonical coefficients obtained for *P. paradoxa* have high internal correlations (r = 0.97, 0.83). Inspection of these coefficients reveals that the first is most heavily weighted by *w/l* for both parents and progeny, reflecting the heritable nature of this character. The second (independent) set of coefficients is dominated by *lwp/l*.

In *P. glabrata*, only the first set of coefficients are highly correlated (r = 0.96), and again are most heavily weighted by the *w/l* ratio for both parents and progeny.

Discussion

The univariate analyses suggest that the three characters (to a greater or lesser extent) are under genetic control in both species. The results for *w/l* in the two species, and for *lwp/l* in *P. paradoxa* are unequivocal. The low level of significance for *ll/l* in both species, and for *lwp/l* in *P. glabrata* suggest that a greater number of progeny would require testing before the heritability of these characters could be adequately demonstrated. However, the situation is clarified by the multivariate analysis. This supports the hypothesis of the genetic basis of inheritance of *w/l* in *P. glabrata* and *P. paradoxa*, and of *lwp/l* in *P. paradoxa*.

The analysis further suggests that the remaining characters have low internal correlations between parent and progeny plants, and thus are not strongly heritable. The significant results obtained in the univariate analyses can be ascribed to the covariation of these characters with the width-length ratio, since this is removed in the multivariate analysis. Furthermore, the multivariate analytical results for *P. paradoxa* indicate that the *w/l* and the *lwp/l* ratios are inherited independently and are therefore presumably controlled by different genes.

The estimation of the degree of heritability from the regression coefficients for the descriptors which gave significant regressions is complicated by the nature of the experimental

situation and the breeding systems of the species. *P. paradoxa* produces flowers on scapes which are sessile at anthesis, so that the plants are largely self-pollinating. In the field, outbreeding can occur by the water-borne transport of pollen between plants, especially since the species usually occupies wet sites. The individuals were grown in separate cans under glasshouse conditions so that self-pollination can be assumed. In this case the degree of heritability of a character is estimated by the offspring-midparent regression, and is equal to the regression coefficient (Falconer, 1970), i.e. $h^2 = 1.0$ for *w/l* and 0.52 for *lwp/l*. The situation is further complicated by the occurrence of ontogenetic drift (Clifford-Evans, 1972) in the species. Thus the parent and progeny plants were of different ages, and might be expected to exhibit age-dependent changes of leaf-shape, in addition to effects caused by small changes in light and temperature regimes not controllable under glasshouse conditions. Nevertheless, such effects are apparently genotype specific, since the analysis demonstrates a high correlation in leaf-shape between parent and progeny, even though the actual leaf-shapes of the two are different. In the *w/l* comparison, for example, the slope of the regression line is unity, whilst the value for a (the intercept) is 8.31, suggesting that in the progeny, the leaves are much broader related to their length, compared with the parent plants. If, on the other hand, the leaf-shapes of the progeny had been identical with the parents, then the intercept would pass through the origin.

P. glabrata is wind-pollinated, but plants grown in isolation appear to be fully self-compatible. Under the conditions of the

present experiment, it is most likely that both selfing and cross pollination have occurred. In this case, the model for estimating the degree of heritability of leaf-shape is confounded between that of offspring with one parent ($h^2 = 2b$) and offspring with mid-parent ($h^2 = b$). All that can be ascertained is that for *P. glabrata*, the shape character *w/l* has a high genetic component, but the degree of heritability remains unresolved.

The results reported here show that, in a common environment, there is a high correlation between the parent and progeny plants of *P. paradoxa* in respect of the *w/l* and *lwp/l* ratios of leaves. The same situation applies to *P. glabrata* in respect of the *w/l* ratio. These results and the results obtained earlier (pp. 113-140) suggest two avenues for further research. Firstly, the common environment studies which were reported in the earlier chapters indicate only the averaged response of each species to the different environmental conditions associated with seasonal change. These studies have shown also that populations of a species may have a differential response to altered environments. However, they do not give any information about the responses of particular individuals within any one population, i.e. an estimate is needed of the genotype-environment interactions within each species. Such an estimate would indicate the relative developmental flexibilities of genotypes within species (Westerman and Lawrence, 1970), and, therefore, whether individual populations differ in their adaptive strategies. Secondly, controlled crosses are needed to establish the formal genetics of leaf-shape inheritance in the two species. Both of these avenues for further research work were considered to be beyond the scope of the present work.

8. The Correlation Between Leaf-Shape and Habitat in *P. paradoxa*

Introduction

The usual habitats occupied by *P. paradoxa* on the Central Plateau are the closed grassland and herbfield communities which border the lakes and streams. At these sites the plants typically have leaves which are broadly ovate, ± petiolate, denticulate-dentate. The leaves have bands of hairs on wart-like thickenings across the upper surface. It has been established that this general pattern of leaf-development is subject to modification both by genotypic changes and, within a genotype, by plastic responses to particular environmental regimes.

At two locations on the Central Plateau, plants were found growing in habitats which differed considerably from that described above. The locations were at Canal Bay on the Great Lake, and on the banks of the Ouse River immediately to the east of Lake Augusta (Fig. 4.1,p.53). At each location three distinct habitats could be distinguished, and the plants occupying each of these had leaves which differed from the typical form of *P. paradoxa*, and also differed from each other. Indeed, the plants were not immediately recognised as belonging to the same species.

The habitats and corresponding leaf-forms at the two locations were as follows:-

1. Soaks: These are depressed areas having a high water table and where fresh water seepage is a year-round phenomenon. The leaves of plants in these habitats were typically large, ± entire, glabrous and ovate-elliptic (Fig. 8.1a).

2. Mudflats: Small areas of drained mud on the shores of the lake or river. The water level at these sites fluctuates in

all seasons,so that the plants are subject to inundation of varying frequency and depth. The leaf-shapes here were elongate-petiolate and linear (Fig. 8.1b).

3. Cushions: At each location diminutive plants were found growing in bolster moor communities dominated by the composite, *Abrotanella forsterioides*. This species forms a tightly compacted cushion which may act as a seed bed for an epiphytic flora of those species capable of colonizing its surface (Jackson, 1972). The *P. paradoxa* plants growing on these cushions bear a strong resemblance to *P. glacialis* (Briggs, Carolin and Pulley, 1973) and to Curtis' (1967) description of *P. muelleri*. The plants are small, with imbricate, coriaceous leaves which are usually glabrous, entire or minutely denticulate and linear elliptic (Fig. 8.1c).

At both locations, the three morphs had inflorescences typical of *P. paradoxa*. The occurrence of such a pronounced morphological differentiation provided an excellent situation in which to study the relative contributions of genetic and plastic response to habitat variation.

Two experiments were undertaken. The first consisted of provenance trials. The second experiment was set up to examine the possibility of hormonal control of leaf-shape in the species.

Provenance Trials

Materials and Methods

At each location, 20 plants of each morph were sampled during January 1973. A single mature leaf of each plant was excised and pressed for later measurement. The plants were

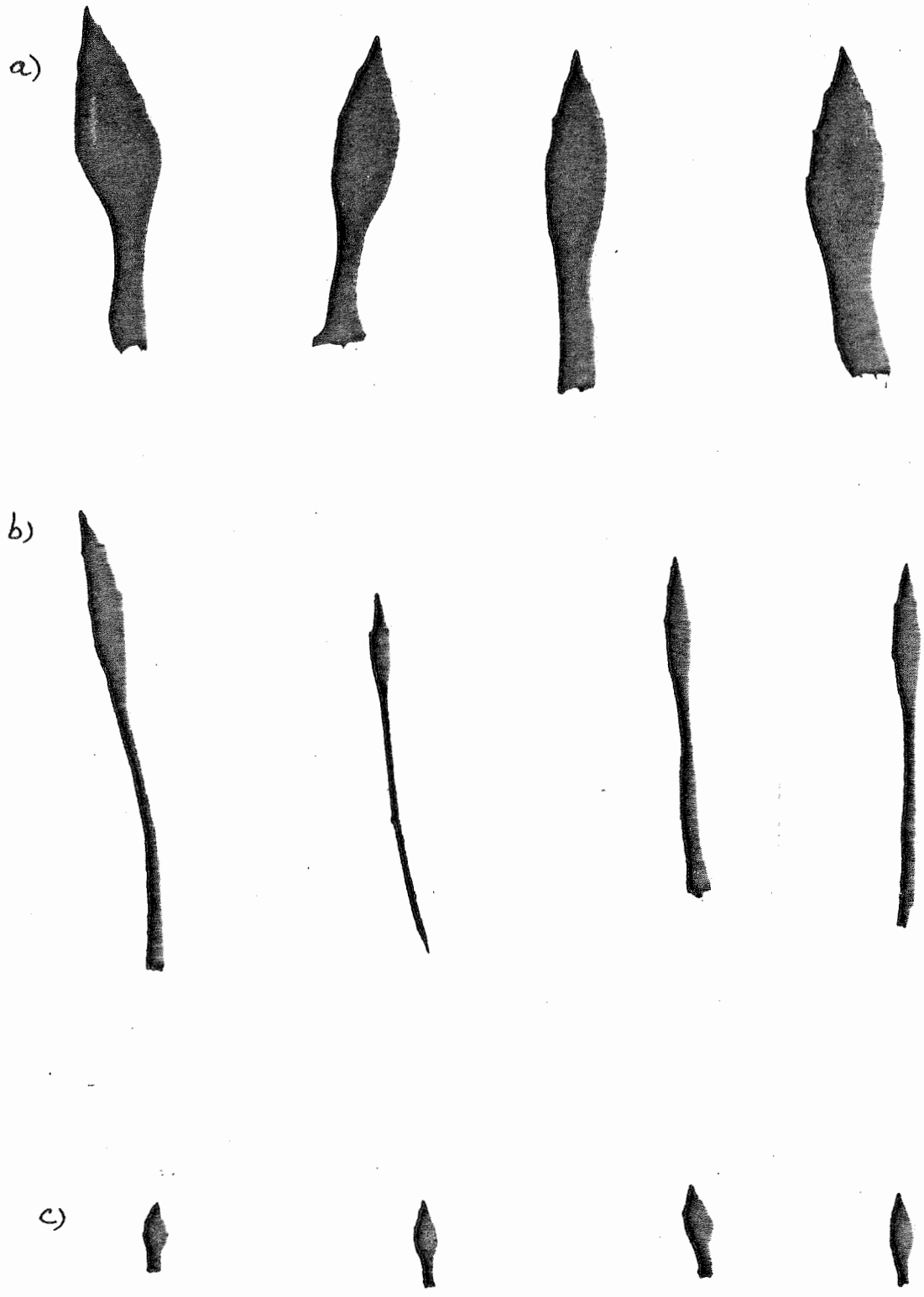


Fig. 8.1. Leaf shapes of *P. paradoxa* plants growing in a) soak; b) mudflat and c) cushion plant communities at Canal Bay.

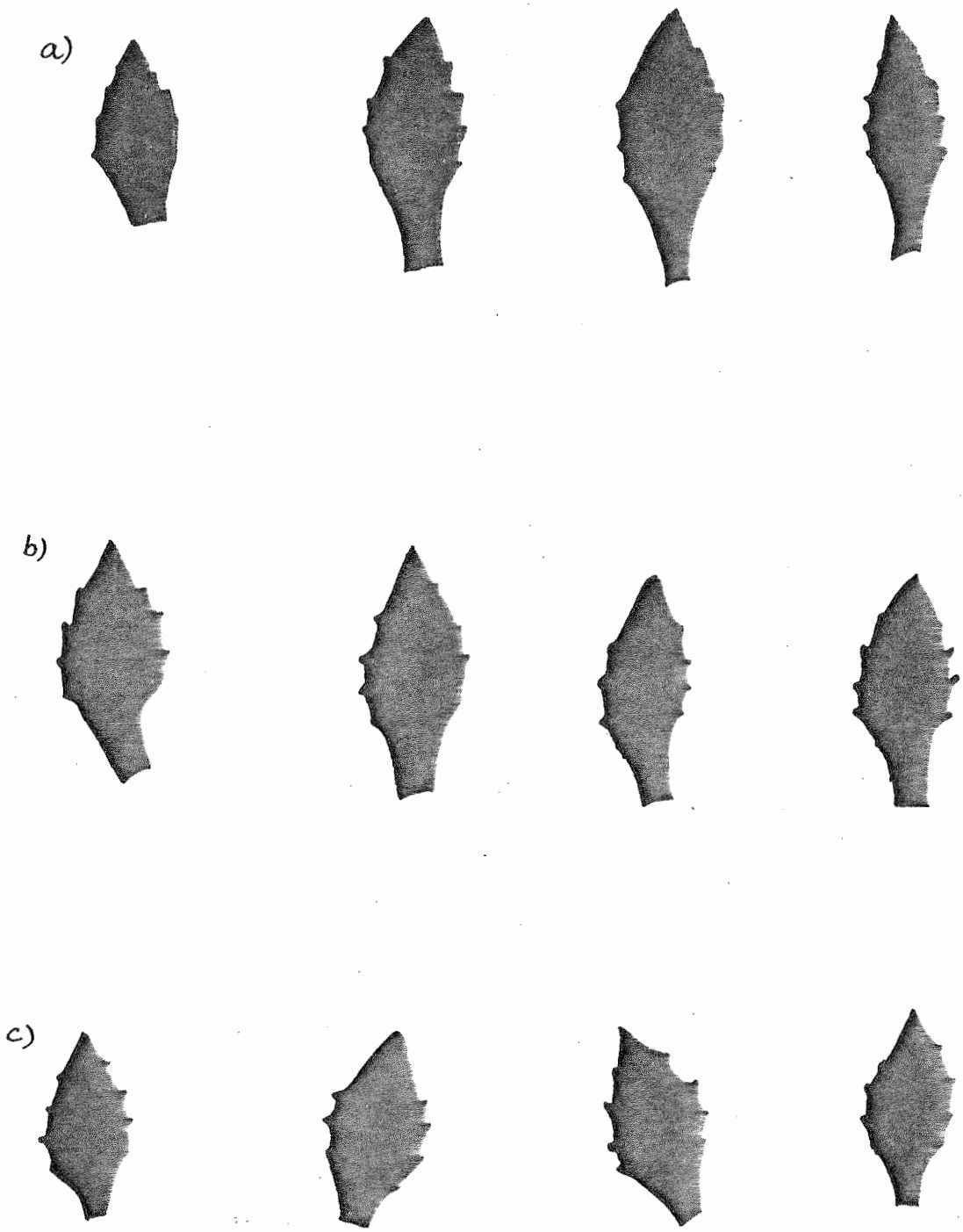


Fig. 8.2. Leaf shapes of *P. paradoxa* transplants from Canal Bay after one year's growth in a common environment.
a) soak; b) mudflat; c) cushion.

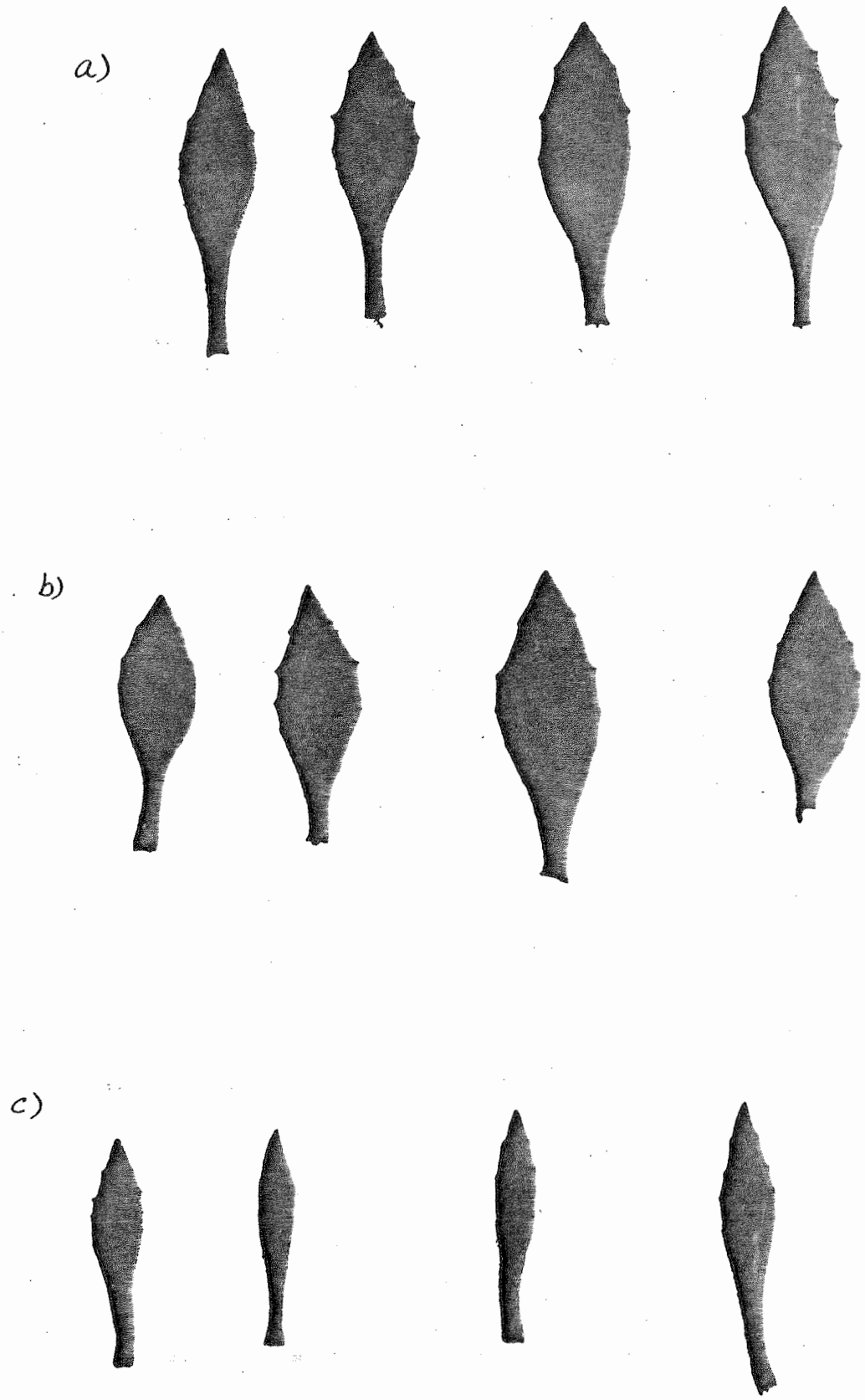


Fig. 8.3. Leaf shapes of *P. paradoxa* transplants from Ouse River after one year's growth in a common environment.
a) soak; b) mudflat; c) cushion.

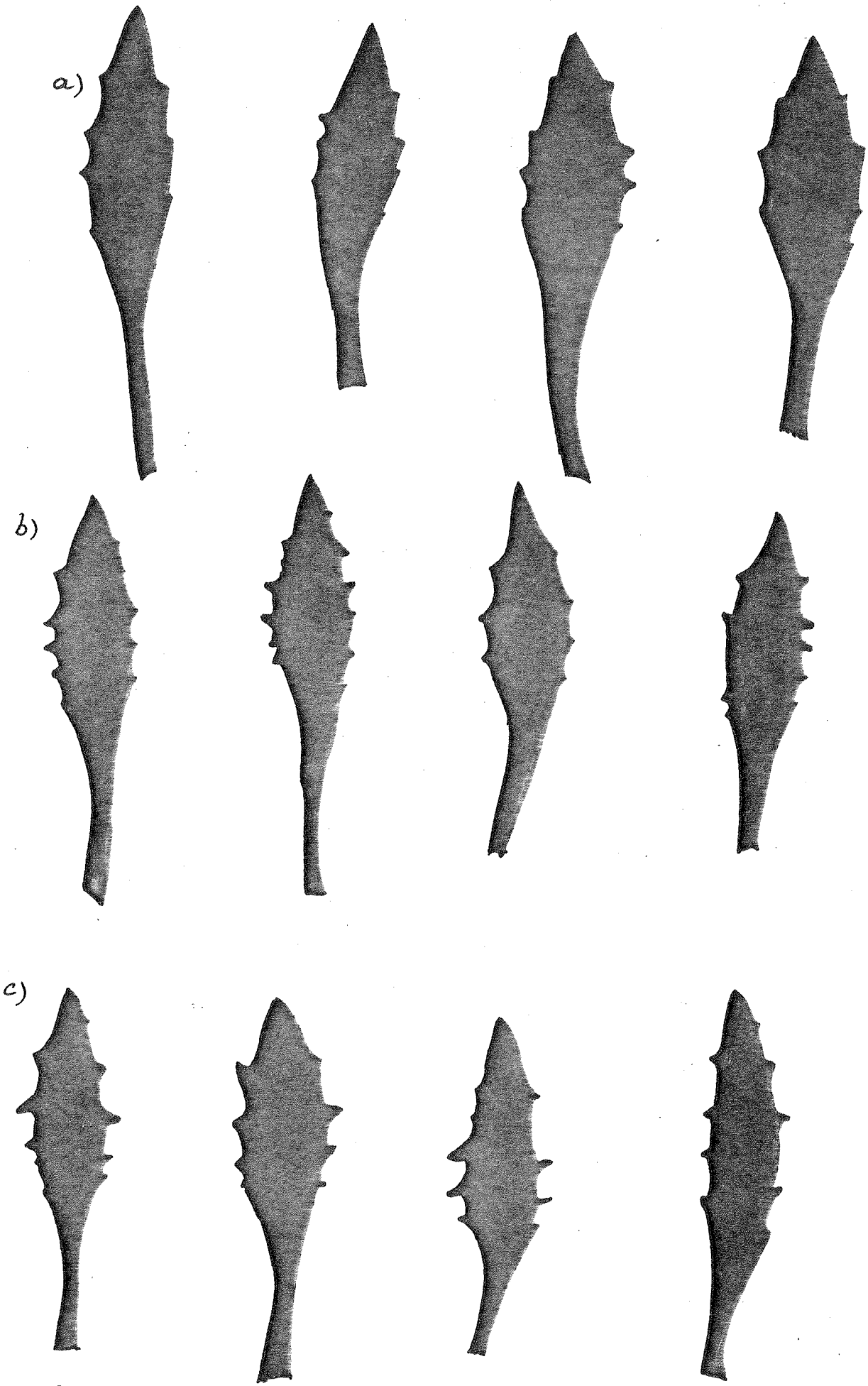


Fig. 8.4. Leaf shapes of progeny plants of *P. paradoxa* from Canal Bay. a) soak; b) mudflat; c) cushion.

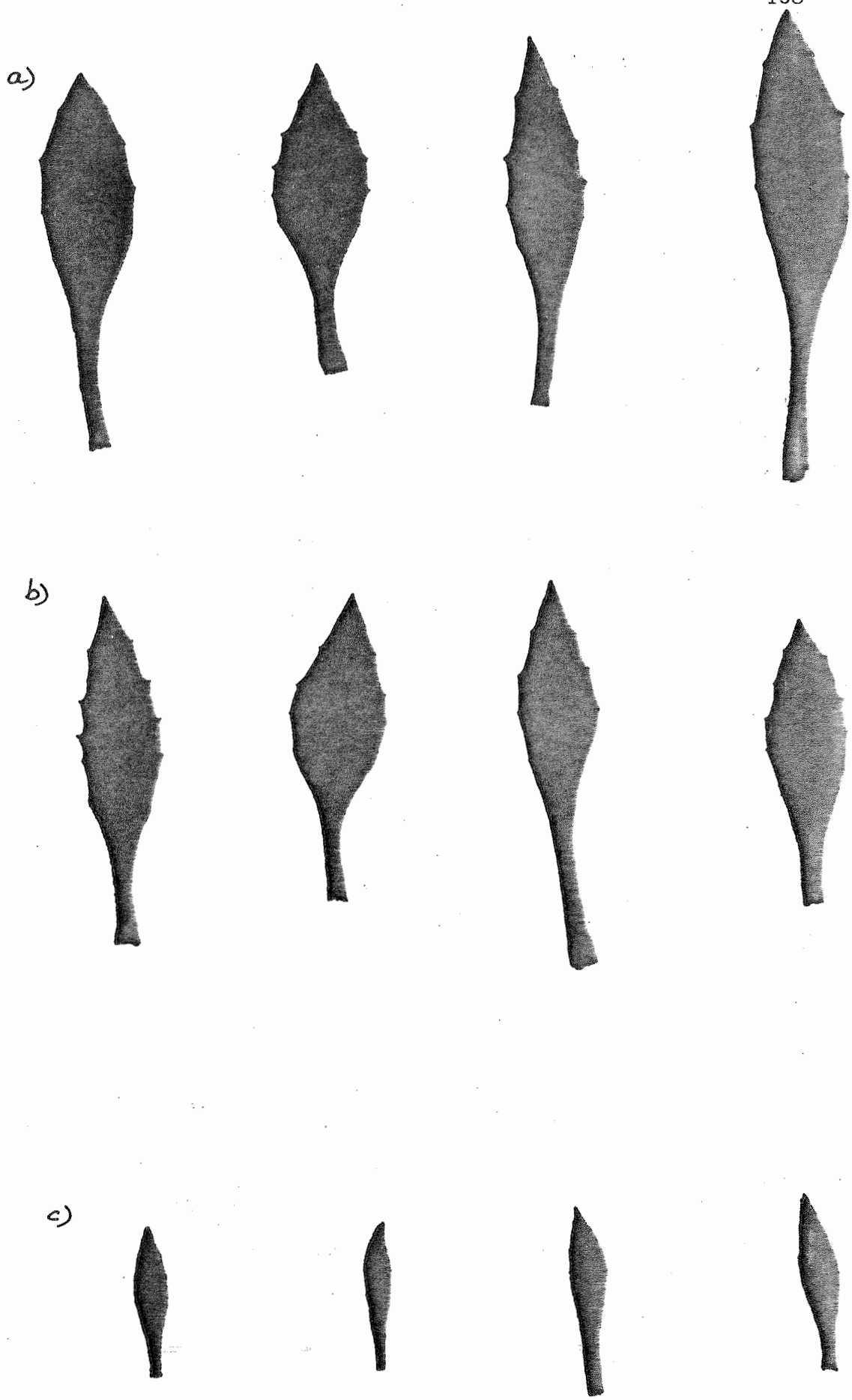


Fig. 8.5. Leaf shape of progeny plants of *P. paradoxa* from Ouse River
a) soak; b) mudflat; c) cushion.

transplanted into cans containing a vermiculite-soil mixture and grown in a randomized block outside the Botany Department glasshouse. It could be assumed that the plants were predominantly self-pollinated, since inflorescences are sub-sessile at anthesis, and each plant was grown in isolation, 3-5 cm below the top of the can. A year later, seed was collected from these plants and bulked within each of the 6 habitat-location combinations to randomise genotypes within each combination. The parent plants were grown in a common environment for a year prior to seed collection, to minimize any maternal effects on seed imposed by the previous habitats. The seed was germinated in petri dishes. Twenty seedlings from each combination were planted out in the glasshouse into cans containing a vermiculite-soil mixture and grown in a randomized block to maturity. A single mature leaf was picked from each progeny plant and pressed for later measurement.

The leaf-characters measured were *l*, *lwp*, *ll* and *w*. The logarithms of the data collected from the plants growing in the field were incorporated in a CVA. A second CVA was conducted on the logarithms of the leaf-measurements of the progeny plants.

Results

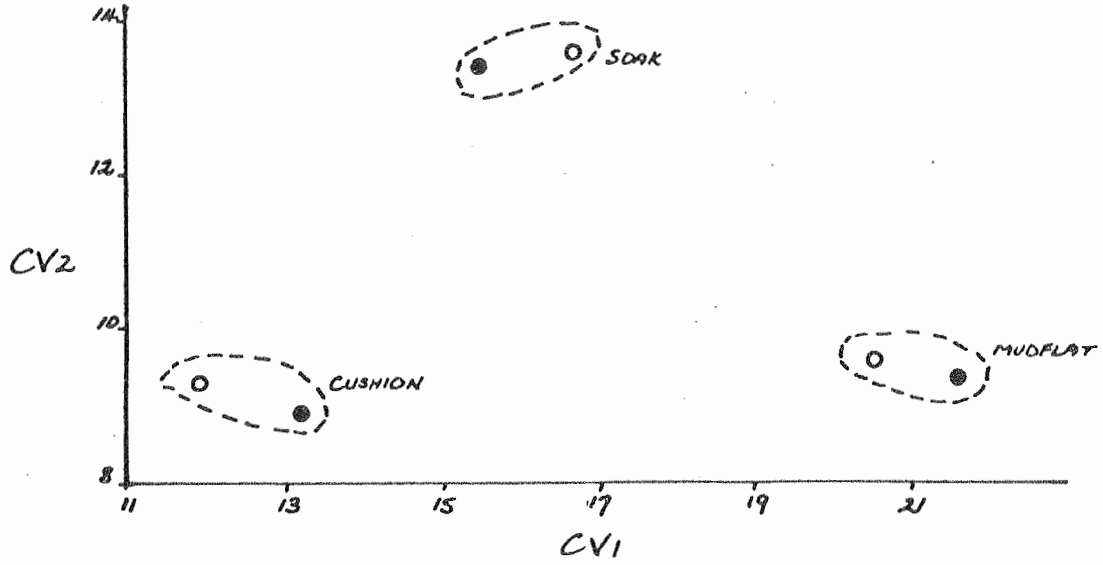
Some typical leaf-shapes of plants from the different habitats at Canal Bay are shown in Fig. 8.1. Differences between locations were negligible. The corresponding leaf-shapes of transplants from both localities after one year's growth in a common environment are shown in Figs. 8.2. and 8.3.

A clear convergence of leaf-form has been elicited in the plants from all three habitats at Canal Bay (Fig. 8.2). The plants from the soak and mudflat on the Ouse River have also converged. However, those from the cushion community at Ouse River remain distinct (Fig. 8.3). These differences in behaviour between locations are found also in the progeny plants (Figs. 8.4 and 8.5).

The mean values of the logarithms of the four leaf-characters and the results of the canonical variates analyses are given in Appendix F, Tables 1, 2 and 3 respectively (pp. 334-336). The CVA of the field data resulted in three highly significant eigenvalues, the first two of which accounted for 98% of the total variation. The positions of the mean vectors of each habitat-location along the first two canonical axes are shown in Fig. 8.6.1. It is evident from this diagram that there is an overall similarity of form within each habitat, irrespective of location, whilst between habitats, leaf-form is widely disparate.

The first two canonical variates obtained from the analysis of the progeny accounted for 97% of the total variation. There is a clear separation of the Canal Bay plants from those of the Ouse River along the first of the axes (Fig. 8.6.2). The analysis also demonstrates the overall similarity of leaf-form in the progeny of the Canal Bay plants. This contrasts with those from the Ouse River, where the progeny of the soak and mudflat plants are similar but are quite distinct from the progeny of plants taken from the cushions.

1.



2.

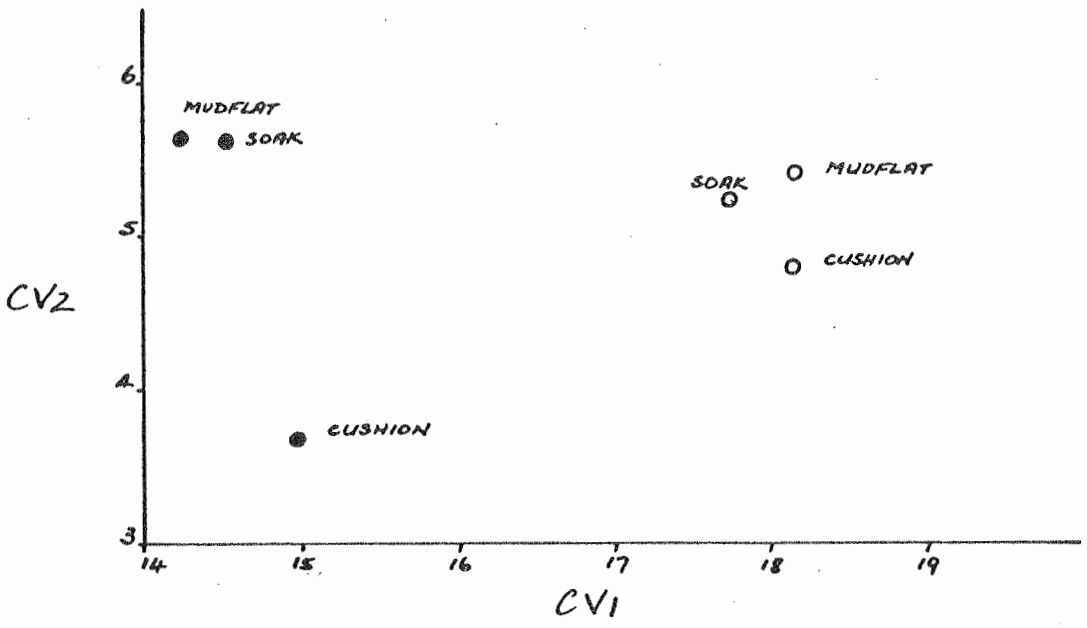


Fig. 8.6 Canonical variates analysis of leaf characters of *P. paradoxa* plants growing in three different habitats at Canal Bay (○) and Ouse River (●).

1. Plants grown in the field.
2. Progeny plants raised in the glasshouse.

The Effect of Gibberellin on Leaf-Shape in *P. paradoxa*

Materials and Methods

In order to examine the possibility of hormonal control of the differences in leaf-shape, an experiment was set up to observe the effects of gibberellic acid (GA_3) on leaf-shape in the species. Seed collected in bulk from the Ouse River population was germinated in the laboratory. Seedlings were grown in the glasshouse in 8 plastic boxes - 24 plants per box, to give a total of 192 plants. Treated plants were given 100µg GA_3 in 10µl ethanol, applied to the first fully expanded leaf below the apex in the ninth week after imbibition. Control plants were given 10µl ethanol. The plants were treated on a randomized block design, half the plants of each box being treated with GA_3 whilst the remainder acted as controls. The plants were scored for the presence of recently expanded dentate, pubescent leaves at zero and 5 weeks from treatment. Chi-square tests were applied to the control and treated groups independently, using the zero week scores as expected values. The GA_3 dose level was deliberately high. Preliminary experiments had shown that a response was initiated with only 20µg GA_3, but the leaf-shapes reverted to the dentate-pubescent form after the expansion of only one or two leaves.

Results

The frequency of dentate-pubescent leaves in the control plants was not significantly different at 5 weeks ($X_1^2 = 0.05$, $0.8 < P < 0.9$), but there was a highly significant difference among the treated plants ($X_1^2 = 77.57$, $P < 0.001$) (Appendix F,

Table 4, p. 337). The foliar application of GA_3 results in the production of plants which are phenocopies of the glabrous entire leaf-forms. In both the control and treated plants, the proportion of dentate to entire leaf-forms was about 2:1 at zero weeks. About one third of the dentate plants remained so after treatment with GA_3.

Discussion

It is evident that *P. paradoxa* exhibits extensive habitat induced modification of leaf-form. The phenotypic expression of leaf-shape within each habitat is closely similar, indicating that expansion by the species to colonize new habitats is accompanied by a rigid canalization of leaf-development, and that this is habitat specific.

An examination of the leaf-silhouettes of the progeny shows that within each group there is an underlying variability of form which is masked by the changes wrought by the particular habitat. It appears that the plasticity may act to buffer the underlying genetic variability, thereby enabling its maintenance in the new environment. This parallels the situation described in *P. lanceolata* by Gregor and Watson (1961).

Some preliminary observations of stomatal number in leaves of the three types from Canal Bay (Appendix F, Table 5, p. 338) show that the differences in leaf-form are accompanied by structural differences which may be of adaptive significance. Thus the number of stomata per unit area is much greater in plants from the cushion community compared with leaves from the other habitats. This might simply reflect an overall reduction in cell size in

these leaves. However, it is interesting to note that the total number of stomata per leaf is in fact greater in the plants from the soaks. In comparison, plants from the other two habitats, have a lower total number and would be subject to (periodically) greater transpirational stress.

High levels of phenotypic plasticity are characteristic of colonizing species (Baker, 1965), but there may often be a physiological cost to the species in making an adjustment at the behest of the environment. Heslop-Harrison (1964) states that there may be selective value in a physiological economy of adaptation, and points to Waddington's (1953) process of genetic assimilation as one means by which this may be achieved. The mechanism could be invoked in the present case to explain the difference in adaptive strategies between the plants of the cushion communities in the two locations. Thus the phenocotype (Davis and Heywood, 1963) observed at Canal Bay corresponds to a genetically fixed form at Lake Augusta.

Whilst recognizing the problems inherent in the extrapolation of controlled environment studies to the field (Daubenmire, 1974), it is interesting to speculate on the control of leaf-form exerted by GA_3 in the present study. The ability of GA_3 simultaneously to induce the production of leaves which are both glabrous and entire, suggests a possible model for the differentiation observed in the field. If the species is capable of regulating the level of GA_3 at the apex in response to the external environment, then leaf-form will undergo plastic modification to 'suit' the new environment. It is possible also that the modification by dominance or penetrance modifiers of an allele at perhaps a single locus

might be sufficient to maintain higher levels of GA_3 production so that leaf-form becomes genetically fixed. On the other hand, while it might be economic for the species to become genetically adapted to the stable environment posed by the cushion community, it would be more advantageous for the plants growing in the mudflat to retain the ability to respond to changes of the environment which are less predictable. Since about one third of the GA_3 treated plants did not alter their leaf-form, the response appears to be genetically limited, so that within a population, individual plants differ in their ability to produce the phenotypic modification.

It is possible that the leaf-form produced in the cushions at Canal Bay is 'mimicking' a different species which occurs in a similar habitat at Ouse River. This situation would create problems for taxonomists and ecologists in the field, because the identity of the particular plants would need to be confirmed by transplanting or by growing from seed. The taxonomic difficulties raised by phenecotypic and genecotypic variation have been discussed at length by Davis and Heywood (1963), who conclude that the range of variation should be included in the description of the taxon, even to the extent of specific mention of the conditions under which the different forms may be produced.

Differences of leaf-shape within the dentate types were observed between Canal Bay and Ouse River. This is possibly the result of selection, but perhaps could also be ascribed to a founder effect. The species is self-compatible, and in many situations may have only limited opportunity for cross-

pollination. But given the high levels of phenotypic plasticity of which the species is capable, the chance establishment of one or a few genotypes could result in a viable population. However, the level of variation between the progeny plants from the two locations appeared to be similar so that the alternative of a founder effect seems remote.

9. A Study of Variation in *P. glabrata* and a Morphometric Comparison of *P. glabrata* and *P. antarctica*

In studies of the seasonal convergence of leaf-shape between *P. paradoxa* and *P. glabrata* (Chs. 5, 6), it was found that some populations of *P. glabrata* had very different leaf-shapes. The differences were apparent in the field, and were maintained when plants were grown in a common glasshouse environment. This section reports on a more detailed examination of the variation shown by some populations of *P. glabrata* on the Central Plateau and the morphometric relationships of these populations to *P. antarctica*.

The descriptive account of the taxonomy of the genus in Tasmania (pp. 36-40) noted that, although *P. glabrata* and *P. antarctica* have quite distinct floral features (and different chromosome numbers), the two species may be confused in the vegetative state, especially when the key character (number of leaf-veins) of Curtis (1967) is used to separate them. At most sites on the Central Plateau, *P. glabrata* shows a range of leaf-shapes from narrowly elliptic-lanceolate to broadly ovate-obovate. The broad leaf-forms of *P. glabrata* key to *P. antarctica*.

Both species occur on the Central Plateau, where *P. antarctica* is 'local in montane grassland' and *P. glabrata* is 'widespread and frequently growing with *P. paradoxa* in montane grassland' (Curtis, 1967). During the course of this work, Interlaken was the only place on the Central Plateau where *P.glabrata* and *P. antarctica* were found growing in the same vicinity.

The following experiments were undertaken.

1. A hybrid index (Anderson, 1954) was constructed using those characters which supposedly differentiate the two species

(Curtis, 1967; Pilger, 1937) and was applied to *P. glabrata* plants from three populations grown in a common environment.

2. Germination rates of seeds of *P. glabrata* collected from four populations were tested at five different temperatures.

3. An examination was made of the flowering times of *P. glabrata* plants from three localities grown in common environments.

4. A multivariate analysis was undertaken to assess the degree of inter- and intra-population differentiation in *P. glabrata* and to compare the morphometric relationship of this species with *P. antarctica*.

Materials and Methods

1. Construction of the Hybrid Index

An examination of published descriptions (Curtis, 1967; Pilger, 1937) of the two species indicated twelve characters which might usefully separate the two taxa (Table 9.1). Accordingly, a hybrid index (Anderson, 1954) was calculated on the basis of these characters for some of the plants grown under long days in the photoperiod experiment (p.116). The numbers of plants scored were 17 from Arthur's Lake, 18 from Kannaleena, and 17 from Pine Creek.

For each character, plants which were similar to *P. antarctica* were assigned the value 0, and those similar to *P. glabrata* the value 2; intermediate characters were scored as unity. The values so obtained were summed over all twelve characters to obtain a hybrid index score for each plant. The index can theoretically range from 0 for 'good' *P. antarctica*, to 24 for *P. glabrata*. Frequency histograms of hybrid indices for the three samples were constructed from these data.

Table 9.1: Characters Used in the Construction of the Hybrid Index between *P. antarctica* and *P. glabrata*.

Character	*P. antarctica*	*P. glabrata*
1. leaf shape	broadly ovate	lanceolate
2. venation	markedly 3-5-nerved	midrib distinct
3. dentation	entire or small distant lobes	lobes present
4. hairs tufted on wart-like thickenings	absent	present
5. leaf indumentum	scattered on both surfaces	upper surface glabrous
6. scape length	± twice leaf length	≃ leaves
7. bract length	< calyces	= calyces
8. bract indumentum	glabrous	fimbriate-ciliate
9. sepal length	*c.* 2 mm	*c.* 2.5 mm
10. sepal indumentum	glabrous	tipped by hair
11. corolla lobe length	≃ tube length	$^2/_3$ tube length
12. scape indumentum	pubescent	pilose

2. Seed Germination Studies

Seed collected in bulk from Interlaken, Kannaleena, Pine Creek and Lake Augusta were sown in eight replicates of 25 seeds per population and placed in controlled temperature cabinets at 5, 10, 15, 20 and 25°C (see Ch. 3 for experimental details). The numbers of seeds germinated were scored daily for 14 days. The rate of seed germination for the four populations was calculated as the inverse of the number of days to 25% germination.

3. Flowering Time

The number of weeks to flowering from sowing date was scored for the plants from Arthur's Lake, Kannaleena and Pine Creek grown under long (24 hour) and short (8 hour) day lengths (p.116). The plants were examined weekly for the presence of floral spikes in the leaf-axils. The experiment was discontinued after 25 weeks.

4. Morphometric Analysis

Six locations were selected to encompass the range of habitats occupied by *P. glabrata* on the Central Plateau. The locations were Clarence Plains, Interlaken, Arthur's Lake, Kannaleena, Pine Creek and Lake Augusta(Table 8, p.345). The populations present at five of these locations exhibited (to a greater or lesser extent) a bimodal distribution of leaf-shape. The plants at each of the five locations were subdivided subjectively into two groups having 'broad' or 'narrow' leaves. At the sixth location (Interlaken), the *P. glabrata* plants were treated as a narrow-leaf sample, and plants of *P. antarctica* were used as the broad-leaf sample. Thus a total of twelve groups were specified for the subsequent

analysis. The sampling structure and sample sizes are given in Table 2 of Appendix G (p.340).

A total of 21 characters were measured for the analysis (Appendix G, Table 3, p. 341). The suite of characters were selected with three *a priori* motives:-

a. To include those characters which traditionally have been used to separate the two species.

b. To provide a sufficiently diverse assemblage as to be meaningful taxonomically, i.e. characters which might reasonably be expected to result from the expression of different genes or gene complexes, and thus not simply be related to each other through the effects of a common selective factor.

c. To obtain transformed characters which might represent viable contrasts of form within a single organ between taxa, i.e. differences in leaf, sepal and petal shape.

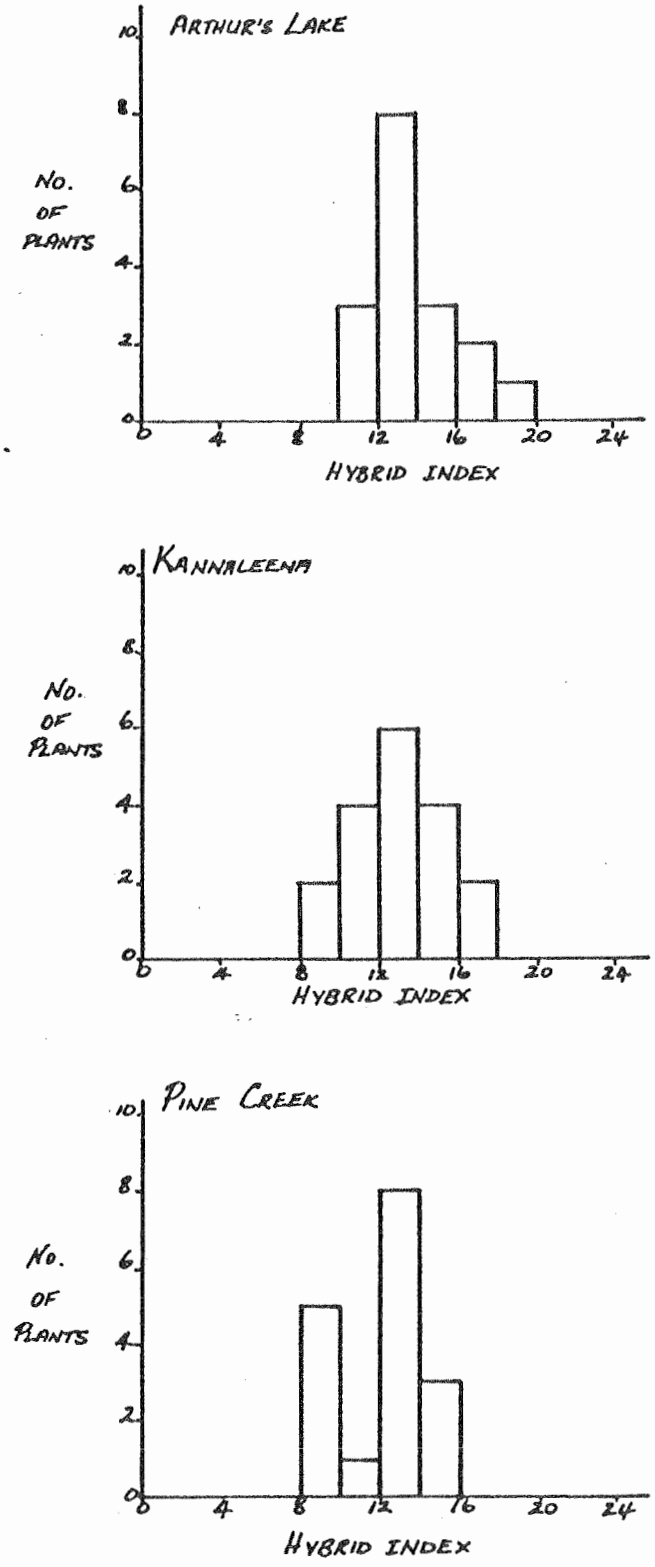
The data matrix obtained was analysed by canonical variates. Only two broad-leaf plants were available from Lake Augusta. These were not included in the calculation of the canonical variates; the position of their mean vector was interpolated after analysis. Standardized values of the coefficients obtained for the first three canonical axes were calculated by weighting the coefficients by their corresponding 'within groups' standard deviations (Phillips *et al.* 1973). To further simplify reification, the resulting values were normalized, so that their sums of squares totalled unity.

Results

1. The Hybrid Index

Frequency histograms of the hybrid indices obtained for the *P. glabrata* plants from Arthur's Lake, Kannaleena and Pine Creek

Fig. g.1. Frequency histograms of hybrid index scores for plants of the *P. antarctica-P. glabrata* complex, grown in a common environment.



are shown in Fig. 9.1. The histograms show that the plants from the three sites are intermediate in character between *P. glabrata* and *P. antarctica*. It is notable that the frequency distributions are not markedly bimodal, i.e. the broad and narrow leaf-types of *P. glabrata* do not appear to be associated with other characteristics which are typical of one or other species.

However, when the scores of the plants for the individual characters are examined, it is apparent that the intermediate hybrid index values result from the high variability exhibited by *P. glabrata* with respect to the leaf and scape characters (i.e. characters 1-6, 12 of Table 9.1).When the plants are scored only on floral characters 7-11 (i.e. plants ascribable to *P. glabrata* score 10, whilst 'good' *P. antarctica* plants score 0), then all 52 plants have values between 8 and 10, i.e. are 'good' *P. glabrata*.

Some of the variability observed in the different leaf and scape characteristics of *P. glabrata* in the glasshouse, is apparent also in the field (e.g. leaf-shape, venation, leaf-indumentum, scape-indumentum), but some of the apparent similarity to *P. antarctica* probably results from phenotypic modification. For example, the dentation (Character 2) and presence of hairs tufted on wart-like thickenings (Character 3) of leaves of *P. glabrata* are distinctive in the field, but are much less obvious or even absent from plants grown in the glasshouse.

The difference of scape length compared with leaf length (Character 6) is of very limited taxonomic use. All of the plants (both in the field and in the glasshouse) had scapes which were 1½ to 2 times as long as the leaves, a characteristic

which is supposedly more typical of *P. antarctica* (Curtis, 1967).

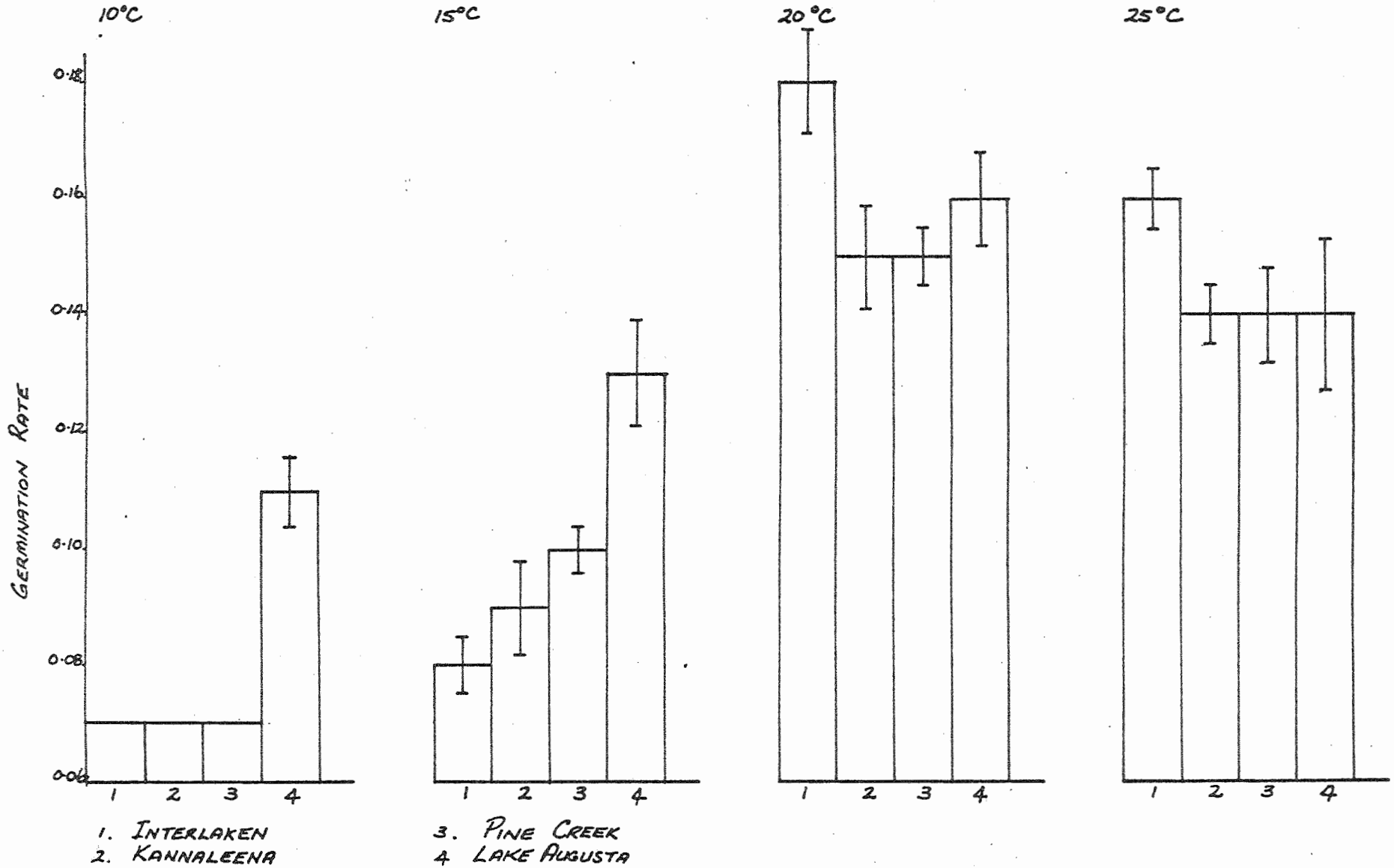
This study has shown that superficially, the plants of *P. glabrata* have some characteristics which are intermediate between the two species. However, the *P. glabrata* plants exhibit a high constancy in their floral features and their apparent intermediate nature probably results from an inherent variability in the vegetative characteristics of *P. glabrata*, rather than from hybridization with *P. antarctica*, especially since the two species have different chromosome numbers. The two species are distinguished best by the characteristics of their flowers.

2. Seed Germination

No seed germinated at 5°C during the experimental period. A two-way analysis of variance of the germination rates observed for the four populations over the remaining four temperatures showed highly significant differences for this character between temperatures, populations and their interaction (Table 1 of Appendix G, p. 339). Mean germination rates in the four populations at each temperature are presented in Fig. 9.2.

Seeds from Lake Augusta (the site experiencing the coldest conditions (p. 57)) were the only ones to germinate at 10°C during the observation period. Seed from all populations germinated at 15°C; the rates of germination were significantly different among three of the four populations, that from Kannaleena not being different from Interlaken or Pine Creek. At this temperature the germination rate appears proportional to altitude and distance west of Interlaken (see p. 57) of the population. The maximum rate of germination for all populations occurred at 20°C. At this temperature, the Interlaken population

Fig. 9.2. The germination rates at four different temperatures of seed collected from four populations of the *P. antarctica-P. glabrata* complex.



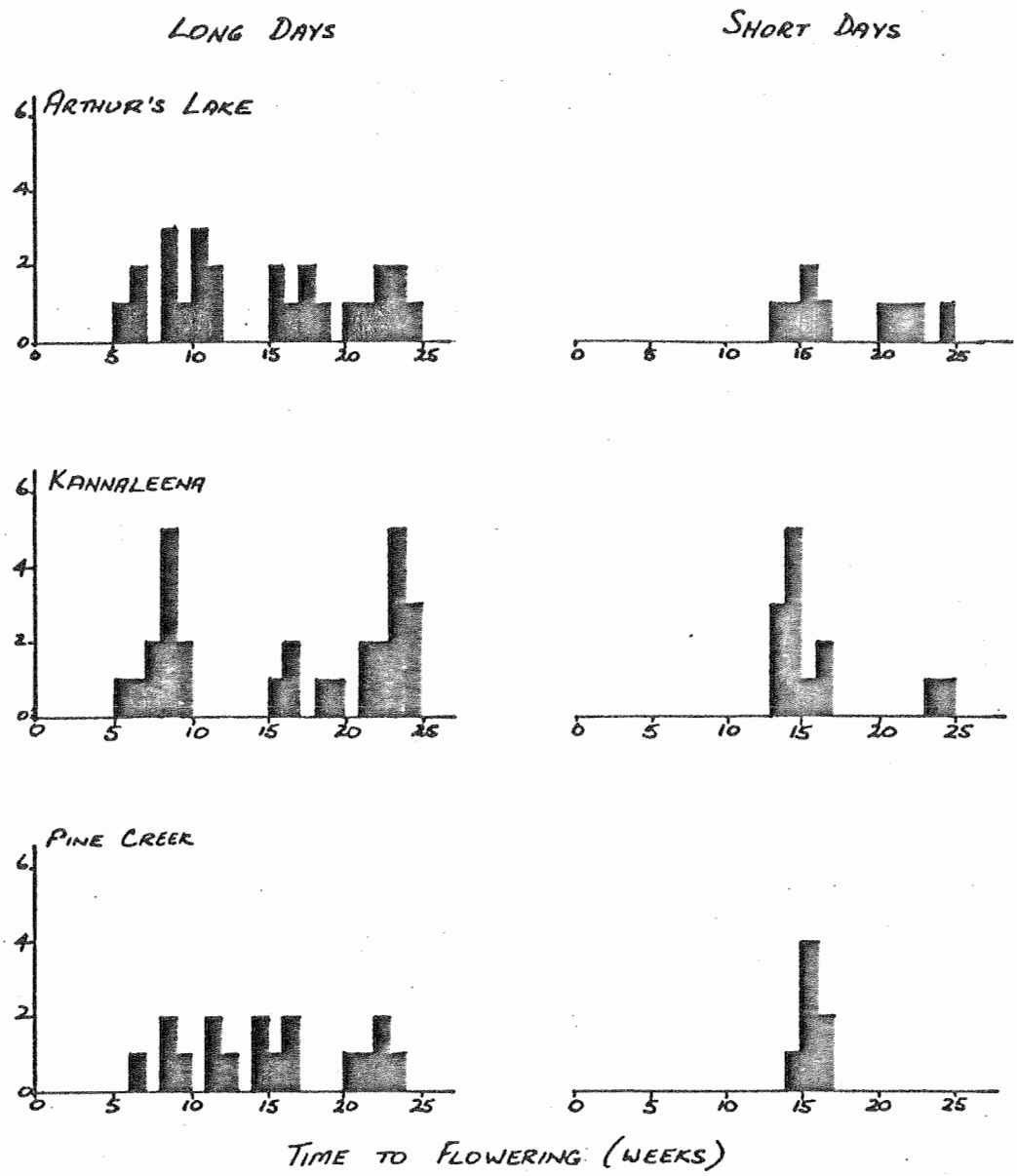


Fig. 9.3. Frequency histograms of time of flowering of three populations of the *P. antarctica-P. glabrata* complex, grown under long days (24 hour) and short days (8 hour).

germinated significantly faster than the other populations. The rate of germination was less at 25°C for all populations, and only the extremes (Interlaken and Lake Augusta) were significantly different.

3. Flowering Time

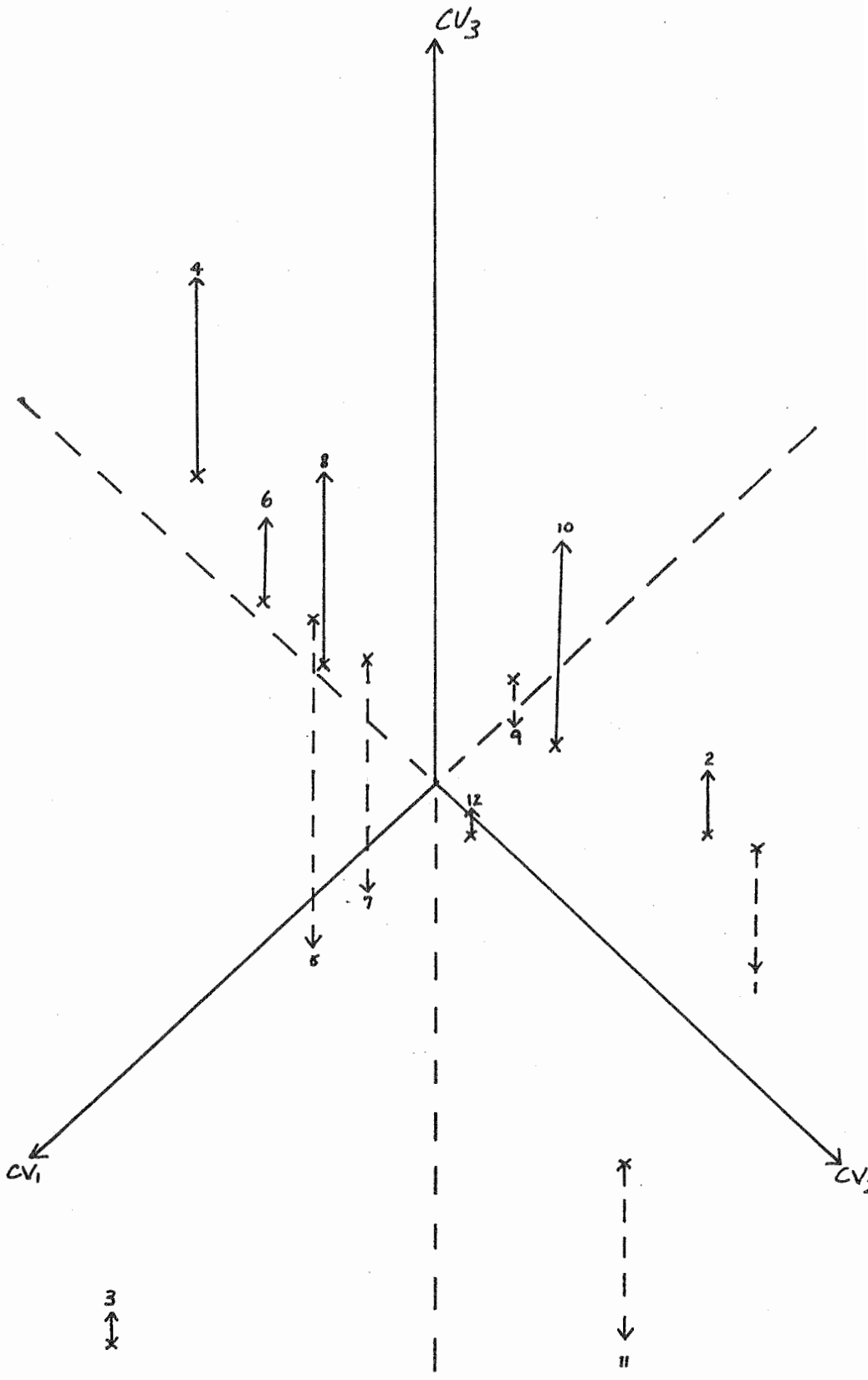
Frequency histograms showing the numbers of plants from the three locations which flowered under the different photoperiods are given in Fig. 9.3. The histograms obtained for long day plants are multi-modal, indicating that each of the three populations is polymorphic for flowering time. This phenomenon did not appear to be related to plant size and was also uncorrelated with leaf-type, since within any population, both broad and narrow leaf plants were observed to flower in the early and late classes.

Flowering was delayed under short days in all populations and the within population polymorphism was even more pronounced under the shorter photoperiod. The results obtained under short day conditions indicate that the interval between early and late phenotypes differs between populations. Thus, while the mode of the early flowering phenotypes occurred at approximately 15 weeks in all populations, plants from Arthur's Lake in the late class had commenced flowering at 21 weeks, plants from Kannaleena at 23 weeks, and no late plants from Pine Creek had flowered at the termination of the experiment.

4. Morphometric Analysis of Variation in the Complex

The locations, leaf-type and number of plants scored in the analysis of population differentiation are given in Table 2 of Appendix G (p. 340). The mean values of the 12 groups for each

Fig. 9.4. Canonical variates analysis of the morphological variation in the *P. antarctica-P. glabrata* complex. Numbering of samples follows Table 2 of Appendix G (p. 340).



of the 21 characters, and the within groups covariance matrix are presented in Tables 4 and 5 of Appendix G (pp.342-343).

The first three eigenvalues obtained from CVA of this data accounted for 73% of the total variation (Appendix G, Table 6). The mean values of the 12 groups along the first three canonical axes are given in Table 7 of Appendix G (p. 345). These axes effectively separate the various groups into biologically meaningful arrays (Fig. 9.4). However, their manifestation is underlain by relatively complex character sets. Inspection of Table 6 (p. 344) reveals that the characters of most weight on CV1 are diverse, and include those characters traditionally used in the separation of *P. antarctica* and *P. glabrata, viz.* leaf width, scape length, bract length, bract indumentum and sepal length. CV2 is more heavily weighted on leaf-characters (length, width, lamina length and tooth number), although scape length, the three sepal dimensions and petal width also enter upon this axis. CV3 is predominantly a contrast of leaf length with lamina length, with lesser contributions from leaf-indumentum, bract length, petal width, leaf width and leaf venation.

The first canonical variate accounts for 31% of the total variation and separates the broad leaf-type from Interlaken (*P. antarctica*, group 3) from all other samples. The (interpolated) broad leaf plants from Lake Augusta appear intermediate in character between *P. antarctica* and the other groups. The mean values of the original characters (Table 4 of Appendix G, p. 342) show that the floral characters which best distinguish the two species are bract length, width of bract keel, sepal length and petal length. The range of means of each of these characters for

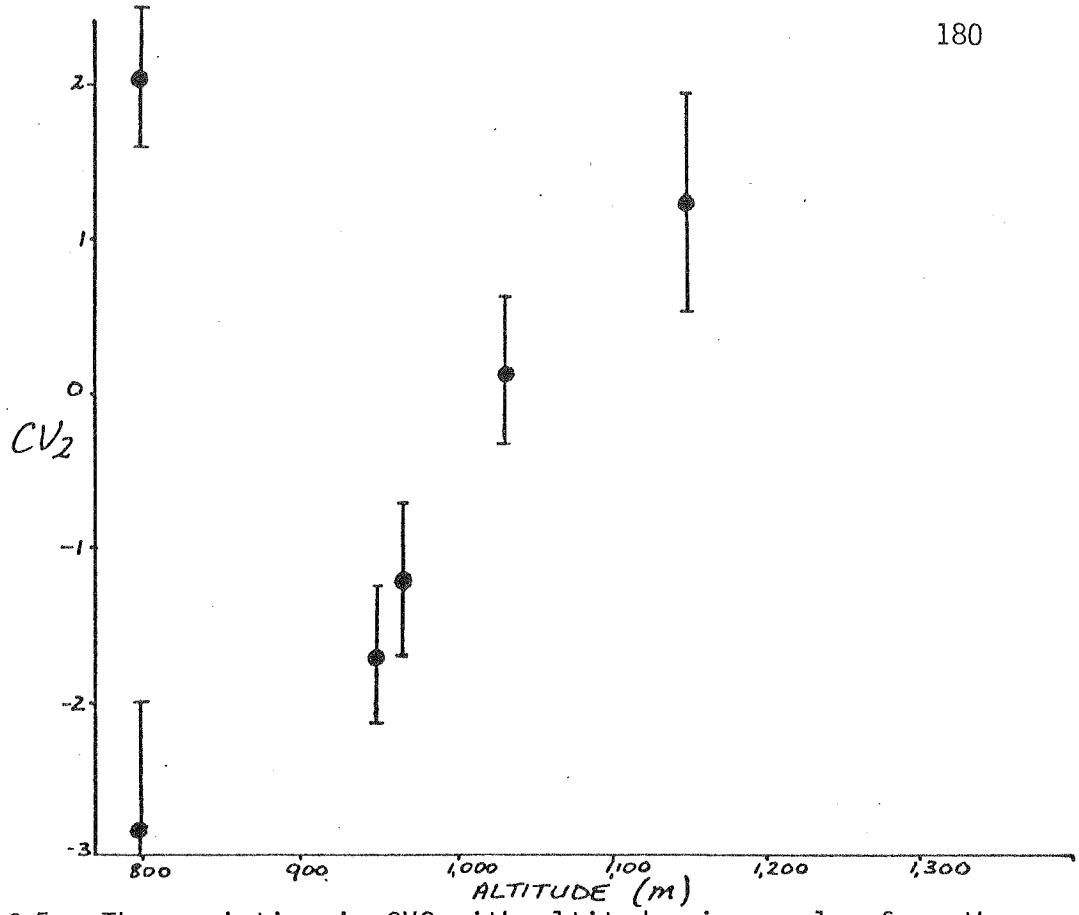


Fig. 9.5. The variation in CV2 with altitudes in samples from the *P. glabrata* complex.

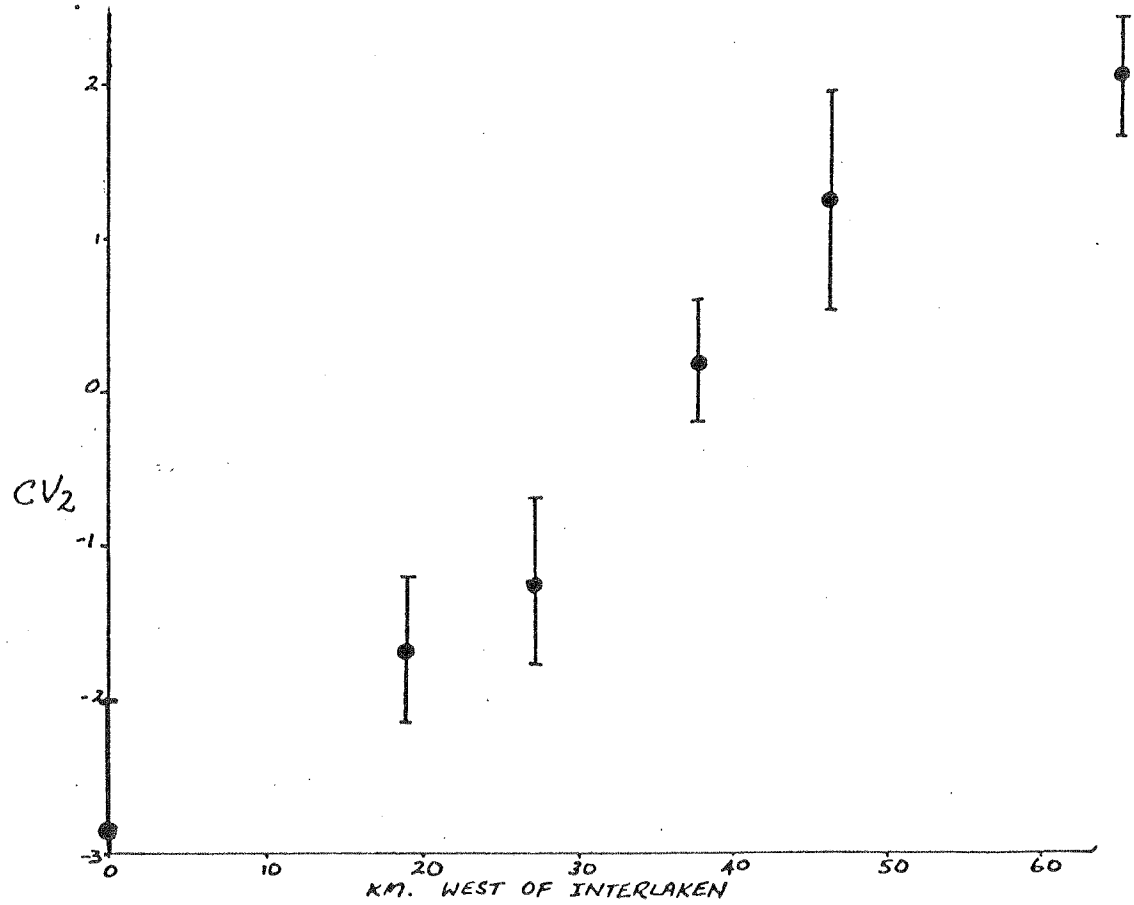
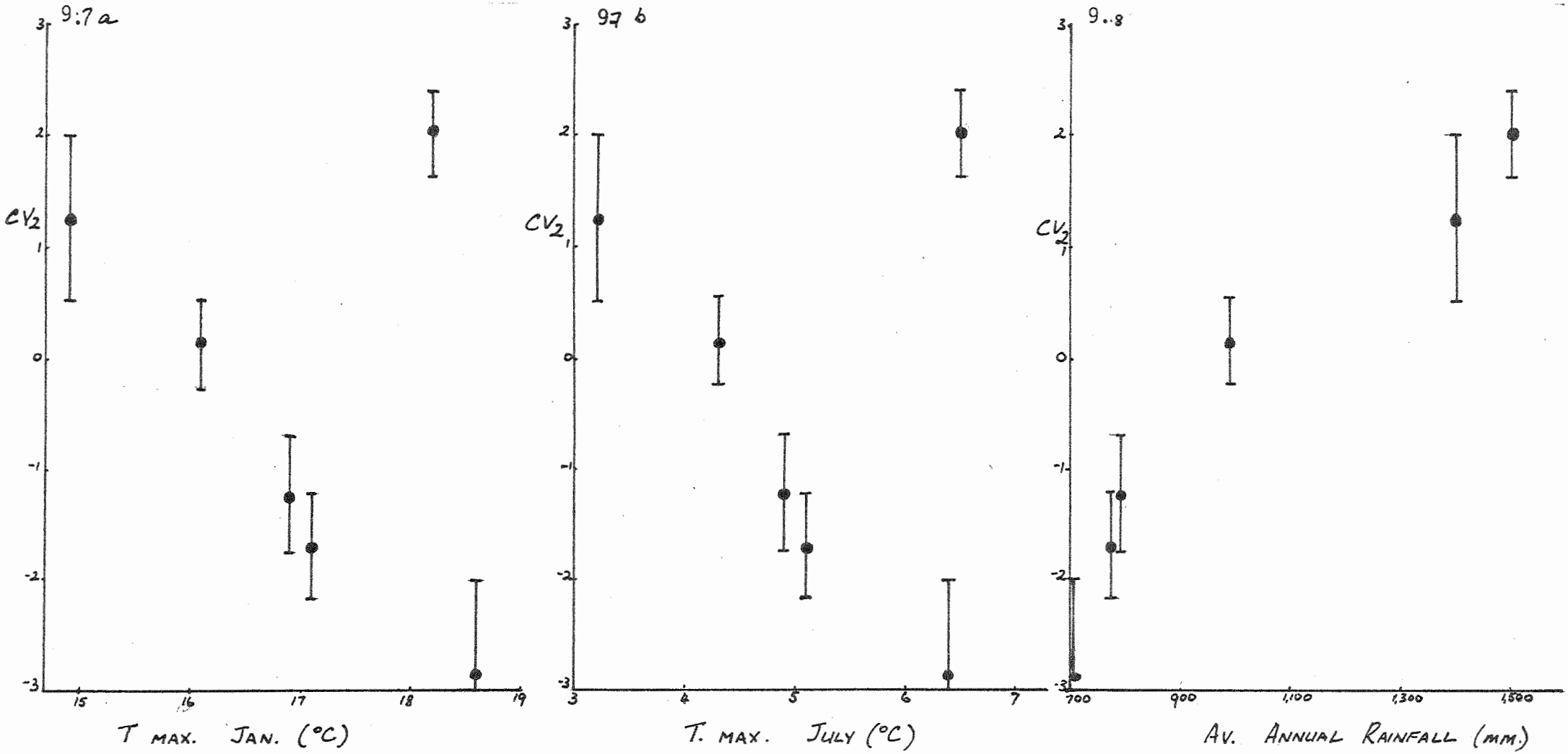


Fig. 9.6. The variation in CV2 against distance west of Interlaken in samples from the *P. glabrata* complex.



Figs. 9.7 and 9.8: The variation in CV2 against average maximum temperatures in January and July (9.7a and 9.7b) and against average annual rainfall in samples from the *P. glabrata* complex.

concomitantly with rainfall. However, the results do suggest that further investigations into the maintenance of the cline might better be directed towards a study of the water relationships within the species, rather than its response to temperature stress.

Discussion

The morphometric analysis has revealed that three biologically distinct patterns of variation are exhibited by the complex.

1. The Separation of the broad-leaf Interlaken Sample (*P. antarctica*) from the Remainder

The *P. antarctica* plants, used in this experiment, have features which are very similar to those represented in holotype photographs of *P. antarctica*, and the plants closely resemble specimens of *P. antarctica* held in mainland Australian herbaria (NSW, CANB, MEL). They were separated in the analysis from the *P. glabrata* complex by a character suite which compares favourably with that listed in the taxonomic literature as differentiating *P. antarctica* and *P. glabrata*. There can be little doubt that this group represents 'typical' *P. antarctica*.

On the other hand, the variation exhibited within the *P. glabrata* complex is orthogonal to that which distinguishes *P. antarctica* and *P. glabrata* and the two species have different chromosome numbers. From these results, it appears unlikely that any of the morphological variability within *P. glabrata* has arisen from introgression between the two species.

2. The Clinal Variation Exhibited by *P. glabrata*

The variation exhibited by the second canonical variate is apparently ecoclinal, and is correlated with the increasingly harsh environmental conditions experienced in a westerly direction

on the Central Plateau. The second variate is the compound of a diverse array of characters, and the cline thus involves the simultaneous variation in a set of correlated morphological characters.

Supportive evidence of clinal variation in *P. glabrata* was obtained in the studies of both the germination and flowering responses of the species. While it has not as yet been possible to demonstrate that these facets are amenable to selection and thus genetically based, it is interesting to speculate on the adaptive advantages they confer.

The germination studies showed that the taxa growing under the harsher environmental conditions exhibited higher germination rates at low temperatures than the taxa growing in more moderate conditions. The early germination of seed at higher altitudes would provide an increased period for both seedling establishment and for plant growth, providing the season did not revert to unfavourable conditions. These plants would, therefore, be at a selective advantage under continuing favourable spring conditions. This contrasts with the plants resulting from late germinating seed. These would be at a selective advantage under changeable spring conditions, but would be selected against in those years when unseasonable cold spells occurred in early autumn, or during periods of prolonged summer drought. Under the more equable conditions prevalent at the lower altitudes, the rate of germination was much reduced at the low temperature (to zero for the observation period), but the germination rate was higher at the optimum temperature.

Similar arguments are applicable to the polymorphism observed in the flowering times of the three populations studied. Further studies (involving progeny testing) are required to determine whether this intra-population differentiation represents a genetic polymorphism, i.e. is due to the presence of alternative alleles of a single locus or loci, or whether it is ascribable to an impenetrant gene system. Progeny tests are needed also to determine whether the differences in the intervals between flowering modes are amenable to selection.

Some preliminary studies have shown that the polymorphism in flowering time is confined to members of the section *Mesembrynia*. Thus, *P. varia, P. bellidiodes, P. hispida* and *P. debilis* were found to exhibit the same type of response as *P. glabrata*, but the introduced species (*P. lanceolata, P. major, P. australis*), and members of the section *Oliganthos* (*P. paradoxa, P. triantha, P. daltonii*) did not respond in this way. Furthermore, the interval between the flowering modes differed between the species of section *Mesembrynia*.

3. The Within Population Differentiation

The subjective division at each sampling site into samples of 'broad' and'narrow' leaf phenotypes was affirmed by the subsequent analysis. The two classes of leaf-phenotypes exhibited similar trends of geographical variation with respect to the second canonical variate. The intra-population differentiation may represent two species which have the same range and which independently have evolved the same internally correlated character set (as measured by CV2) in response to directional selection. It may also reflect secondary disruptive forces

which are common to all locations and which are superimposed on a single species which is subject to directional selection. Another possibility is that the intra-population differentiation is a plastic response (albeit adaptive) to a local heterogeneity of ecological conditions which are recurrent at the different sites. This situation has been discussed by Westerman and Lawrence (1970) who feel that the optimum adaptational strategy in a heterogeneous environment is likely to be one of developmental flexibility (i.e. adaptive phenotypic plasticity). The results of an investigation into these alternatives within one of the above populations is presented in the next chapter.

10. The Interrelationship between Habitat, Water Balance and Leaf-Shape in a Population of *P. glabrata*

Introduction

This chapter describes an investigation of the variation of leaf-form in *P. glabrata* within a single population at Lake Augusta.

In this population a complex range of leaf-forms occur. The two extremes of leaf-shape encountered are a form with broad leaves which are markedly 5-7-nerved and which may be runcinately lobed, and a form with narrow leaves which is (1-)3-nerved and runcinately lobed (Fig.10.1). The broad-leaf-form tends to occur under bushes and in sheltered sites, whilst the narrow-leaf-form occurs in the open, more exposed areas. Intermediate forms occur in habitats which are intermediate in exposure, although some of these extend to the extreme habitats.

These two forms are found over much of the range of *P. glabrata* (Chapter 9). The wide disparity in form necessarily makes the character description very loose (Curtis, 1967). In the population at Lake Augusta, the mixture of the two forms is very striking. There are at least three ways in which the differentiation of leaf-shape might arise:-

(a) The two forms and the intermediates represent a plastic response to differing environmental conditions.

(b) The variability arises from two distinct (but possibly introgressant) species, each adapted to a separate habitat.

(c) The differences in leaf-shape are the result of disruptive selection acting on a single species.

A series of experiments was designed to test these alternatives,

A. P. glabrata : NATURAL HABITATS.

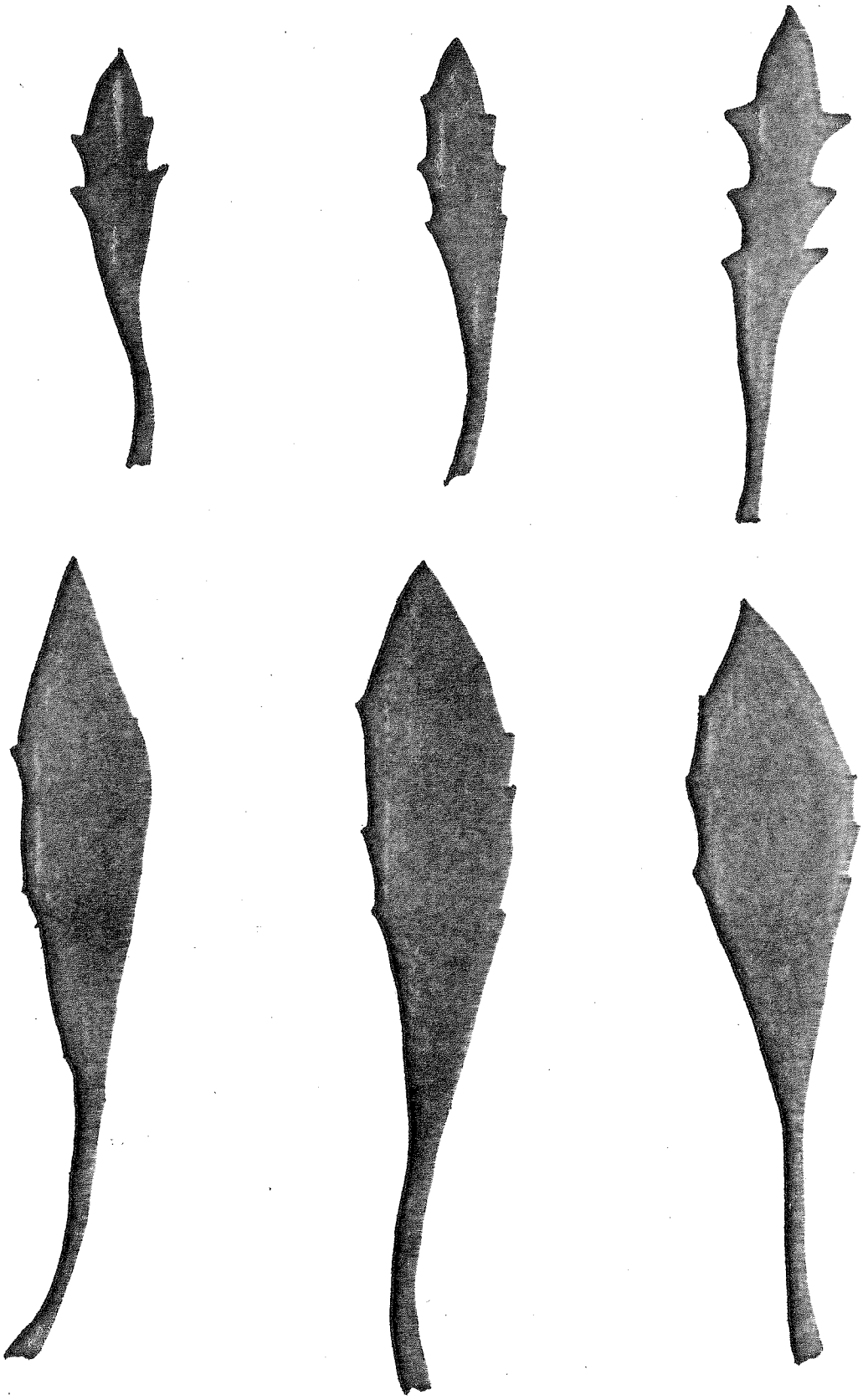
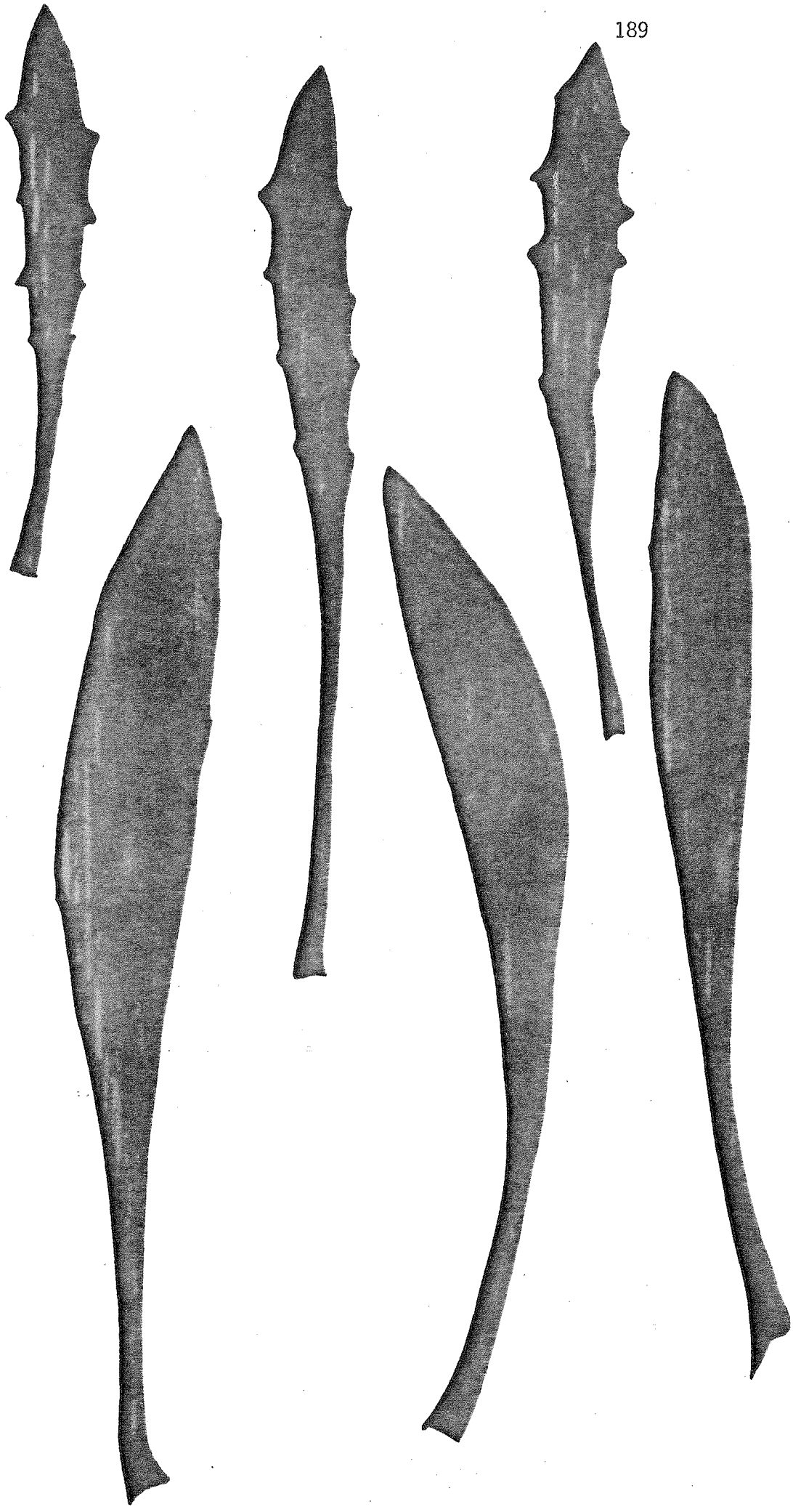


Fig.10.1. The extremes of leaf form encountered in *P. glabrata* at Lake Augusta. A, leaves collected in the field; B, leaves from plants grown in the glasshouse (over page).

B. *P. glabrata* - GLASSHOUSE GROWN



and to examine the possible adaptive significance of the observed differences in leaf-shape.

The experimental layout was as follows:-

(i) A comparison was made between plants:-

(a) in the field,

(b) on transplanting to a common environment in the glasshouse,

(c) grown in the glasshouse from open-pollinated parents,

and, (d) grown from a cross between the two extreme forms.

(ii) Plants were assessed on:-

(a) their leaf-morphometry,

and, (b) the water potential of their leaves.

(iii)Field sites were assessed on:-

(a) the degree of exposure,

(b) the soil moisture percentage,

and, (c) the degree of soil compaction.

Habitat Description

In common with much of this region of the Central Plateau, the vegetation at the eastern end of Lake Augusta is a mosaic of proteaceous shrubbery (mainly *Orites* spp.), closed herbfields and bare ground (Jackson, 1972).

The lake lies at an altitude of 1200 m. Its geological origins are uncertain, but it is underlain by Jurassic dolerite, and lies beyond the known limits of the Pleistocene glaciation (Banks, 1972b). Soils in the area are mainly clays derived from the parental dolerite, intermixed with aeolian sands, and exhibit the results of periglacial solifluction.

The lake has an area of approximately 12 sq. km, and consists of two arms. The eastern arm is artificial and was formed by a dam placed across the Ouse River. During summer, the lake level is much reduced in the eastern arm, and water occupies only the old stream course of the Ouse River. The remainder of the eastern basin becomes a large 'dust bowl' of sand fines deposited the previous winter. The exposed sands are readily transported by the strong prevailing westerly winds. This aeolian material tends to be trapped by projecting objects such as bushes and rock outcrops to the east of the lake, so that in areas of shrubland, the clay soils are overlain by a silty sand of variable depth, whilst the exposed areas are relatively sand free.

Annual rainfall in the area is approximately 1300 mm, with a winter maximum. However, effective rainfall probably occurs in all months. Furthermore, Edwards (1973) has demonstrated the efficiency with which the shrubby vegetation can strip water droplets of small diameter, both liquid and frozen, from the low cloud, mist and fog prevalent in the area in all seasons, thereby locally increasing effective precipitation.

Materials and Methods

1. Leaf-Morphometry

The different leaf-types were initially classified by their *w/l* ratio, but this proved quite inefficient. The forms were more effectively separated by a discriminant function calculated between the extremes of leaf-shape, based on the four variables, *l*, *lwp*, *w* and *ll*, and the discriminant scores obtained from this were used to describe differences in leaf-form in all further calculations (Appendix H, Table 1, p. 346). All measurements

were made on fresh leaves. Leaf-areas were measured with an electronic planimeter, which has a variability of 0.3% over ten replicates.

2. Controlled Growth Studies

(a) Transplants

Eight plants from each of the extreme broad and narrow leaf-forms were removed from the field and grown in a common environment in the glasshouse. Plants were grown individually in cans containing a mixture of vermiculite and gravel, watered daily and given a modified Hoagland nutrient solution once weekly.

(b) Progeny

Seeds were collected in the field from the open-pollinated mother plants of the extreme forms, and progeny were grown from these in the glasshouse, under the same conditions as the transplants.

(c) The Cross between the Narrow-Leaf and Broad-Leaf Forms

A cross was made between two extreme types which were kept isolated from other plants. Seed was obtained by growing a narrow-leaf maternal parent and a broad-leaf pollen donor together in a glass cabinet inside the glasshouse. Flowering spikes which had formed prior to the isolation of the plants were removed and the plants were left to grow new inflorescences. It was not practical to emasculate the plants because the individual flowers on each spike are small and densely packed (*cf.* Rahn, 1974). The flowers are protogynous and the anthers are not exserted until 2-3 days after the style. Cross-pollination was achieved by dusting the newly exserted styles of the mother with pollen from the father.

This procedure was repeated periodically until the styles had withered. However, some self-fertilization may have occurred under these conditions.

3. Measurement of Leaf-Water Potentials

Water potentials in leaves were measured using the sap pressure bomb technique outlined by Boyer (1966). Leaves were picked from the plants and their bases were cut to give an even surface. A single leaf was placed in the bomb and sealed in position by a heavy guage rubber washer cut to the approximate shape of the leaf petiole. Pressure was then applied by the release of nitrogen into the chamber until xylem sap appeared at the cut end of the petiole.

The pressure reading at this point is related to the water potential of the leaf cells by

$$\psi w = P + \psi s_{,xylem}$$

where P represents the negative component of the water potential of the xylem sap measured as a positive pressure in the bomb. $\psi s_{,xylem}$ is the osmotic effect of solutes in the xylem sap. The osmotic effects are usually small in relation to the other forces in non-saline environments, and were ignored in the present study. According to Boyer (*loc. cit.*), the sap pressure bomb is well-suited to comparisons of water potentials in which absolute values are not required, and Tyree *et al.* (1973) affirms that the initial 'balancing pressure' of a shoot or leaf collected in the field is equal to minus the water potential at collection time, provided the turgor pressure remains unchanged.

4. Field Measurements

(a) The Association of Leaf-Shape with Habitat

Fifty plants within the field population were selected at random and designated as having 'shrubby' or 'open' sites, depending on the associated vegetation. The 4 metric leaf-characters were measured on one mature leaf of the plant occurring at each site and the plant was assigned a discriminant score. A t-test was used to test the degree of association between the broad and narrow leaf-types and the shrubby and open habitats. In the field, the plants are subject to grazing by herbivores, and it was not always possible to obtain more than one undamaged mature leaf.

(b) Leaf Measurements

A further twenty five plants were selected subjectively to sample the range of habitats. At each site a single mature leaf was excised from the plant and the four variables *l*, *lwp*, *w* and *ll* were immediately measured. The lower (5-10mm) portion of petiole was then excised and the water potential of the leaf was measured. The pressure bomb technique is suited best to woody plants. If too much pressure is applied when sealing fleshy *Plantago* leaves, the rubber washer cuts through the petiole and a tight seal is not maintained. Two plants were damaged in this way and had to be discarded.

(c) Soil Sampling

A cylindrical galvanised iron tube of 50 mm diameter was driven into the ground next to each plant to a depth of 125 mm, and the core of soil so obtained was placed into a plastic bag and sealed. The soil samples were removed to the laboratory, weighed and oven dried at $105^{o}C$.

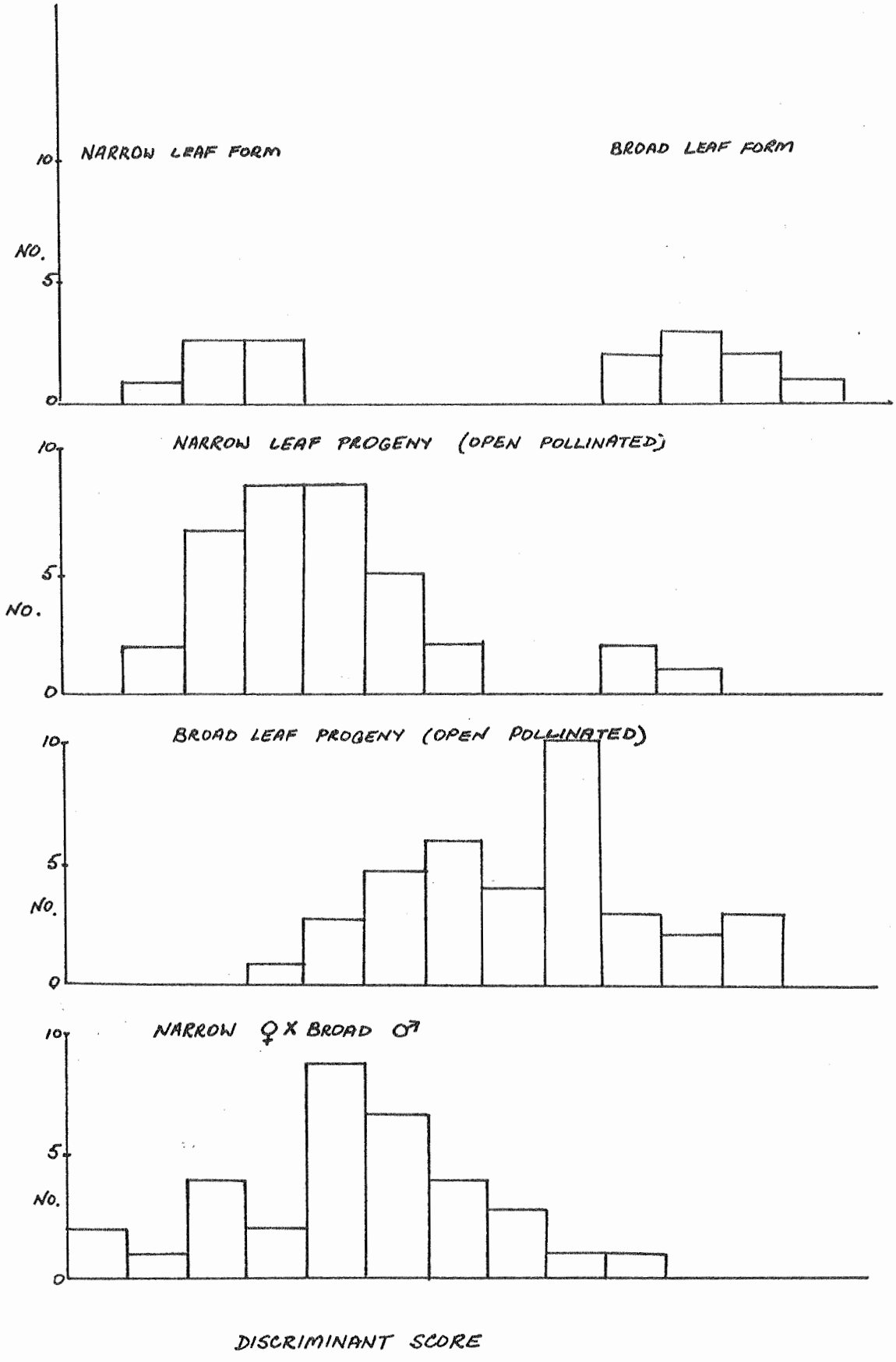


Fig. 10.2. Frequency histograms of the discriminant scores obtained for the narrow and broad leaf forms of *P. glabrata* and their progeny.

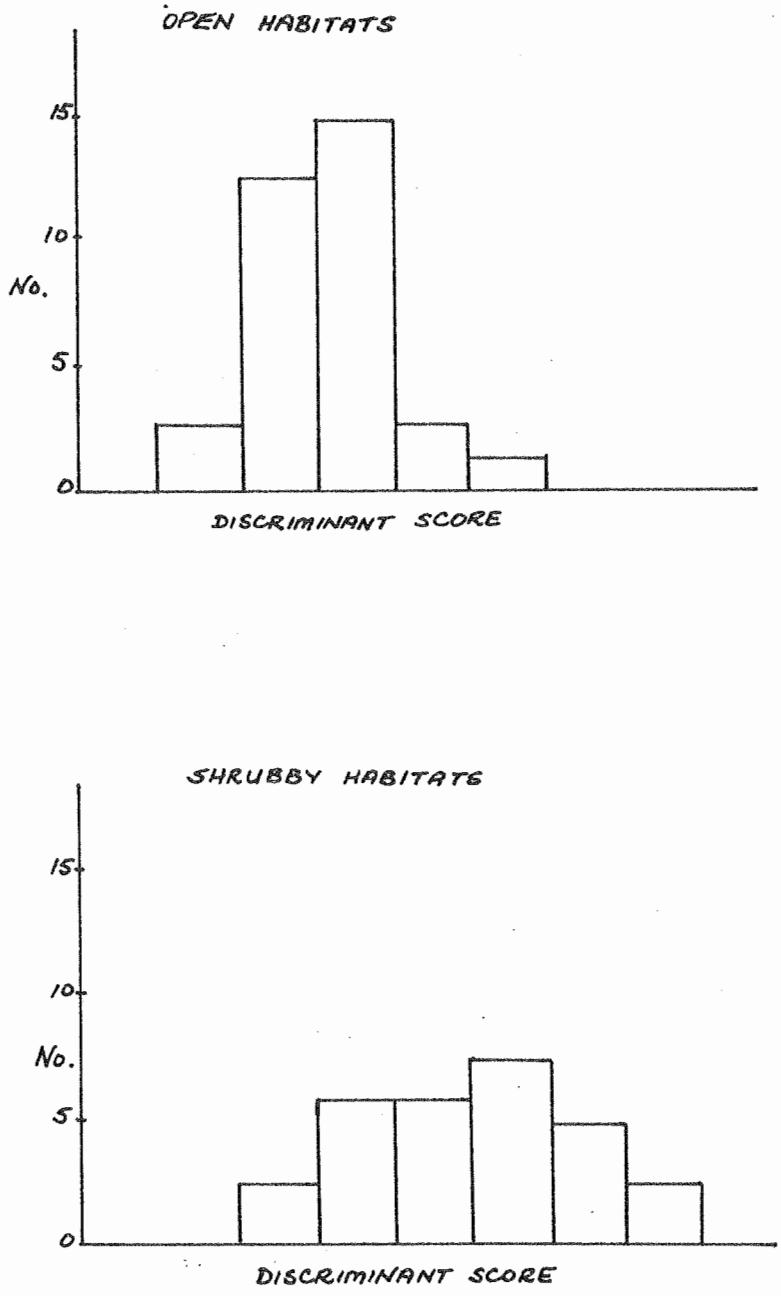


Fig. 10.3. Frequency histograms of discriminant scores of leaves taken from plants growing in either open or shrubby habitats.

Soil moisture content was calculated as:-

$$\theta = \frac{Wwet - Wdry}{Wdry} \times 100$$

where W is the weight of soil in gm (Leeper, 1964). The dry weight of soil obtained was divided by the initial core volume to obtain an averaged estimate of the soil bulk density (Rose, 1966). The technique provided a reasonable measure of the relative proportions of clay to sandy soil in the core. In the absence of any measurement of the soil matric potential, it was assumed that the differing physical properties of the clay and sand were such that the bulk densities obtained were correlated with the availability to the plant of water included in the soil volume.

Results

1. Glasshouse Studies

Preliminary transplants of the two types into a common environment indicated that although they are plastic in their response to the environmental change, the observed field differences in leaf-shape are maintained, i.e. may well have a genetic origin (Fig. 10.1.B).

The mean values of the variables *l*, *lwp*, *w* and *ll* obtained for the broad and narrow leaf-types, and the associated variance-covariance matrix are given in Table 1 of Appendix H (p. 346). The discriminant function obtained was

$$2.30\ l - 7.04\ lwp + 64.27\ w - 11.30\ ll$$

The discriminant function effectively separated the two forms ($D^2 = 80.27***$) (Fig. 10.2).

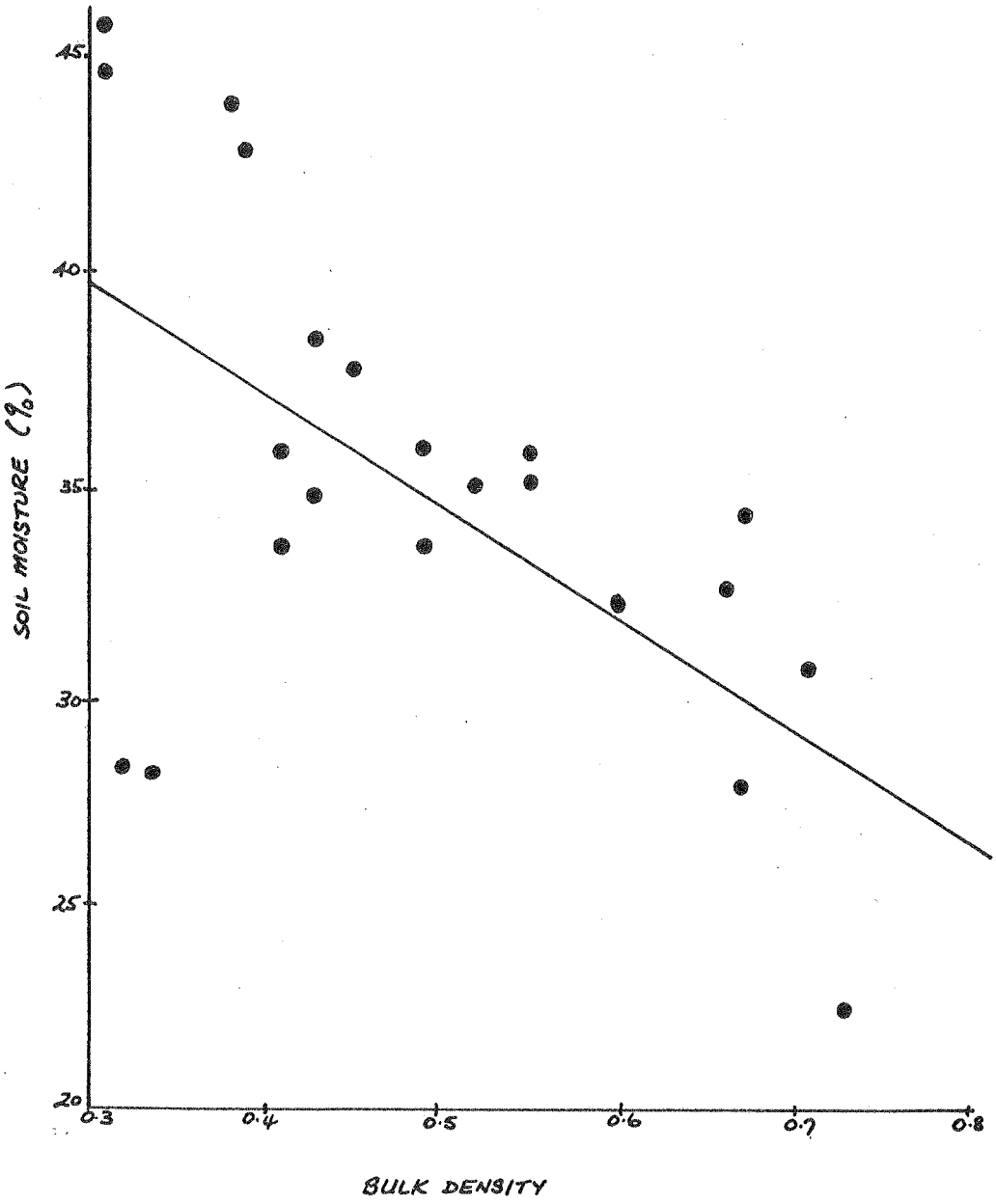


Fig. 10.4. The relationship between soil moisture and bulk density of soil samples collected at Lake Augusta.

$y = 47.69 - 26.21x$, $r = 0.59^{**}$.

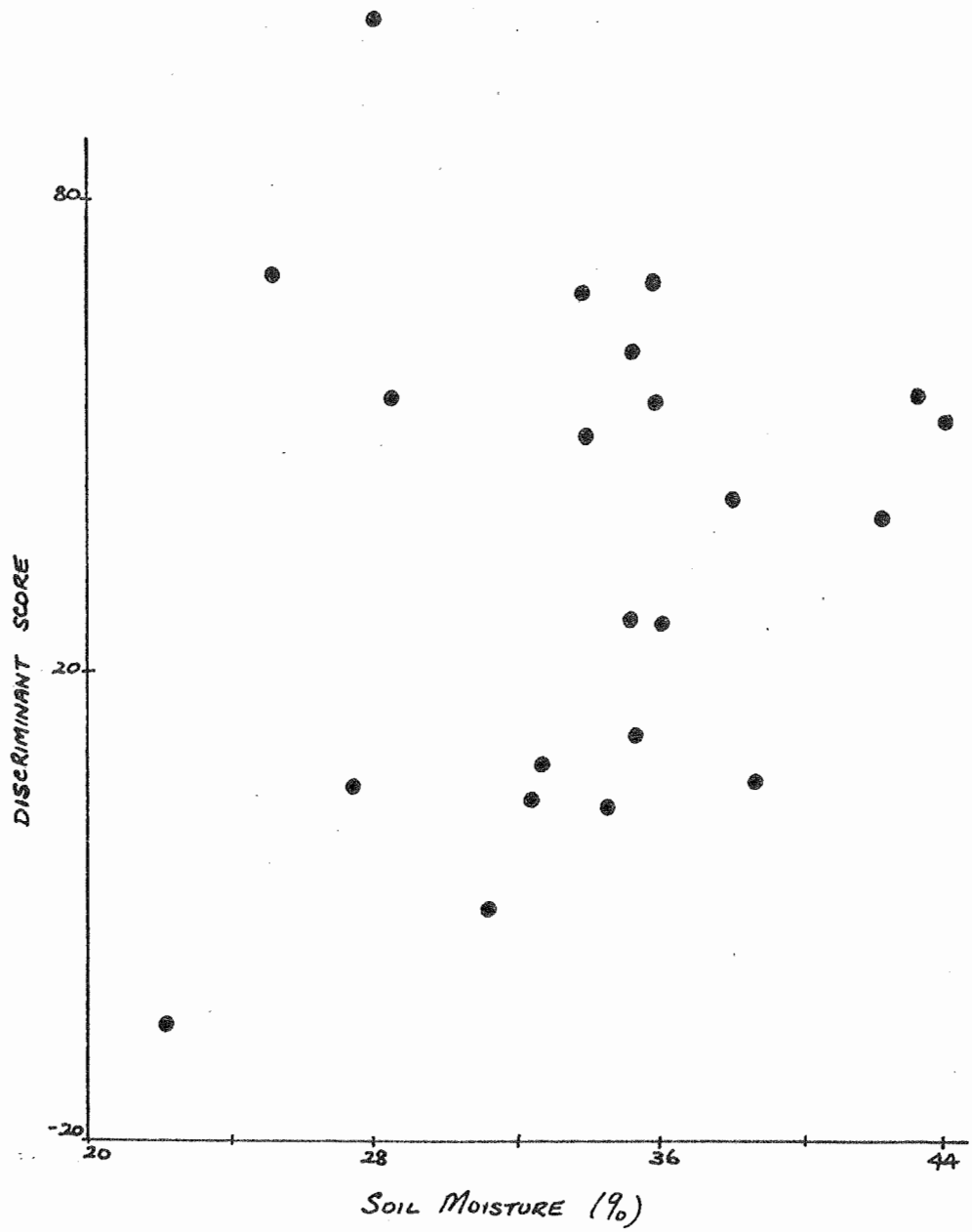


Fig.10.5. The relationship between the discriminant scores of leaves and the soil moisture at Lake Augusta.
r = 0.112 (N.S.).

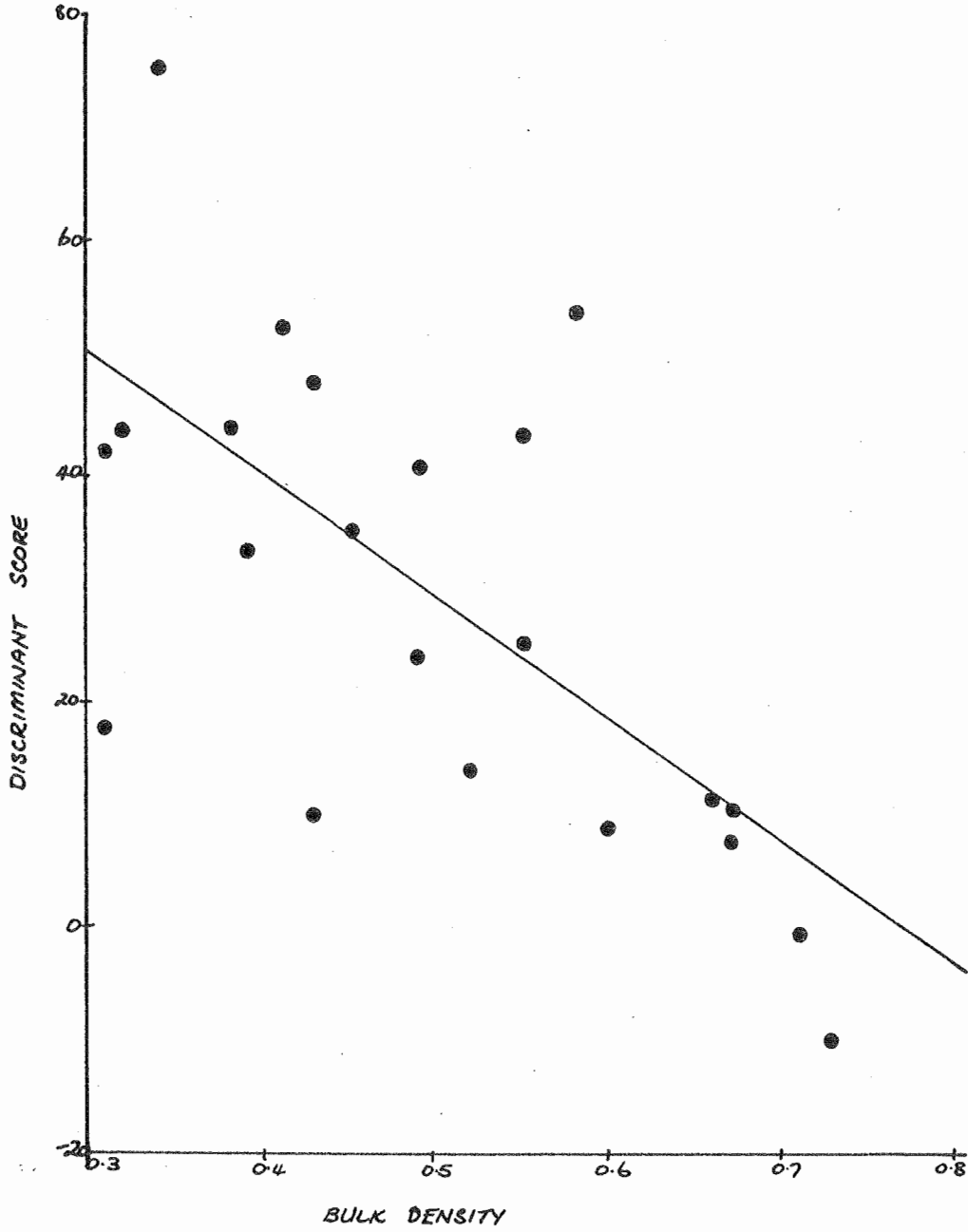


Fig. 10.6. The relationship between the discriminant score of leaves and the bulk density of the soil at Lake Augusta. $y = 83.57 - 107.86x$, $r = 0.672^{***}$.

The progeny grown from seed collected from open-pollinated mother plants of each type exhibited a range of leaf-shapes (Fig. 10.2), and the progeny plants all appeared fully fertile. Artificial crossing of the two also gave a range of fertile intermediate types (Fig. 10.2).

2. The Association of Leaf-Shape with Habitat

The leaves of plants growing in 'shrubby' or 'open' habitats were assigned discriminant scores, and, whilst the frequency distributions overlap (Fig. 10.3), the t test confirmed that habitat correlated polymorphism is pronounced ($t_{48} = 7.90$, $P < 0.001$). The narrower forms tend to predominate at open sites whilst broader forms are more common in shrubby habitats. However, it is apparent from Fig. 10.3, that the differences of leaf-form are quantitative.

The moisture content of the soil samples collected was found to be correlated with their bulk density ($r = 0.59$, $P < 0.01$) (Fig. 10.4). The discriminant scores were unrelated to soil moisture at each of the twenty three sites (Fig. 10.5), but were highly correlated with the bulk density of the soil ($r = 0.645$, $P < 0.001$) (Fig. 10.6).

In general, the soil moisture content tends to increase with the sandiness of the site, but would be subject to more rapid fluctuation and local variation than the bulk density. The leaf-form adopted by a plant at a particular site is the product of growth over a relatively long period and therefore would not necessarily be correlated with a sampling of soil moisture made at any one time.

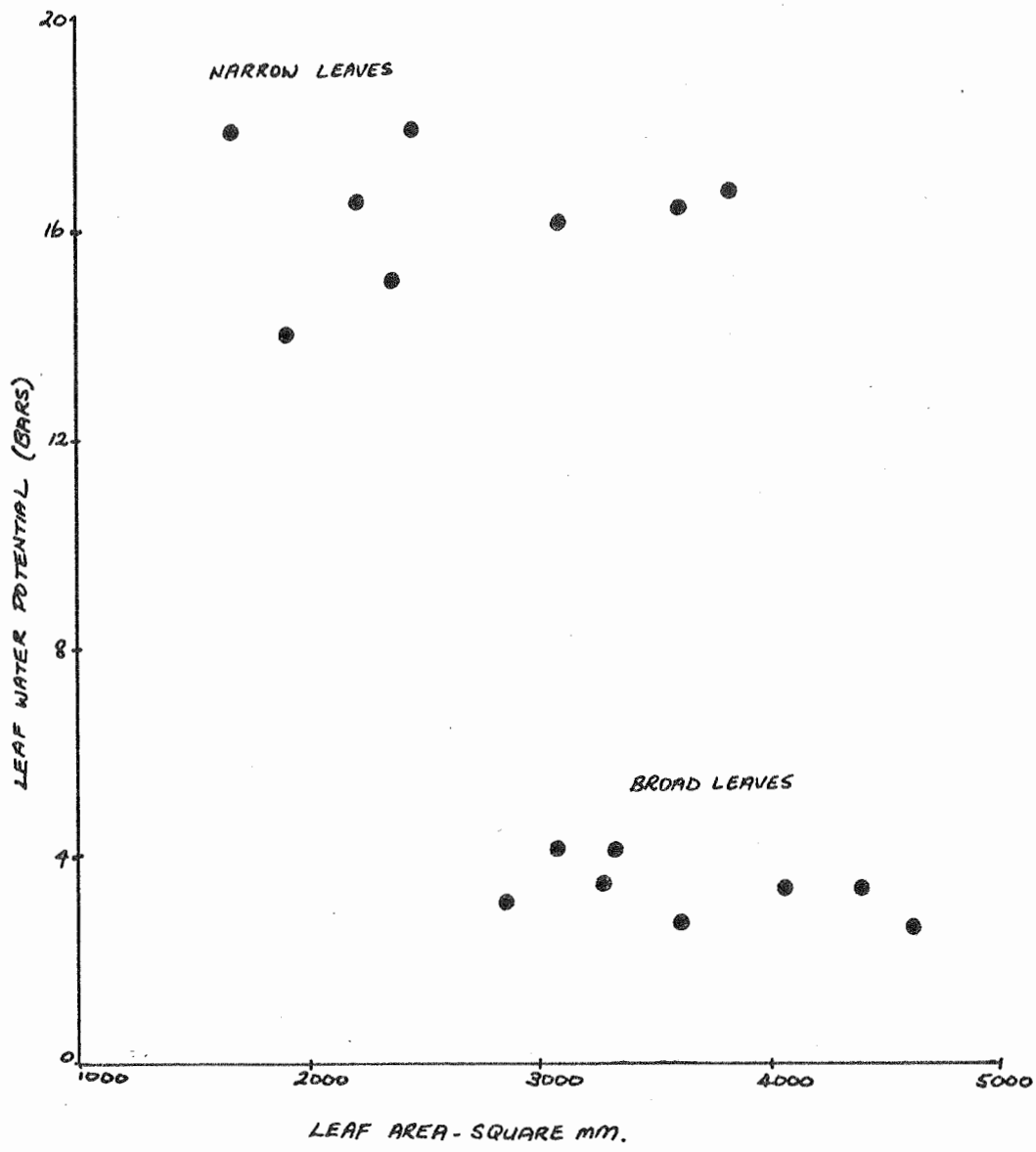


Fig. 10.7. The relationship between leaf water potential and leaf area in the broad and narrow leaf forms of *P. glabrata*, grown in the glasshouse.

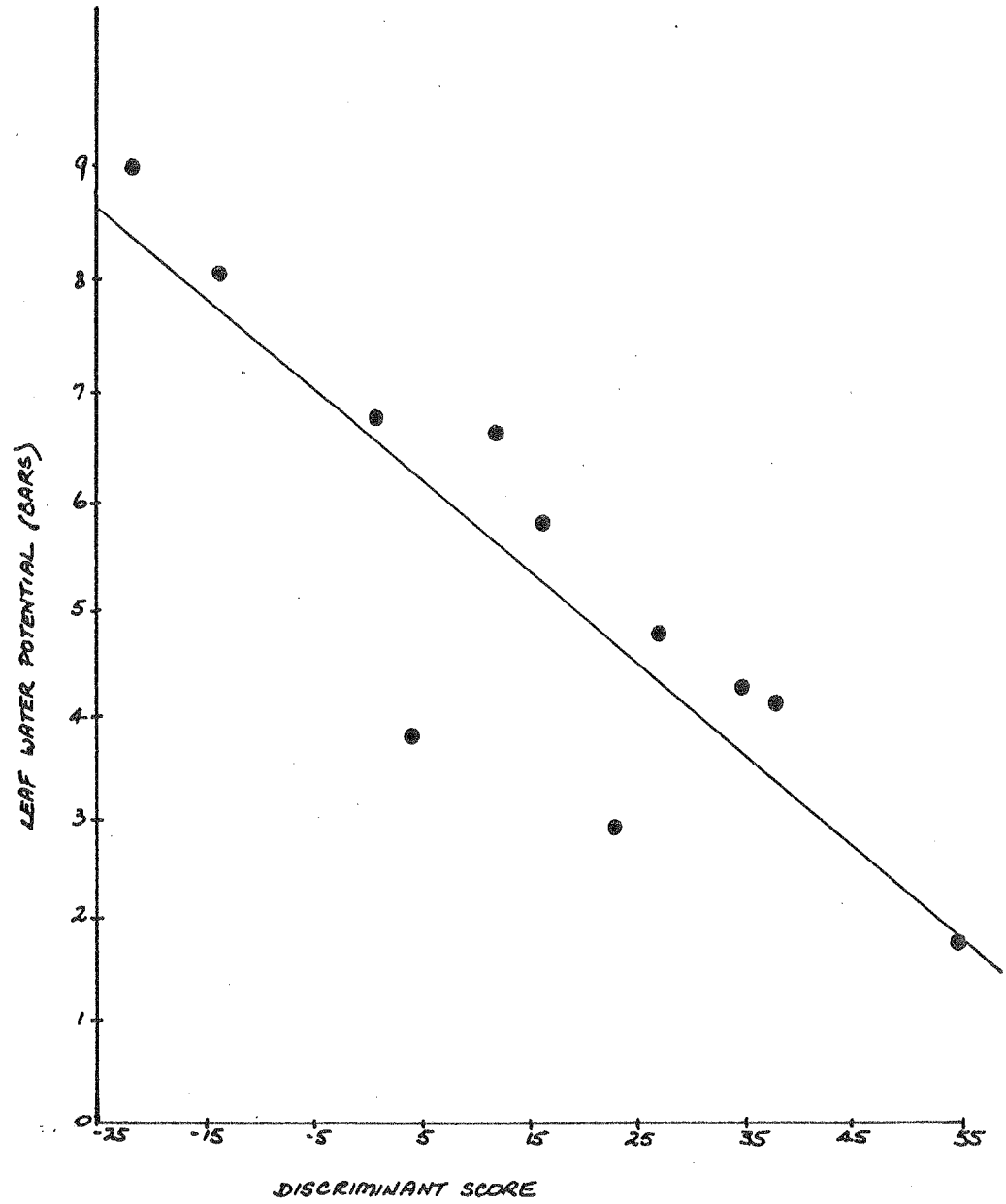


Fig.10.8. The relationship between the leaf water potential and discriminant score of leaves taken from the progeny plants of the narrow - broad leaf cross.

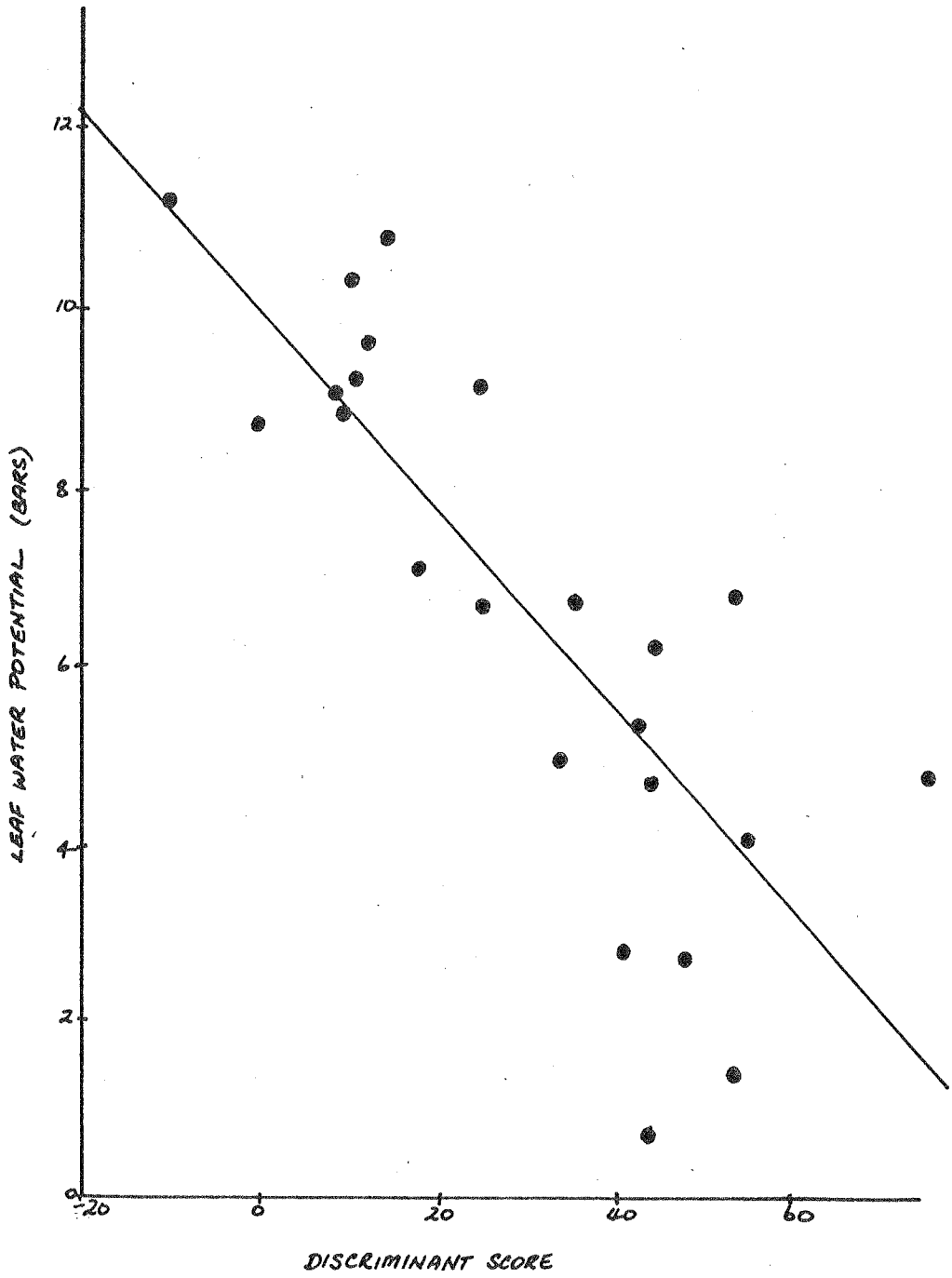


Fig.10.9. The relationship between leaf water potential and discriminant score of leaves taken from plants growing in the field. $y = 10.04 - 0.11x$, $r = 0.791^{***}$.

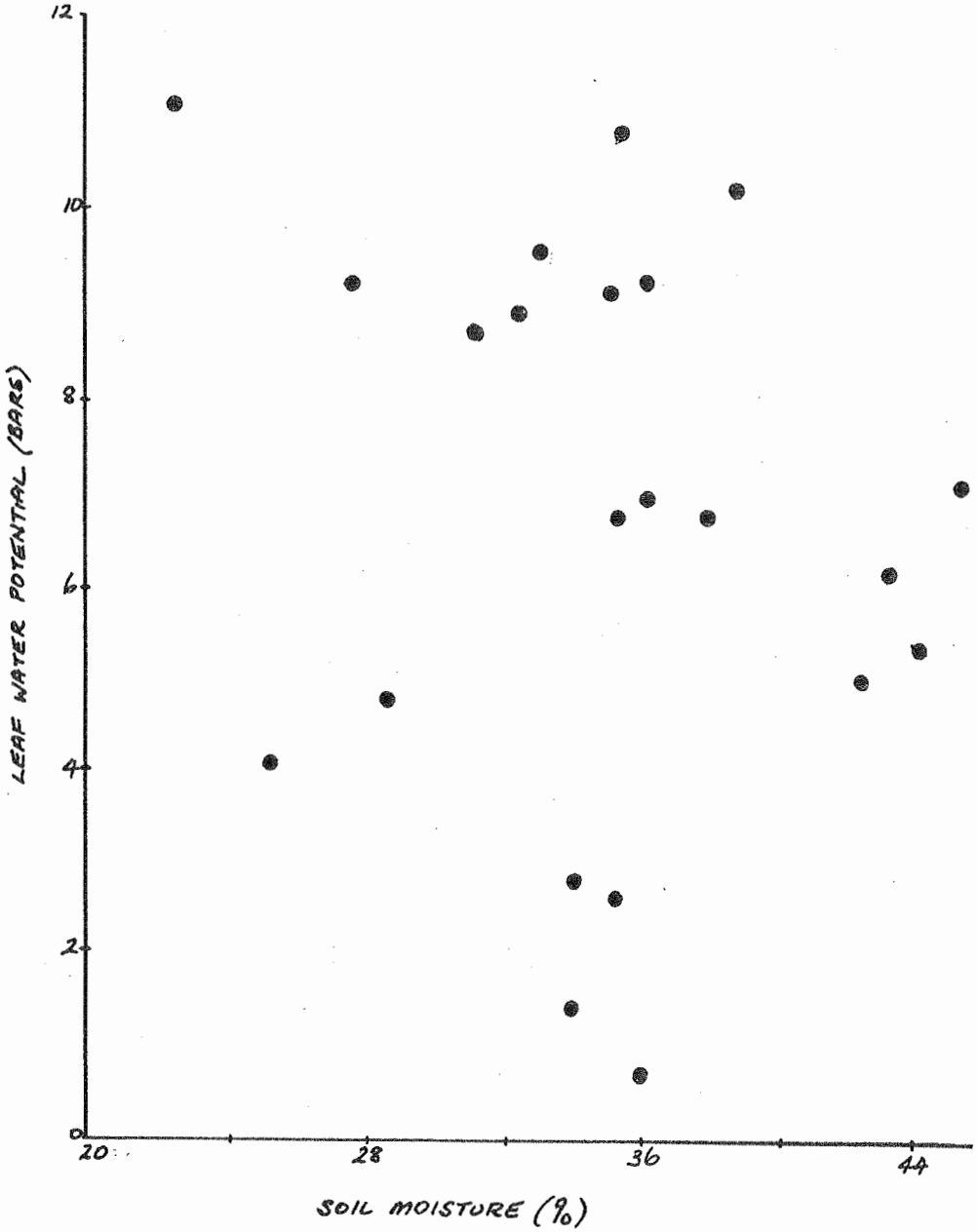


Fig.10.10. The relationship between leaf water potential and soil moisture content at Lake Augusta.
r = 0.125 (N.S.).

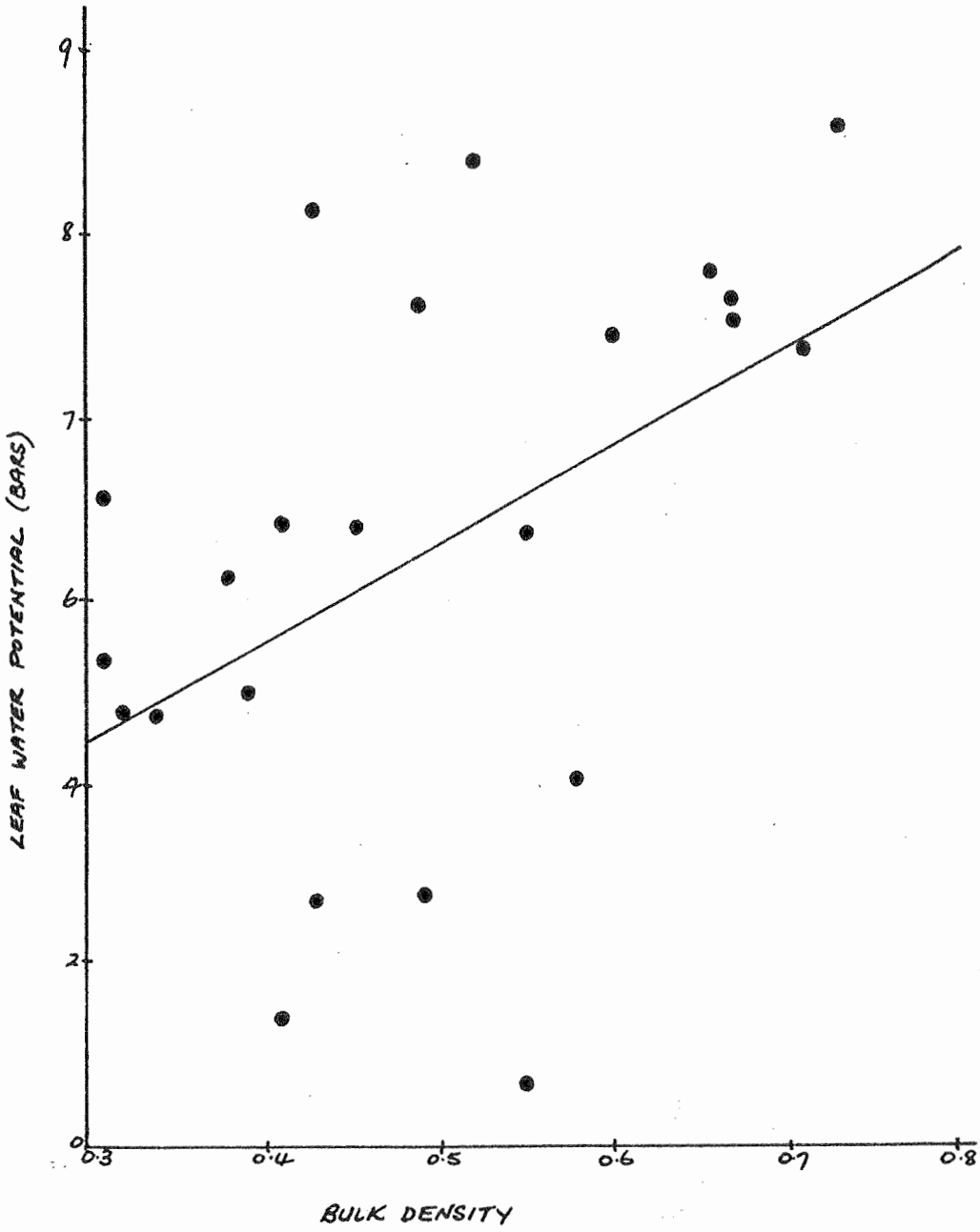


Fig.10.11. The relationship between leaf water potential and bulk density of soil at Lake Augusta.
$y = 1.38 + 10.64x$, $r = 0.467^*$.

3. Leaf-Water Potential and Leaf-Shape

Preliminary measurements made on the leaves of plants grown in the glasshouse showed large differences in leaf-water potential between the broad and narrow leaf-forms. This difference was uncorrelated with leaf-area (Fig. 10.7), but was highly correlated with leaf-shape as measured by the discriminant score calculated among the progeny plants ($r = 0.867$, $P < 0.001$) (Fig. 10.8). The discriminant scores obtained from plants growing in the field were also highly correlated with their leaf-water potentials ($r = 0.782$, $P < 0.001$) (Fig. 10.9), and leaf-water potential similarly was unrelated to soil moisture (Fig. 10.10), but correlated with soil bulk density ($r = 0.467$, $P < 0.05$) (Fig. 10.11).

Discussion

The results of the transplant experiments and the progeny testing suggest that the differences in leaf-shape have a genetic origin. It could be argued that the artificial glasshouse conditions might maintain leaf-types which are inviable in the field, and that the two forms are distinct species. However, the fertility of the progeny plants, and the range of leaf-shapes found - both in the field and among progeny grown in the glasshouse - indicate that the differences obtain within a common gene pool.

It was shown in Chapter 9, that *P. glabrata* exhibits clinal variation across the Central Plateau. This cline involves a complex of characters. If the within population variation in leaf-shape arose from the hybridization of two distinct species, then each of the species must also independently have evolved a cline in this character set in response to directional selection.

From these considerations, it seems more probable that disruptive selection rather than introgressive hybridization is the originating mechanism.

The interrelationships between leaf-shape, leaf-water potential and soil bulk density suggest that the physiological water balance of the plant is partly reflected in the leaf-shape adopted, and that where (as in the present case) there exists a 'patchy' environment, disruptive selective forces might be expected to control the distribution of the different phenotypes.

The extreme forms maintained their relative differences in leaf-water potential when grown in a common environment, and the difference was highly correlated with leaf-shape, so that it too is probably under genetic control. The adaptive significance of this can best be ascertained by a consideration of the differences in the environments in which the two types grow. The broad-leaf-form has a high water potential, and grows where water availability is not limited by the physical nature of the soil. Situated under bushes, the humidity, boundary layer resistance to diffusion, and effective precipitation are increased, whilst the incident radiation would be reduced. This contrasts with the narrow-leaf-form of low water potential, growing on soils where the water availability is decreased, and where the incident radiation and potential evapotranspiration are high.

Differences in leaf-water potential exert a partial control on stomatal closure, and the habitats occupied by the different leaf-forms would have very different light regimes. These facts suggest that the different forms may have differing photosynthetic responses. However, facilities to test this hypothesis were not available.

A number of workers have demonstrated intercorrelations between the particular leaf-shape adopted by a species, the environment in which it grows, and with physiological factors such as differential heat transfer and photosynthetic rate (Holmgren, 1968; Raschke, 1960; Lewis, 1969). Ladiges (1974) has shown that regionally, the length-breadth ratio of the leaves in populations of *Eucalyptus viminalis* is correlated with annual rainfall. However, there appears to be no previous account of an association between leaf-shape and leaf-water potential and their concomitant association with differential water stress occuring over short distances in a single population.

Generally, the degree of contrast which occurs in such a case will be a resultantof the interaction between the magnitude of the difference in selection pressure, and the degree of reproductive isolation occurring between environments (Jain and Bradshaw, 1966). For example, Snaydon (1970, 1973), Snaydon and Davies (1976) and Davies and Snaydon (1973, 1974), have demonstrated that markedly distinct populations of the grass *Anthoxanthum odoratum* have evolved over very short distances in contrasting environments. These populations are both morphologically and physiologically adapted to the conditions pertaining at the source site, and are only partly genetically isolated at the boundary by differences in flowering time.

In the present case, soil factors which determine water availability appear to provide the component of selection. The distribution of soil types forms a continuum rather than a sharp disjunction, and this is reflected in the array of leaf-shapes

occurring at the site. The species is wind-pollinated and although it is polymorphic for flowering time, this is not related to leaf-morphology, and thus this factor does not provide a barrier to gene-flow.

The morphological variation described here is also apparent in other populations on the Central Plateau (Chapter 9), although it is usually less pronounced. However, the narrow leaf-forms tend to occur on sites of soil compaction such as old roads, or sites seasonally subject to drought stress, whilst the broader leaf-forms are restricted to sheltered sites or sites where water is not limiting.

11. A Morphometric Analysis of Variation in the *Plantago tasmanica* Complex

Introduction

Plantago tasmanica and *Plantago daltonii* are perennial rosette herbs occurring naturally in alpine Tasmania. The two species have similar floral characteristics and have the same chromosome number ($2n = 12$, p. 44). The distinguishing features of the taxa are leaf characteristics. This had led to a degree of taxonomic confusion, especially since intermediate forms occur, and these are often difficult to classify. Furthermore, it is evident that leaf-form in the genus *Plantago* is highly responsive to environmental fluctuation.

The purpose of the present study was to undertake a multivariate morphometric study of the interspecific variability of leaf-form of *P. tasmanica* and *P. daltonii*, in an attempt to:-

1. clarify the interspecific differences, and to
2. determine whether the differences of leaf-form are related to habitat.

Taxonomic History

In his description of *P. tasmanica*, Hooker (1860) recognized three varieties: *P. tasmanica* var. L., *P. tasmanica* var. *glabrata* and *P. tasmanica* var. *daltonii*. The var. *glabrata* had been described previously as *P. daltonii* by Decaisne (1852) and included *P. leptostachys* (Hooker, 1847). In addition to the above varieties, Hooker (1860) recognized *P. archeri* as a distinct species.

Later treatments (Bentham, 1870; Rodway, 1903) subsumed all the above taxa in *P. tasmanica*. Pilger's (1937) monograph of the

family places *P. tasmanica* in section *Oliganthos*. *P. archeri* was included in the section as a species of uncertain affinity. Pilger maintained the varietal ranks of var. *glabrata* and var. *daltonii* and suggested *P. tasmanica* var. *eutasmanica* as an alternative to Hooker's *P. tasmanica* var. L.

Curtis (1967) describes two varieties of *P. tasmanica:* the var. *tasmanica* syn. *eutasmanica* Pilger, and *P. tasmanica* var. *archeri*. *P. daltonii* and *P. glabrata* are given specific rank.

Due to the co-extensive nature of the occurrence of *P. daltonii* and *P. tasmanica* in the Victorian highlands, Willis (1973) has resumed these taxa under *P. tasmanica*. However, Briggs (*pers. comm.*) feels that *P. daltonii* is endemic to Tasmania, and that reports of the occurrence of the species in Victoria have resulted from confusion with the closely related *P. alpestris*, a species which does not occur in Tasmania.

The nomenclature used in the present discussion follows Curtis (*loc. cit.*). The three named taxa which are studied are:-

P. tasmanica var. *tasmanica*
P. tasmanica var. *archeri*
P. daltonii

For convenience, the two varieties of *P. tasmanica* will be referred to as *P. tasmanica* and *P. archeri* respectively.

Description of Morphology and Habitat

With the exception of *P. archeri*, the taxa are differentiated by leaf-morphology and this appears to be loosely correlated with habitat (Table 11.1). *P. tasmanica* occurs throughout a wide range of habitats from *Astelia alpina* wet closed bogs, to exposed dry slopes having a sparse ground cover. Its leaves are hispid, broadly lanceolate to oblanceolate and narrowed into a broad

petiole, which is quite short in dry habitats, but elongate in soaks and shady places. *P. archeri* occurs through a similar range, but differs from *P. tasmanica* in having densely tomentose, obovate-elliptic leaves, and scapes scarcely longer than the leaves. *P. daltonii* is restricted to more or less permanently wet situations on creek edges and in soaks, where it may be covered by water for long periods. The species has glabrous, linear-lanceolate to elliptic leaves, borne on narrow petioles as long as or longer than the blade. In habitats such as sheltered rock crevices, and ditches and pools subject to alternating periods of flooding and dryness, a range of intermediate forms occurs. These are similar to *P. tasmanica* and *P. daltonii* in scape and floral characters, but have lanceolate-oblanceolate leaves, which are (glabrous-) sparingly hispid, and borne on broad petioles which are usually slightly shorter than the blade.

Preliminary observations from a wide range of habitats and localities indicated a high degree of homogeneity in floral characters between the taxa. The only exceptions were the relative scape length of *P. archeri* and the degree of anthocyanin pigmentation in seed testas among the taxa. The colour of the testa in seeds of *P. tasmanica* and *P. archeri* was much darker than *P. daltonii*.

Materials and Methods

A series of four experiments was conducted; these were:-

(a) An examination of the field variation

(b) Glasshouse studies on *P. tasmanica* and *P. daltonii* material from the Hartz Mountains

(c) Glasshouse studies on *P. tasmanica, P. daltonii* and intermediate material from the Central Plateau

(d) A preliminary study of germination characteristics in *P. tasmanica* and *P. daltonii*.

Table 11.1: Qualitative Differences Among the Populations

Taxon	No.	Population	*i*	*Sc/l*	*t.p.*	Habitat Notes
P. archeri	1	Projection Bluff	++	1	+	*Astelia* alpine bog
	2	Mt. Field	++	1	+	" " "
	3	Mt. Anne	++	1	+	?
P. tasmanica	4	Crystal Waters	+	2	+	*Eucalyptus coccifera* woodland
	5	Scenic Point	+	2	+	*Restio australis* sedgeland
	6	Mt. Wellington	+	2	+	± bare solifluction soil
	7	Hartz Mountains	+	2	+	Alpine herbfield
Intermediate	8	Projection Bluff	(+)	2	(+)	Roadside drainage ditch, peak flows infrequent in summer
P. daltonii	9	Pine creek	-	2	-	Stream bank subject to inundation in all seasons
	10	Hartz Mountains	-	2	-	" " "
	11	Gordon River	-	2	-	" " "

i = indumentum ++ densely tomentose; + hispid; - glabrous

Sc/l = Scape to leaf length ratio: 1 Scape < leaves; 2 Scapes >> leaves

t.p. = testa pigmentation: + present; - absent

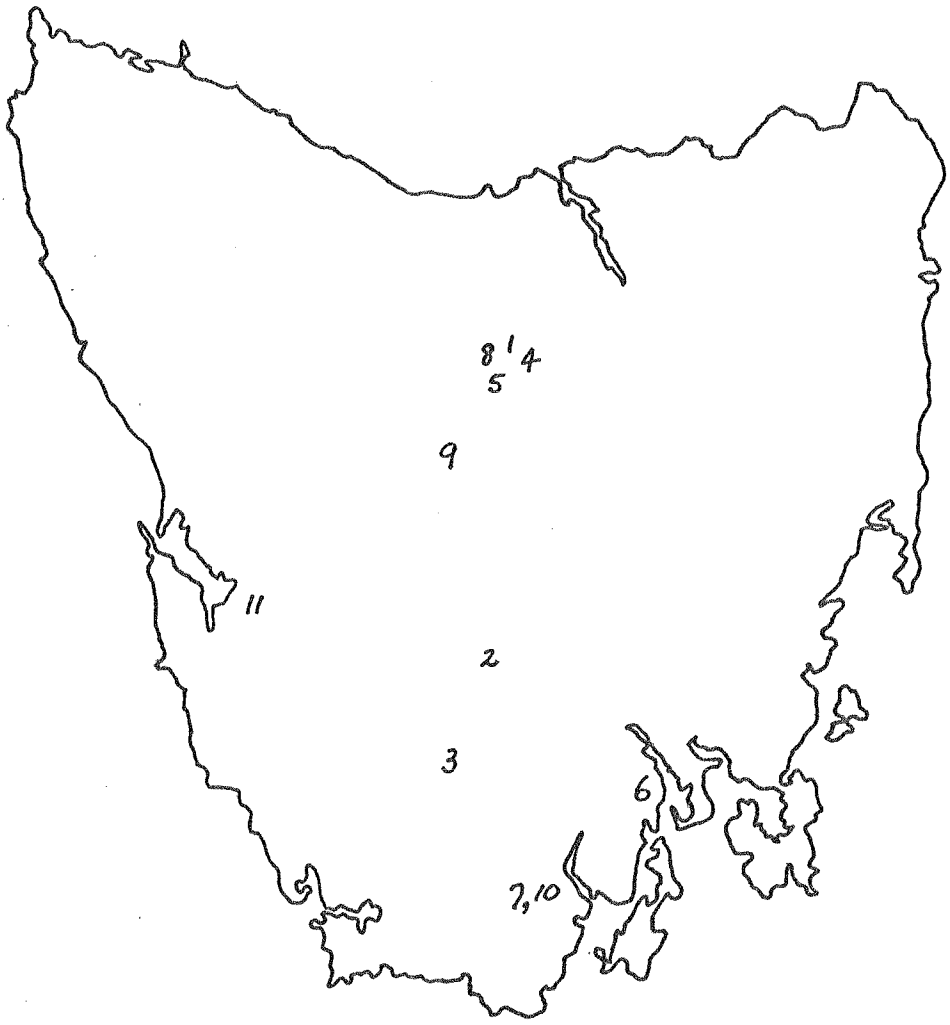


Fig. 11.1. The locations of sites sampled for the analysis of *P. tasmanica* and *P. daltonii*; numbering of sites follows Table 11.1.

(a) Field Sampling

Nine localities were sampled to include the range of habitats and morphological variants encountered within the complex (Table 11.1, Fig. 11.1). In order to allow for heteroblasty, only plants in flower were collected. The six leaf characters measured were:-

length	*l*
width at widest part	*w*
lamina length	*ll*
petiole width	*pw*
thickness	*t*
length from apex to the widest part	*lwp*

The plants were scored subjectively for the degree of leaf-pubescence, scape length, relative petiole length and anthocyanin pigmentation, and the samples were categorised as one of the three taxa, or as intermediate on this basis. Seed colour in plants of the 'intermediate' population was variable, encompassing the full range of pigmentation from pale to dark.

In addition to the nine samples used in the analysis, two further small samples (collected by other workers) were categorised subjectively as *P. daltonii* (Gordon River) and *P. archeri* (Mt. Anne), and were used to test the efficiency of the analytical technique.

(b) Glasshouse Study of the Hartz Mountains Populations

Twenty plants each of *P. tasmanica* and *P. daltonii* collected from the Hartz Mountains were planted into boxes containing a vermiculite-gravel mixture, and grown under a 24 hour or 8 hour photoperiod for six months. Leaves of each type were removed and scored as above.

(c) Glasshouse Study of the Central Plateau Populations

In order to examine the status of the intermediate types, plants of *P. tasmanica*, *P. daltonii* and the intermediates collected from the Central Plateau were planted out together in the glasshouse under natural light and ambient temperature and scored after six months. Scapes present on the intermediates were removed and the plants were then isolated in a separate glasshouse and allowed to flower and set seed. Progeny were grown from seed collected in bulk from these plants, and scored at flowering.

(d) Seed Germination in *P. tasmanica* and *P. daltonii*

Studies on the germination requirements of *P. tasmanica* and *P. daltonii* were made using bulked samples of seed collected from the Central Plateau. Freshly collected seed was sown in petri dishes on germination pads supported by filter paper wicks above a supply of water.

Preliminary studies had shown that *P. daltonii* germinated readily under ambient laboratory conditions (temperature 20 ± 2°C). Seeds from the intermediate population germinated less readily than *P. daltonii*. *P. tasmanica* did not germinate under these conditions (*cf.* Fig. 11.6). Accordingly, two experiments were designed to test the germination requirements of the taxa. In the first experiment, seed of *P. tasmanica* was stratified in the cold room at 4°C for periods of four, six, twelve and twenty weeks and removed to the laboratory to germinate. In the second experiment, the seed was divided into two groups, one set from each taxa acted as controls, whilst the testas of the other set were pricked with a sterilized, mounted needle. The intermediate population was not tested.

In both experiments, seed was sown in four replicates of twenty five seeds per treatment, and germinated at $20^{o}C$.

Morphometric Analyses

Experiments (a)-(c) above, were analysed by canonical variates using the GENSTAT program on the CDC CYBER 76 (CSIRO) computer.

Sample 3 (*P. archeri* from Mt. Anne) and sample 11 (*P. daltonii* from the Gordon River) were excluded from the analysis of the field data since the numbers of plants in these groups were less than the number of characters. Their mean vectors were located along the canonical axes obtained from CVA of the other nine groups.

CVA of the Central Plateau populations was conducted using a total of six groups. These consisted of the 'pure' field populations of *P. tasmanica* (group 4) and *P. daltonii* (9), the intermediate population (8), and the corresponding populations grown in the glasshouse. Canonical scores for the progeny plants were interpolated into this analysis.

The 95% Confidence Limits were calculated as $1.96/\sqrt{n}$ in all analyses (Seal, 1964).

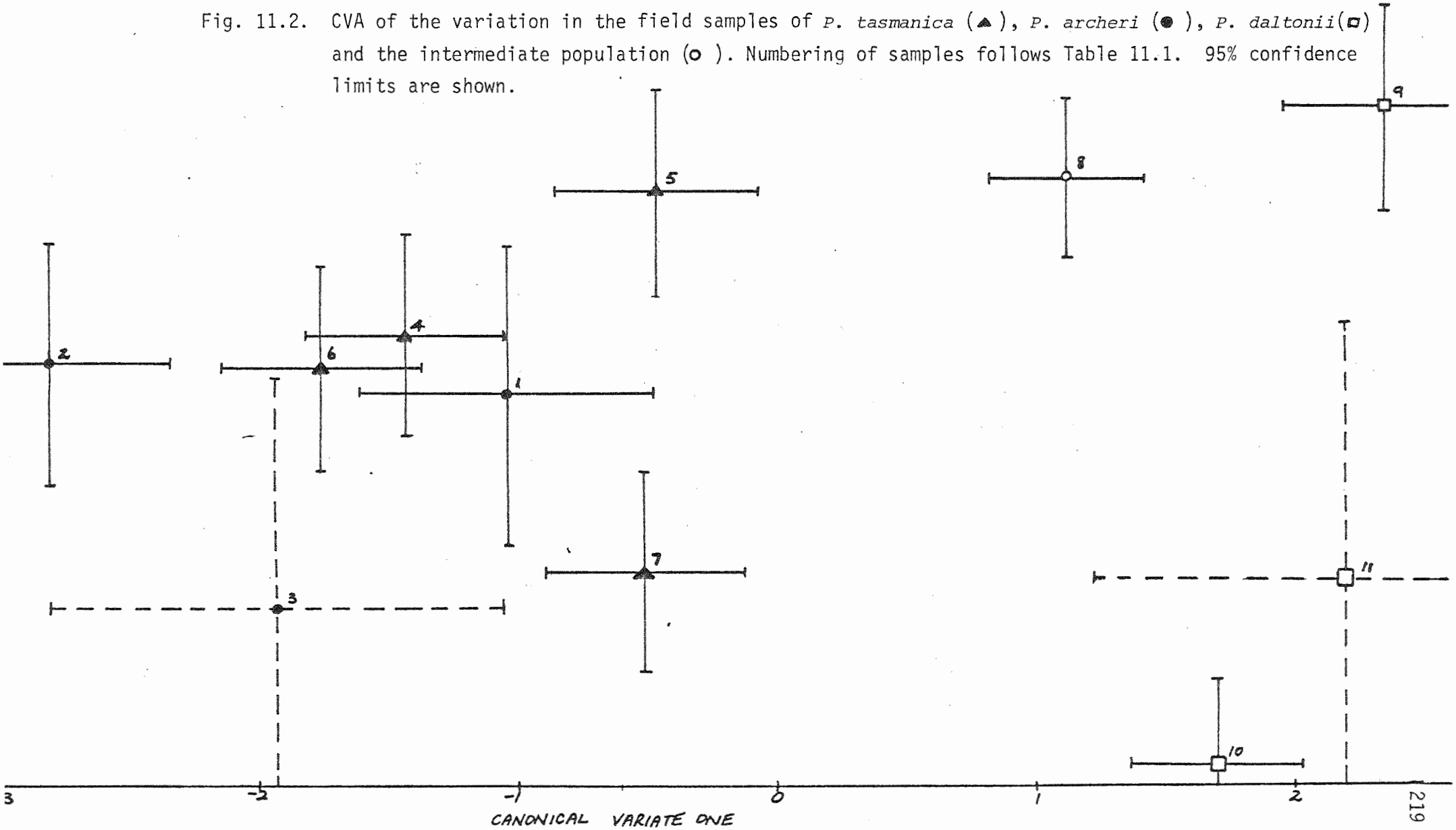
Results and Discussion

Detailed tabulations of results are given in Appendix I.

(a) Variation among the Field Populations

The mean values of the six leaf characters obtained for the eleven field populations are presented in Appendix I, Table 1(a) (p.347). The results of the CVA are given in Table 2 of Appendix I (p.348). The first two canonical variates accounted for 93.5% of the total variation, and 74% of this occurs along the first canonical axis. The mean vectors of the eleven groups were plotted on these axes (Fig. 11.2).

Fig. 11.2. CVA of the variation in the field samples of *P. tasmanica* (▲), *P. archeri* (●), *P. daltonii* (□) and the intermediate population (o). Numbering of samples follows Table 11.1. 95% confidence limits are shown.



The first variate discriminates among the (predesignated) taxa, whilst the second provides evidence of habitat differentiation within the taxa.

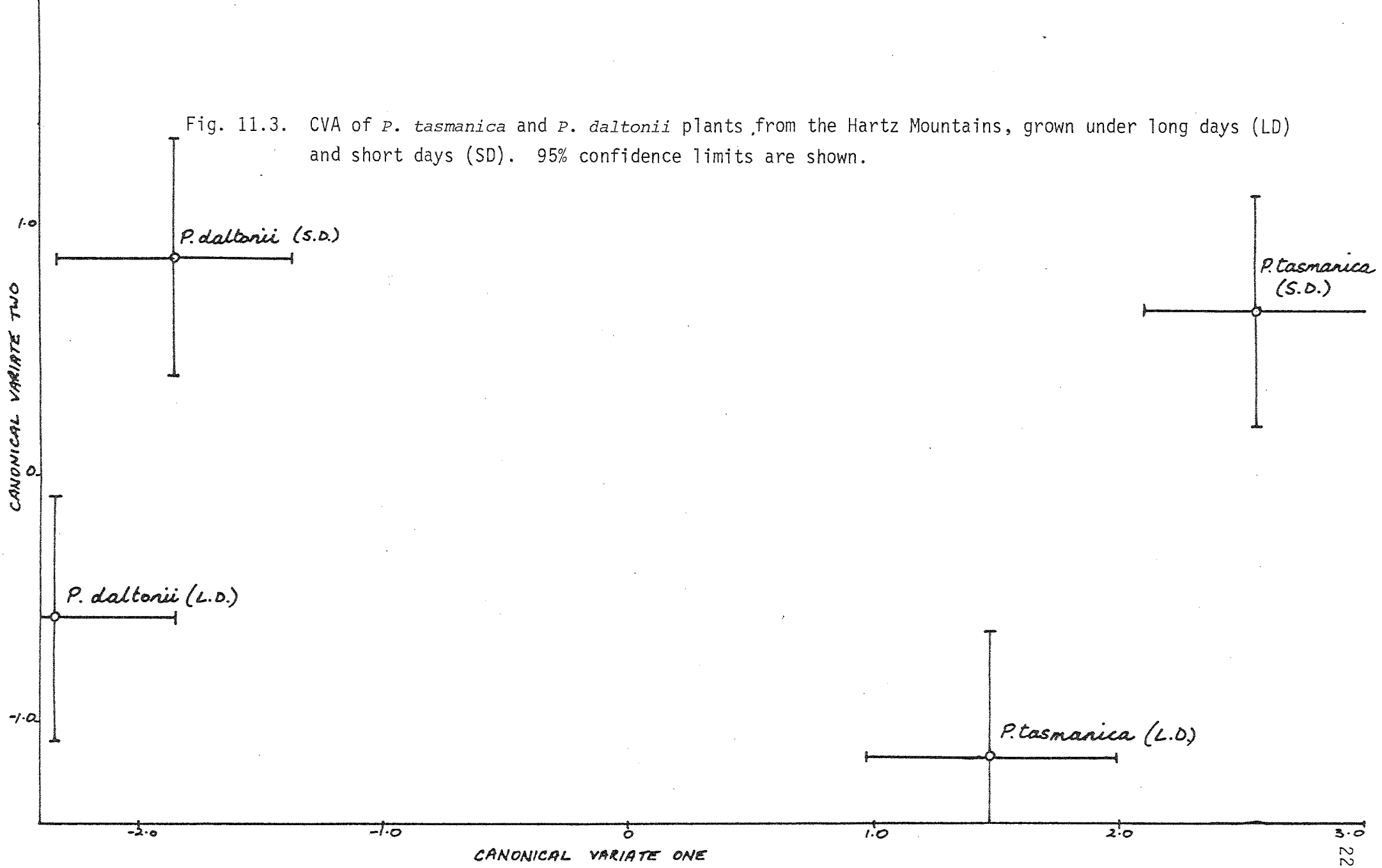
The *P. daltonii* populations (9) and (10) measured on CV1 are disjunct from *P. tasmanica* (4-7) and *P. archeri* (1, 2). The *P. tasmanica* and *P. archeri* populations intergrade, but some differentiation is apparent. Thus *P. archeri* from Mt. Field (2) is distinct from the other populations, and *P. tasmanica* populations (5) and (7) are distinct from (4) and (6). The intermediate population (8) is closer to *P. daltonii* than *P. tasmanica* on this axis. The test samples, which were predesignated *P. archeri* (3) and *P. daltonii* (11) are assigned 'correctly' by the first canonical variate.

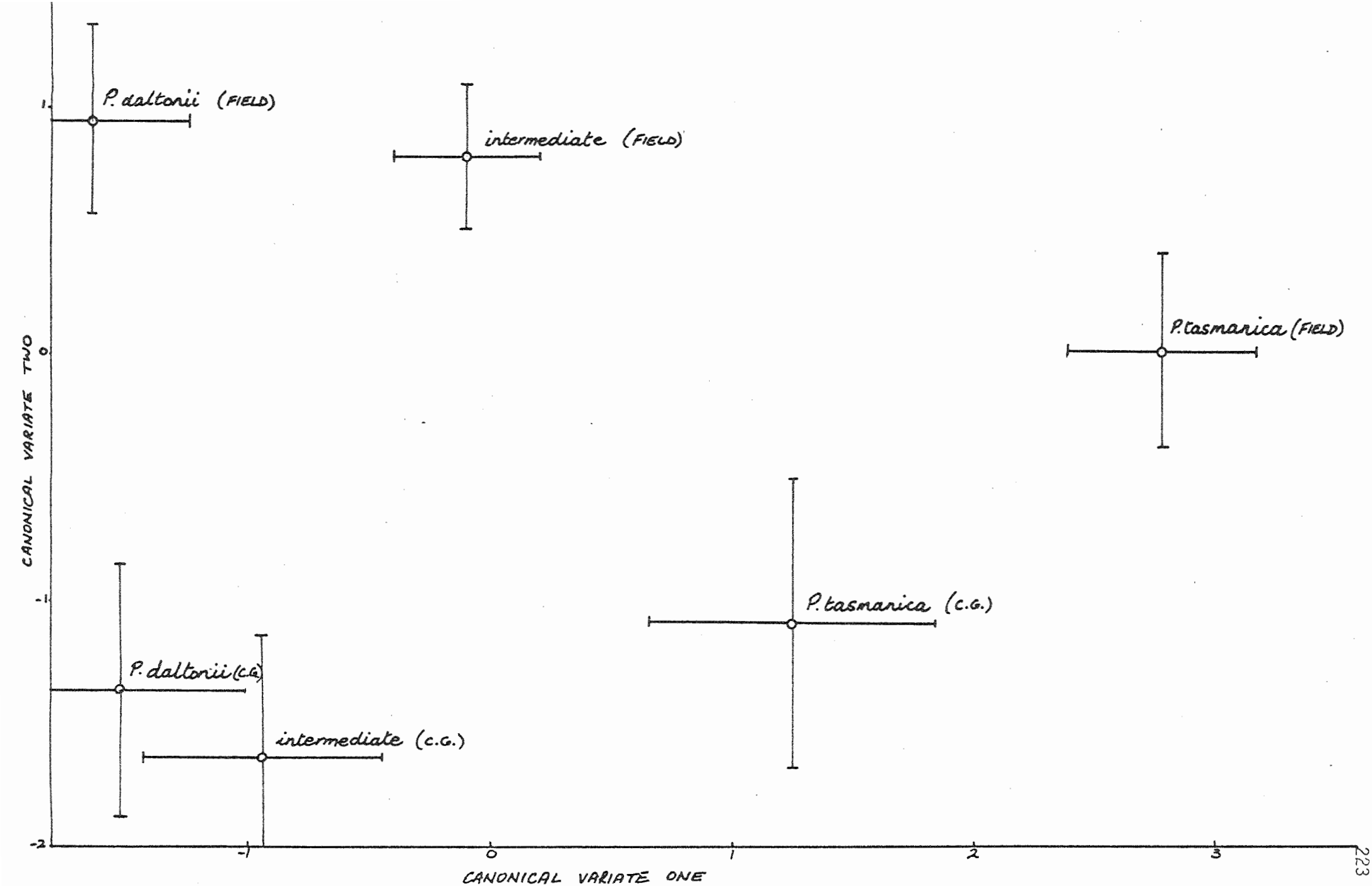
The second canonical variate discriminates between the two *P. daltonii* populations (9 and 10). The *P. tasmanica* and *P. archeri* populations intergrade on this axis, although the extreme populations (5 and 7) are distinct.

(b) Variation in the Hartz Mountains Populations

The mean values of the leaf-characters measured for the Hartz populations of *P. tasmanica* and *P. daltonii* grown under long and short days are given in Table 1(b) of Appendix I (p. 347). The within and between groups SSP matrices and the results of the canonical analysis are given in Appendix I, Table 3 (p. 350). The first canonical axis contains 82% of the total variation and effectively distinguishes the taxa under both photoperiods (Fig. 11.3). The second axis discriminates between photoperiods for both taxa, and accounts for a further 13% of the variation.

Fig. 11.3. CVA of *P. tasmanica* and *P. daltonii* plants from the Hartz Mountains, grown under long days (LD) and short days (SD). 95% confidence limits are shown.




P. daltonii (FIELD)
intermediate (FIELD)
P. tasmanica (FIELD)
P. tasmanica (C.G.)
P. daltonii (C.G.)
intermediate (C.G.)
CANONICAL VARIATE TWO
CANONICAL VARIATE ONE
1
0
-1
-2
-1
0
1
2
3

(c) Variation in the Central Plateau Populations

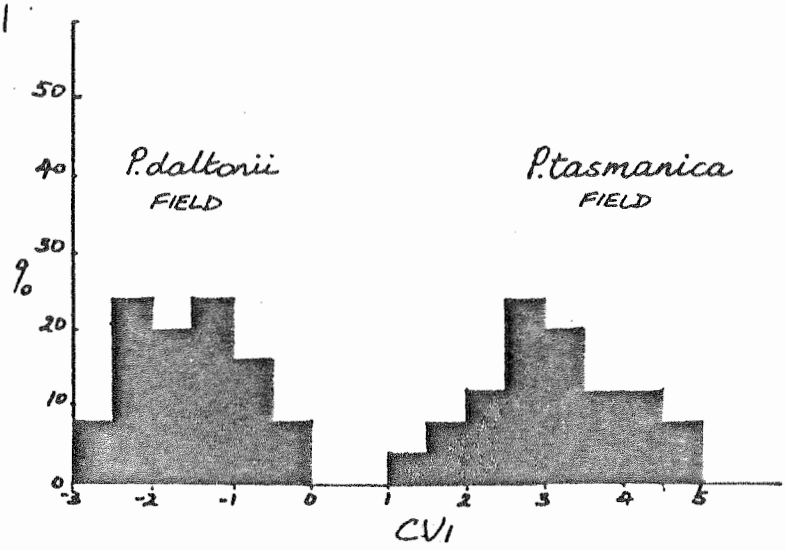
The mean values of the leaf-characters of the Central Plateau samples grown in the glasshouse are listed in Table 1(c) of Appendix I (p.347). The within and between groups matrices of SSP and the results of the canonical analysis of the six groups are given in Appendix I, Table 4 (p.352). The first two canonical vectors take up approximately 96% of the total variance, and exhibit two biologically distinct patterns of variation (Fig. 11.4). The first canonical axis separates the two taxa, whilst the second differentiates among the glasshouse and field environments.

The intermediate population is distinct from both taxa in the field, and occupies a position between them on the first canonical axis, although the mean vector lies generally closer to *P. daltonii*. The glasshouse samples exhibit some convergence of *P. tasmanica* with *P. daltonii* relative to the field populations on this axis, and the intermediate sample is doubtfully distinct from *P. daltonii*.

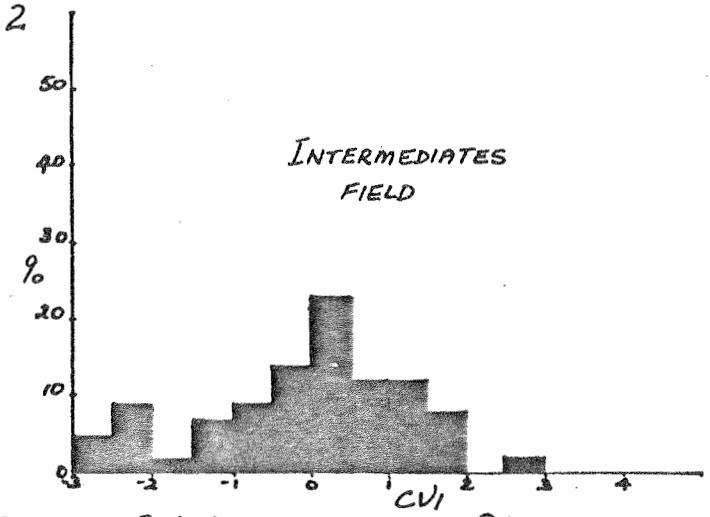
The canonical scores obtained for the six groups along the second axis indicate that the plastic response invoked by the change of environmental conditions is much greater in *P. daltonii* and the intermediate than in *P. tasmanica*.

Frequency histograms were constructed from the scores of CV1 for the various groups (Fig. 11.5). Under field conditions, the distributions of *P. tasmanica* and *P. daltonii* are distinct, whilst individuals from the intermediate population take values which are truly intermediate, i.e. their apparent intermediate status does not result from a bimodal distribution of *P. tasmanica* and *P. daltonii* types.

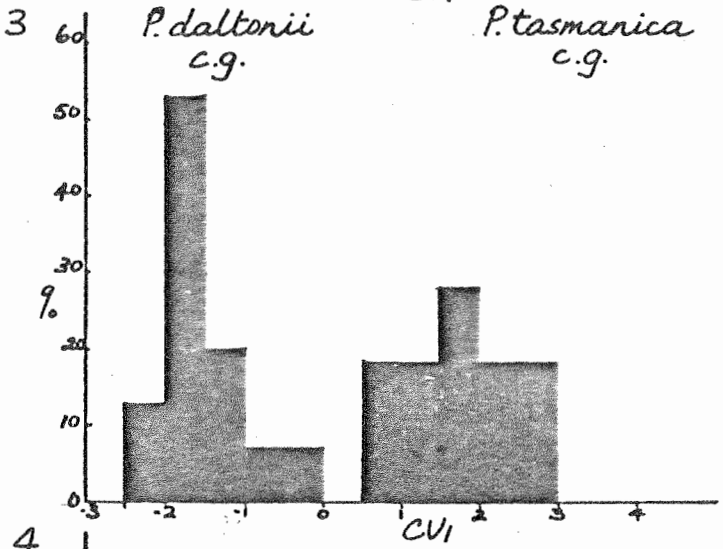
1


P. daltonii
FIELD
P. tasmanica
FIELD
%
CV1

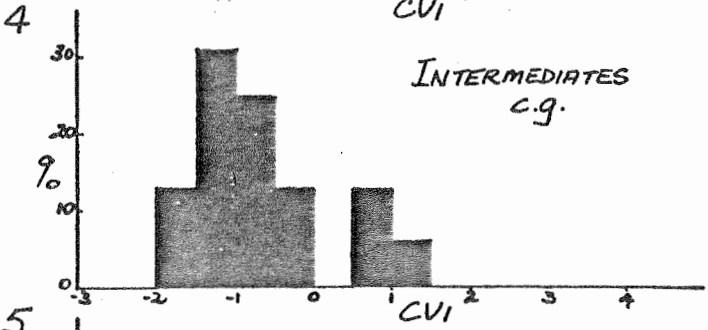
2


INTERMEDIATES
FIELD
%
CV1

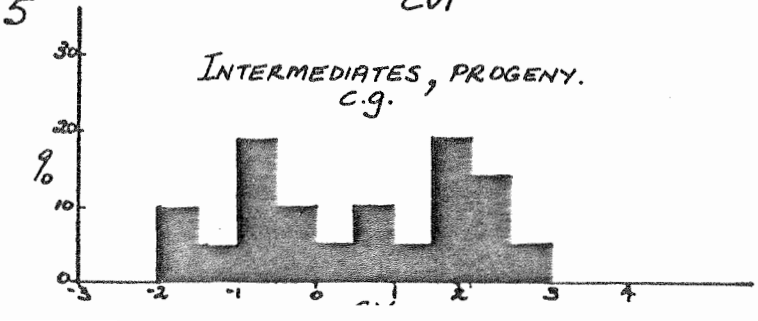
3


P. daltonii
c.g.
P. tasmanica
c.g.
%
CV1

4


INTERMEDIATES
c.g.
%
CV1

5


INTERMEDIATES, PROGENY.
c.g.
%

Under the environmental conditions which prevailed in the glasshouse, *P. tasmanica* and *P. daltonii* remain distinct, but exhibit a degree of convergence. The distribution of the intermediate plants is disjunct, some individuals having values similar to *P. tasmanica* whilst the majority are similar to *P. daltonii*. This disjunction is emphasised in the scores obtained for the progeny plants. These plants have a pronounced bimodal (though overlapping) distribution, the modes lying near those of either *P. daltonii* or *P. tasmanica* grown under the same conditions.

(d) Seed Germination Studies

(i) Stratification

Seed of *P. tasmanica* did not germinate after any of the periods of cold treatment which were tested.

(ii) Scarification

The seeds of *P. daltonii* with disrupted testas, germinated at a marginally faster rate than the control seed lot (Fig. 11.6), although the final percentage of germination was similar in both. The response of the *P. tasmanica* seeds was spectacular. None of the control seeds germinated, whilst 70% and 80% germination of the scarified seeds was achieved after 14 and 30 days respectively. However, the germination rate of the scarified *P. tasmanica* seed was less than that of *P. daltonii*.

The partial inhibition of germination in the intact seeds of the intermediate population, and the complete inhibition in

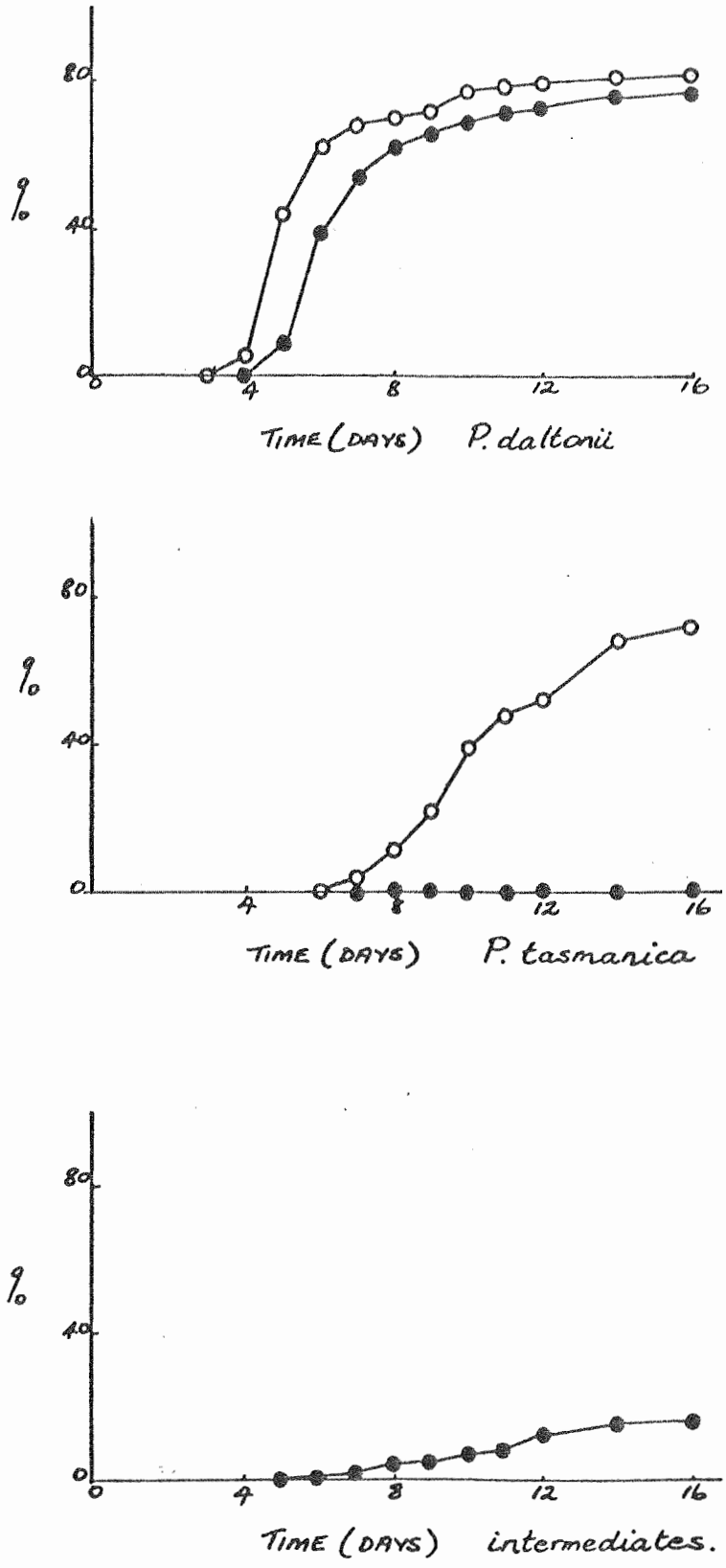


Fig. 11.6. Germination response curves for seeds of *P. daltonii*, *P. tasmanica* and the intermediate population. Each point is the mean of four observations.

● testas intact

o testas disrupted

P. tasmanica, contrast with the response in *P. daltonii*, and appears correlated with pigmentation of the seed testa. The lack of germination does not appear to be the result of inhibition of water uptake, since all seeds imbibed and secreted mucilage, a phenomenon common in many species of *Plantago*. It remains to be tested whether scarification results in the cessation of other germination prevention mechanisms ascribable to an impervious testa, e.g. the prevention of gaseous exchange, or prevention of the leaching of a chemical inhibitor.

Conclusions

The field studies indicated that *P. tasmanica* and *P. archeri* intergrade in the measured leaf characters. However, the two are readily distinguished by their leaf-indumentum and especially by the differences in their relative scape length, so that *P. archeri* should be retained at the varietal rank.

An overlap in leaf-form between *P. tasmanica* and *P. daltonii* was observed in the intermediate population. This cannot be ascribed solely to a morphological convergence resulting from the occurrence of these taxa in an ecologically intermediate (albeit man-made) habitat. Thus, although the frequency distribution of the progeny plants was bimodal, plants having a leaf-morphology intermediate between that of *P. tasmanica* and *P. daltonii* also occurred. The possibility of introgression, therefore, must still be considered.

The degree of plasticity observed in the taxa, and their overall morphological similarity, make it tempting to suggest

that one of the forms has become differentiated relatively recently. This could arise in two ways. It is possible that the two forms arose by a mechanism similar to that apparently occurring in *P. paradoxa* (Chapter 8). Thus *P. daltonii*, for example, might represent an initially plastic form of *P. tasmanica* developed in response to expansion by the species to colonize a new habitat, and which has become genetically fixed. Alternatively, the two forms could equally have arisen more directly through mutation and subsequent fixation by disruptive selection. In either case, the occurrence of intermediate populations suggests two alternatives:-

either (i) the interspecific differentiation is relatively recent, or is still in progress,

or (ii) particular combinations of habitat variables have arisen; these favour the introgressants at the expense of the putative parental species, notwithstanding their potential for plastic modification.

The morphometric analyses reveal that the patterns of leaf-variation in *P.tasmanica* and *P. daltonii* are quite distinct, at least for the six characters measured. While both taxa exhibit plastic responses to environmental change, the overall differences between them are maintained over much of their range. If these differences are considered in conjunction with

(i) the discrete habitats occupied by the taxa,

(ii) the overlap in flowering time between taxa,

(iii) the qualitative differences observable in leaf-indumentum,

(iv) the differences in seed coat pigmentation, and germination response on scarification,

then the taxa probably warrant maintenance at the specific level.

12. A Distance Analysis of Variation in *Plantago* section *Mesembrynia*

Introduction

Most of the intra- and interspecific variability which has been discussed in this thesis has involved only one or two species. The multivariate techniques which were used have provided an effective method of sorting correlated characteristics from those which are independent. This chapter sets out to assess the wider application of multivariate morphometry as a general taxonomic tool. The study involves a number of species of *Plantago* section *Mesembrynia*.

Briggs (1973, 1977) and Briggs *et al.* (1973, 1977) have undertaken a comprehensive review of representatives of *Plantago* occurring in New South Wales. These authors place nine species of *Plantago* into section *Mesembrynia* (Table 12.1). This number includes two new species (*P. turrifera* and *P. cladarophylla*) and several taxa which previously have been included in *P. varia*. Of particular interest to the Tasmanian situation is the separation of *P. hispida* from *P. varia*. This species was included in *P. varia* by Curtis (1967). The authors also have resumed *P. gaudichaudii*, a species not mentioned by Curtis (1967), but which is known in Tasmania from a single record (p. 40). Briggs (*pers. comm.*) has also noted some differences (mainly in characters of the capsules) between the holotype of *P. antarctica* (which was collected in Tasmania) and Australian mainland specimens. In addition to the species discussed by Briggs *et al.*, there are two species of section *Mesembrynia* which are endemic to Tasmania (*P. glabrata, P. bellidioides*).

It was decided to investigate the interrelationships within the whole of the section *Mesembrynia*, or at least of those taxa for which herbarium specimens were available. The revision of herbarium sheets already carried out by Briggs *et al.* provided an excellent basis for a

Table 12.1: The taxa sampled in the analysis of *Plantago* section *Mesembrynia*.

No.	Taxon	Abbreviation	Source	No. available	No. sampled	Chromosome No.	Distribution*
1	*P. debilis*	*deb*	NSW, CANB, HO	128	17	12	Y, Q, N, V, S, T
2	*P. varia*	*var*	NSW, CANB, HO	66	16	24	N, V, S, T
3	*P. glabrata* (SE)	*gSE*	HO	12	12	24	T
4	*P. antarctica* (Tas.)	*aTa*	HO	12	9	12	T
5	*P. antarctica* (Aus.)	*aAu*	NSW, CANB	32	15	12	N, V
6	*P. glabrata* (NW)	*gNW*	HO	12	12	24	T
7	*P. cladarophylla*	*cla*	CANB, NSW	7	6	36	N
8	*P. cunninghamii*	*cun*	CANB, NSW	52	14	12	Y, Q, N, V
9	*P. drummondii*	*dru*	CANB	9	6	12	Y, Q, N, S, V, W
10	*P. bellidioides*	*bel*	HO	6	6	12	T
11	*P. turrifera*	*tur*	CANB, NSW	30	12	12	Q, N, V, S, W
12	*P. hispida*	*his*	CANB, NSW	40	10	12	N, V, S, T, W
13	*P. gaudichaudii*	*gau*	CANB, NSW	49	14	12,24,36	W, N, V, S, T

*Y - Northern Territory
Q - Queensland
N - New South Wales
V - Victoria
S - South Australia
T - Tasmania
W - Western Australia

detailed examination of morphological variation within the section and at the same time provided an assessment of the multivariate analytical techniques.

Materials and Methods

1. Classification of Herbarium Material

Measurements were made on specimens obtained from the herbariums in New South Wales (NSW), Canberra (CANB), and Hobart (HO). The NSW and CANB material had been revised previously by Briggs, Carolin and Pulley. For the purposes of this investigation, the Tasmanian and mainland Australian specimens of *P. antarctica* were treated separately. Two populations of *P. glabrata* were incorporated in the analysis - the narrow leaf-form from the S.E. Central Plateau (*P. glabrata* S.E.) and the broad leaf-form from the N.W. Central Plateau (*P. glabrata* N.W.). In all, there were 13 taxa used in the analysis (Table 12.1).

Table 12.1 also details the distribution, chromosome number and number of specimens sampled for each taxon. Where possible, stratified random sampling of the sheets was used to ensure a broad biogeographic representation within each taxon. Only a few specimens of some taxa were available, and in some cases the number was further reduced by incomplete or poorly pressed material.

2. The Character Suite

In order to facilitate comparison with the previous analysis of *P. glabrata* and *P. antarctica*, the same set of 21 characters (Table 4 of Appendix G, p. 341) was used in this analysis. A preliminary computer run indicated the presence of heterogeneity due to outlying values for some characters, a problem which was not encountered in the earlier analysis. The whole suite was transformed using

$x_i = \log(1 + x_i)$; where x_i is the value of the i^{th} character,

and this resolved the problem (Table 1 of Appendix J, p. 354).

3. The Morphometric Analyses

Two different approaches were adopted; these were:-

(a) A principal components analysis of sepal and petal characters. One of the aims of the study was to determine the extent to which shape characters could assist in the discrimination of the taxa. Accordingly the 3 sepal and 3 petal characters were analysed separately by principal components to obtain variables describing the comparative sizes and shapes of these organs. The mean values of the logarithms of the 3 sepal characters (*sl*, *slwp* and *sw*) and the 3 petal characters (*pl*, *plwp* and *pw*) were used to construct ideograms to represent the forms of organs in each taxon. These were then 'plotted' in the positions of the extracted principal components of each taxon. It was hoped that this procedure would provide a direct comparison of the agreement between the principal components,and the observed shapes of sepals and petals.

(b) A canonical analysis using the 21 characters.

The 13 taxa were analysed by canonical variates. In order to obtain an overall impression of the level of diversification within the section, a minimum spanning tree (Gower and Ross, 1969) was super-imposed on the ordination. The tree was constructed using the Mahalanobis' distances (D^2) obtained between pairs of taxa.

Results and Discussion

Detailed tabulations of results are presented in Appendix J.

(a) The Analysis of Petals and Sepals

(i) Sepal and Petal Size

In both analyses (Tables 2a-c of Appendix J, p. 355) the first (size) component contained the major proportion of the variation (55% in the analysis of the sepals and 74% in the analysis of the petal characters). These values reflect the relatively higher internal correlations among the petal compared with the sepal characters.

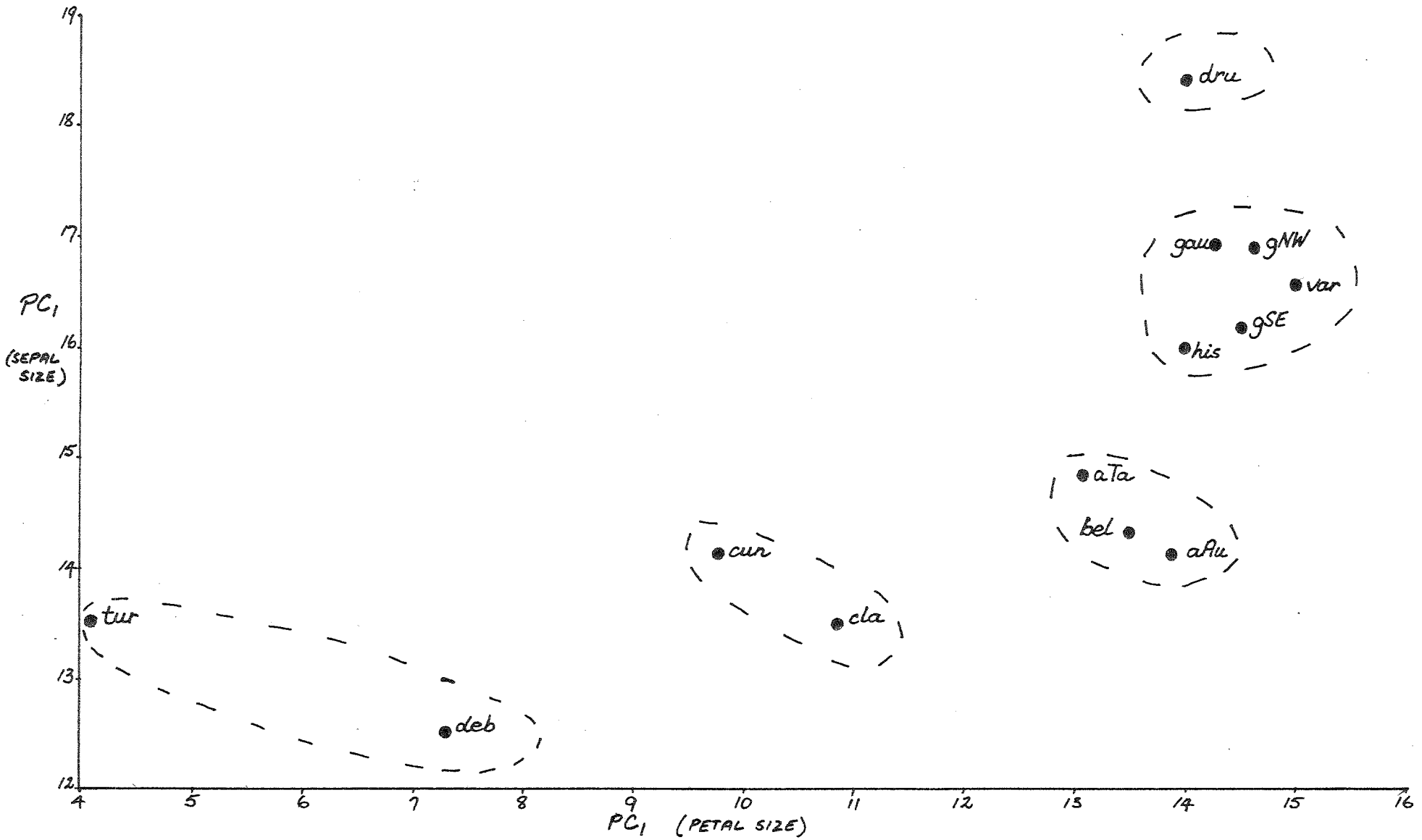


Fig. 12.1. Ordination of the species of section *Mesembrynia* by the principal components which describe the 'size' of sepals and petals.

Fig. 12.1 shows that the taxa can be grouped according to the relative size of their sepals and petals:-

Group 1: Small sepals and petals

P. turrifera, P. debilis

These species have similar sepal sizes, but the corolla lobes of *P. debilis* are somewhat larger than those of *P. turrifera*.

Group 2: Small sepals, intermediate petals

P. cunninghamii, P. cladarophylla

Group 3: Small sepals, large petals

P. antarctica (Tas.), *P. bellidioides, P. antarctica* (Aus.)

The Tasmanian and Australian mainland forms of *P. antarctica* have similar sepal and petal sizes.

Group 4: Intermediate sepals, large petals

P. gaudichaudii, P. glabrata (NW), *P. varia, P. glabrata* (SE), *P. hispida*

The two forms of *P. glabrata* are not differentiated by sepal or petal size.

Group 5: Large sepals, large petals

P. drummondii

This species is separated clearly from the other taxa by its large sepals.

(ii) Sepal and Petal Shape

The remaining principal components describe aspects of shape in both organs. The coefficients of the second and third components are very similar in the two organs. The second component reflects differences in the relative widths of each organ among taxa, and the third contrasts the length with the position of widest part. The overall similarity of the two sets of descriptors may reflect an economy of organisation within the section, or it may be an artefact resulting from the initial selection of characters. However, there is no *a priori* reason why,for example, there should not be concomitant variation of the three sepal characters, contrasting with independent aspects of variation in the

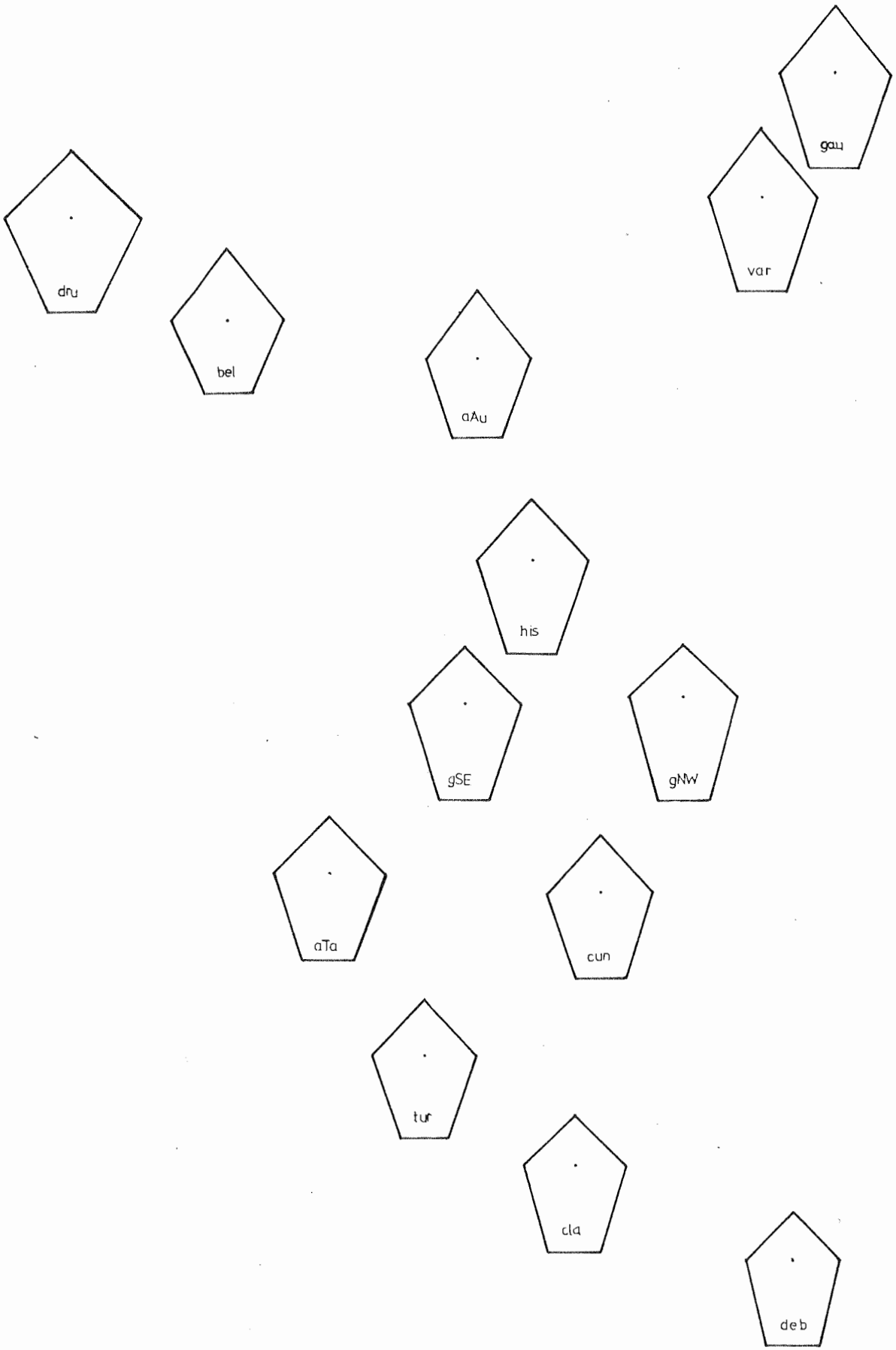


Fig. 12.2. A principal components ordination of section *Mesembrynia* by sepal shape.

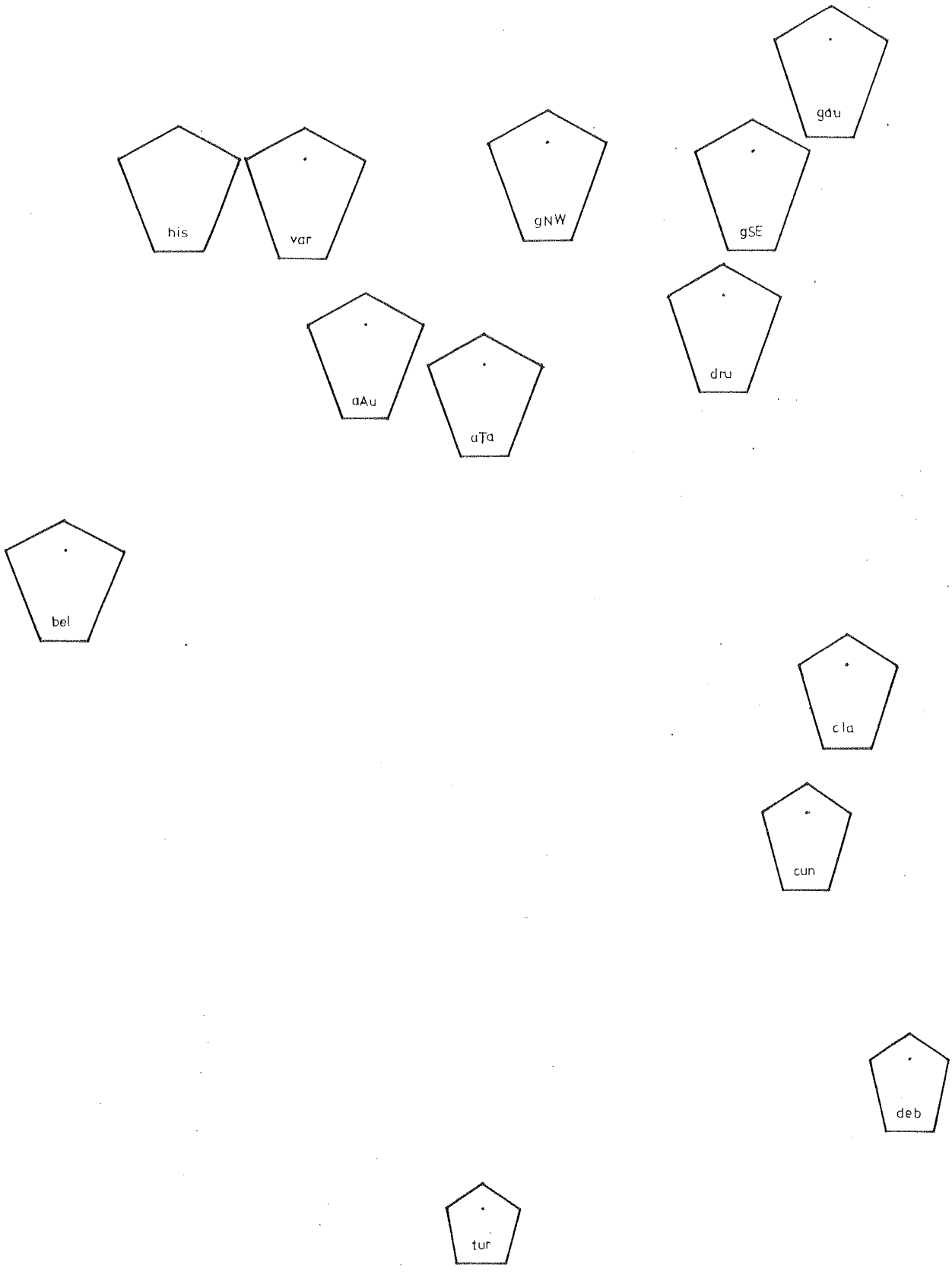


Fig. 12.3. A principal components ordination of section *Mesembrynia* by petal shape.

petals. In any event, plots of the shape components of the sepals (Fig. 12.2) and petals (Fig. 12.3) produce quite different ordinations of the taxa, and these are in agreement with the observed differences of shape.

The sepal shapes of *P. gaudichaudii* and *P. varia* are similar to each other, but they are clearly differentiated from the other species (Fig. 12.2). The other species which are distinct, both from each other, and from the remainder, are *P. debilis*, *P. drummondii* and *P. bellidioides*. There is also some evidence that the Australian and Tasmanian forms of *P. antarctica* differ in the relative position of the widest point of their sepals.

The shape of the corolla lobes (Fig. 12.3) provides further subdivisions of the groups erected on the basis of sepal and petal size. Thus in group 3 (small sepals, large petals), *P. bellidioides* is distinguished from the two forms of *P.antarctica* by the relative width of the corolla lobes. Similarly, the five taxa contained in group 4 (intermediate sepals, large petals) can be subdivided in three subgroups consisting of *P. gaudichaudii*, *P. glabrata* (SE); *P. glabrata* (NW); and *P. varia*, *P. hispida* in order of increasing relative petal width.

(b) The Analysis by Canonical Variates

The CVA resulted in 12 eigenvalues, and the first 8 of these were very highly significant (Appendix J, Tables 3-7, pp. 356-360). The positions of the mean vectors of the taxa on the first two canonical axes is shown in Fig. 12.4. The minimum spanning tree has been super-imposed to clarify the distortion arising from the reduction in dimension. The diagram shows that the taxa fall into five groups:-

(i) Group 1: *P. debilis*, *P. turrifera*, *P. cunninghamii*, *P. cladarophylla*

The first axis clearly differentiates these species from the

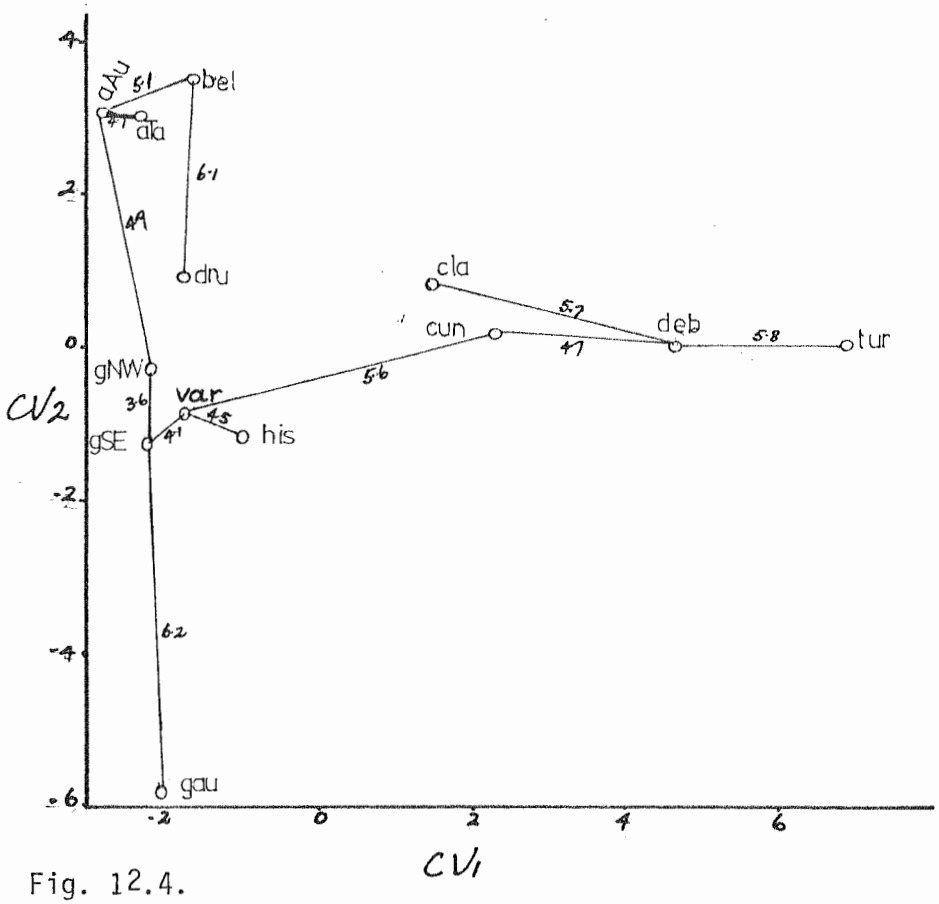


Fig. 12.4.

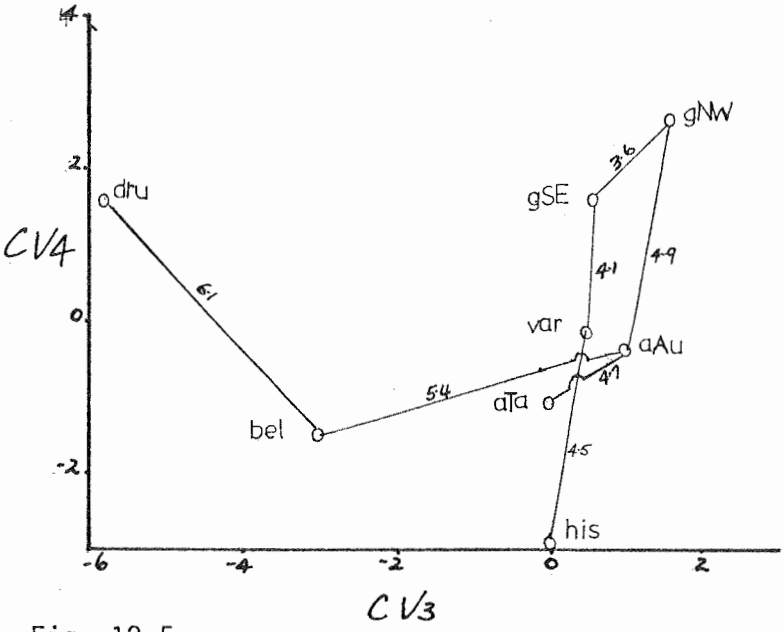


Fig. 12.5.

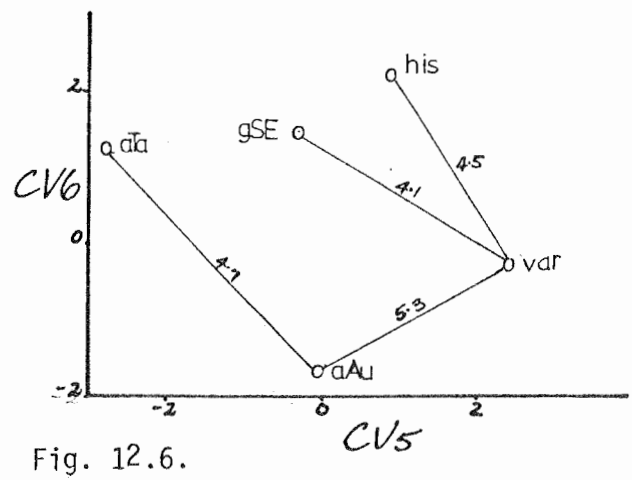


Fig. 12.6.

remainder. The species of this group all have small petals. The group is fairly heterogeneous, since the values of D^2 between each species pair is as great as the values of D^2 which separate the other groups. However, the minimum spanning tree suggests, that for the characters measured, the affinities of *P. turrifera*, *P. cunninghamii* and *P. cladarophylla* are with *P. debilis* rather than with each other or with the remaining taxa.

The large values of D^2 obtained between each of the species of group 1, and their comparatively large distance from *P. varia*, supports Briggs' *et al.* (1977) contention that these taxa are distinct. However, these authors suggest that *P. cunninghamii*, an annual species, has features which are intermediate between two other annual species, *P. turrifera* and *P. drummondii*. On the basis of the present character suite, the distance between these species and *P. cunninghamii* is 6.3 and 6.9 respectively. On these morphological grounds, *P. debilis* (D^2 = 4.7) and *P. varia* (D^2 = 5.6) are the nearest neighbours to *P. cunninghamii*.

(ii) Group 2: *P. gaudichaudii*

The second axis distinguishes *P. gaudichaudii* from all other taxa. The indumentum of the leaf, and the relative leaf-width are two of the characters which enter on this axis. These characters are felt by Briggs *et al.* (1973) and Briggs (1977) to be of significance in the differentiation of *P. gaudichaudii* from *P. varia*.

(iii) Group 3: *P. antarctica* (Aus.), *P. antarctica* (Tas.), *P. bellidioides*

These taxa are characterised by their broad, 5-7-nerved,pubescent leaves, relatively broad bract keels, and small sepals. The two forms of *P. antarctica* are more closely similar to each other than to *P. bellidioides*. The distinction is apparent on the third canonical axis (Fig. 12.5).

The minimum distance between any two of the species studied by

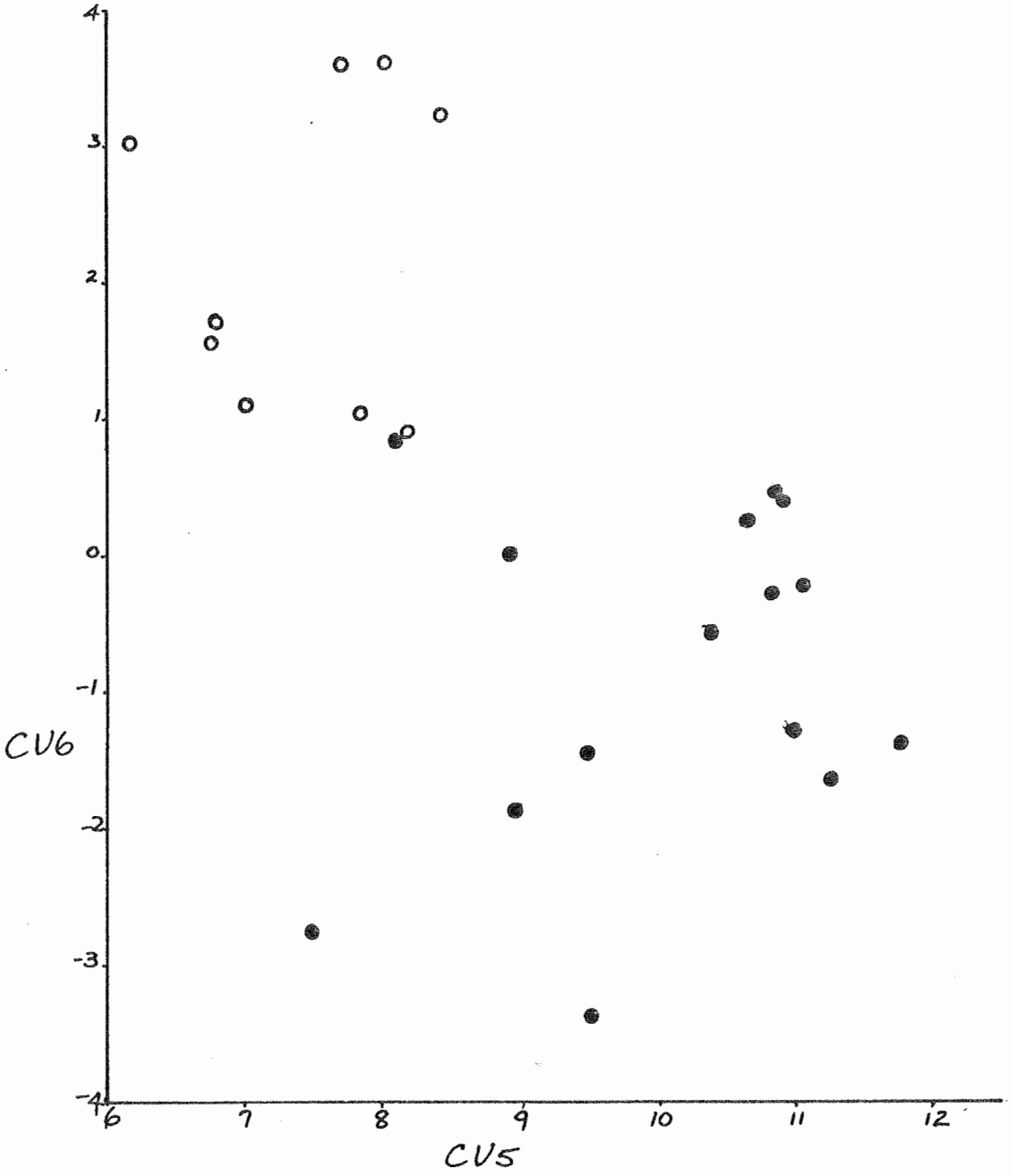


Fig. 127. Scatter diagram of individuals of *P. antarctica* (Tas) ○ and *P. antarctica* (Aus) ● on canonical axes 5 and 6.

Briggs *et al.* (1977) is 4.5 (between *P. varia* and *P. hispida*), with respect to the characters measured for the present analysis. In comparison, the distance between the forms of *P.antarctica* is D^2 = 4.7. Whilst other characters of significance may have been omitted, these values of D^2 suggest that the status of the Tasmanian and Australian forms of *P. antarctica* warrants further investigation.

The major differences between these taxa can be ascribed to the variation along the fifth and sixth canonical axes (Fig. 12.6). Fig. 12.7 shows the distribution of the individual specimens of each taxon along these axes. The scatter diagrams of the two forms are all but disjunct.

The characters which have high loadings on these axes include metric leaf-characters, leaf-indumentum, relative scape length and metric characters of the bracts, sepals, and petals. More plants need to be studied to confirm the constancy of these differences, but the analysis has highlighted some of the characteristics which differentiate the forms.

(iv) Group 4: *P. hispida, P. varia, P. glabrata* (SE), *P. glabrata* (NW).

This group has large petals compared with group 1, but the taxa are intermediate between groups 2 and 3 in the character of their leaves. This grouping is reasonably homogeneous in that it contains the lowest internal D^2 values between pairs of taxa.

The distance between *P. glabrata* and *P. varia* (4.1) is less than the distance between *P. varia* and *P. hispida* (4.5). However, *P. glabrata* and *P. varia* are quite distinct in the combinations of characters measured along the fourth, fifth and sixth canonical axes (Figs. 12.5 and 12.6). The distribution of the taxa on the fourth axis is interesting, since it reflects the ecological-altitudinal gradient

exhibited by the species in Tasmania. The characters which are weighted most heavily on this axis are the leaf-length and width, the relative spike-scape lengths, and the bract length. These characters were also found to exhibit eco-clinal variation within *P. glabrata* (Chapter 9).

The four taxa of this group appear to be the most closely interrelated of all the species in the section. They may also represent an ecologically determined gradient. It has been shown (Chapter 9) that the two forms of *P. glabrata* are samples abstracted from a cline within a single species. The results of the present analysis suggest that this cline may itself be only the upland portion of a more extensive altitudinal cline.

(v) Group 5: *P. drummondii*

This species is clearly distinguished from the remainder by the large value of D^2 between it and its nearest congener (*P. bellidioides*, $D^2 = 6.1$). The species is intermediate between groups 3 and 4 on the second canonical axis (Fig. 12.4), but is manifestly different on the third and fourth axes (Fig. 12.5). The distinction results largely from the relative lengths of scape and spike in the species, its large bracts, and broad bract keels.

A Comparison of the Two Multivariate Analyses

A summary of the groupings obtained by the two multivariate methods is presented in Table 12.2. The table shows the clear similarities among the interspecific groupings obtained by the two techniques:-

Table 12.2. Multivariate groupings of taxa in the section *Mesembrynia*

Method	Species												
	tur	*deb*	*cun*	*cla*	*aTa*	*aAu*	*bel*	*gau*	*gNW*	*gSE*	*var*	*his*	*dru*
PCA	+	+	+	+	+	+	+	+	+	+	+	+	+
CVA	+	+	+	+	+	+	+	+	+	+	+	+	+

Since the principal components groupings were made only on the basis of petal and sepal size, it appears that these characters may be of value in the determination of the overall interspecific relationships within the section.

The major difference between the analyses is the canonical variates separation of *P. gaudichaudii* from *P. varia, P. hispida* and the two forms of *P. glabrata.* All five of these taxa have similar petal and sepal sizes, but *P. gaudichaudii* is readily differentiated from the remainder by the characters of its leaves.

The other difference that arises is the separation of *P. turrifera* and *P. debilis* from *P. cunninghamii* and *P. cladarophylla* by virtue of their petal size in the PCA. For convenience, these species were grouped together after the CVA, to reflect their nearest neighbour relationships to *P. debilis*, rather than to indicate a particular intra-group homogeneity.

The PCA involved the measurement of only 6 characters and was therefore less time consuming than the CVA, in which 21 characters were measured. The results of the PCA indicate that sepal and petal size and shape are useful characters in the delimitation of species within the section. However, a much finer degree of resolution is obtainable using the canonical variate approach, partly because the generalized distance (D^2) between the taxa are obtained concurrently.

An Assessment of the Usefulness of the Multivariate Studies for Taxonomic Research

The multivariate studies have given results which support the taxonomy of the group as established by Briggs *et al.* (1977). However, the two approaches are complementary because the quantification of the established taxa has provided an estimate of the level of diversification within the section. This estimate can then be used to gauge the degree of difference between the established taxa and taxa whose status is less clearly defined.

The characteristics which were measured for these analyses have overlapping ranges among the species and so normally would not be used for taxonomic separation. However, by combining these same characteristics into compounded descriptors of form (i.e. size and shape), the multivariate analyses have provided new sets of characters which can be used to differentiate among (and show the affinities of) the various taxa. At the same time, the analyses have highlighted some areas which will require more detailed studies in the future.

For example, the analyses have supported the contention that there are distinct forms of *P. antarctica* from Tasmania and from the mainland (see p. 244). A closer study is required, because the compounded set of characters which discriminated between the taxa might reasonably be expected to exhibit clinal variation. Whilst more plants need to be examined before any taxonomic decision can be made, the analysis has indicated that this compounded character set can be used to discriminate among the taxa in further studies.

P. hispida, *P. varia* and the two forms of *P. glabrata*, are the most closely related species in the present analysis, and these species occur in an altitudinal gradient within Tasmania. It is possible that *P. varia* (2n = 24) has arisen from a polyploid form of

P. hispida (2n = 12), and subsequently has become differentiated at the upper end of its range to form *P. glabrata* (2n = 24). However, any speculation about the origins of the taxa, and especially about the role of polyploidy in the evolution of Australian *Plantago* species, must be premature because the present analysis includes only species of section *Mesembrynia*. It has been noted previously (p. 36) that Pilger's distinctions between *Mesembrynia* and *Oliganthos* appear to be artificial. Briggs (1973) has shown that the karyotypes are very stable across species of both sections and which encompass the extremes of morphological variation to be found in Australia. Therefore, more detailed phylogenetic speculation must await a joint analysis of both of the sections which occur in Australia.

Conclusion

The multivariate techniques are capable of numerically depicting complexes of characters, for ordination of the taxa. However, the worth of these character complexes for taxonomic weighting must still be decided by the investigator.

In an experimental taxonomic investigation, the real value of the multivariate methods lies not so much in their taxonomic decision-making ability, as in their value for sorting out useful character complexes from background "noise" and for generating hypotheses for genecological research. Thus the techniques may suggest possible clinal trends, indicate a response to particular selective factors, or otherwise imply similarities between the taxa in their ecological requirements.

13. Concluding Remarks

The purpose of this study was to examine the adaptive nature of shape variation in Tasmanian species of the genus *Plantago*. Studies conducted in Europe and America have indicated that high levels of variability are typical of the genus. This was also found to be the case with the Tasmanian species.

The investigation was undertaken using the techniques of multivariate analysis. The advantages of these methods were twofold. Firstly, the techniques freed the investigation from the problems of covariation among the original variables. Secondly, they were to some extent heuristic (*cf.* Sneath and Sokal, 1972) in that they generated viable inter-relationships from the original characters for further investigation. These inter-relationships were found to provide reasonable descriptors of form. Furthermore, ordination of the data by these compounded descriptors frequently suggested modes of plant-environment interaction.

The maintenance of variability in populations of *Plantago* was found to result variously from phenotypic and/or genetic modification. Usually these mechanisms were found to act in concert. In particular, the phenotypic plasticity in leaf shape was found to provide a reliable means of offsetting the effects of seasonal and other environmental change on the extant genotypic variation. The plasticity may occur as a more or less immediate response to a particular environmental trigger, or it may be auto-regulatory, and tied to an indirect, but more predictable, factor. Furthermore, it is possible that, with continued selection, these responses may become genetically fixed. Hence, the maintenance of variability in leaf shape has provided a major evolutionary strategy for the colonization of new environments, and in the occupation of

unpredictable and frequently disruptive habitats.

The investigation has highlighted two significant areas for further research. Evidence supporting the hypothesis of the adaptive significance of phenotypic plasticity has accumulated in the course of the study. The arguments favouring this hypothesis would have a firmer footing if the propensity for selection of plasticity in particular characters was known. Also the adaptive nature of the observed differences has been inferred largely from their correlation or association with particular features of the environment. The inference would be enhanced if both the environmental factors and the external morphology could be shown to be related to physiological differences.

BIBLIOGRAPHY

Allan, H.H. (1961). Flora of New Zealand. I. 780-786. Govt. Printer, Wellington N.Z.

Anderson, E. (1954). Efficient and Inefficient methods of Measuring Specific Differences. Ch. 6. IN "Statistics and Mathematics in Biology", ed. Bancroft, T., Gowen, J.W. and Lush, J.L. Iowa State College Press.

Ashby, E. (1948a). Studies in the morphogenesis of leaves. I. An Essay on Leaf Shape. New Phytol. 47 153-195.

Ashby, E. (1948b). Studies in the morphogenesis of leaves. II. The Area Cell Size and Cell Number of Leaves of *Ipomoea* in Relation to their Position on the Shoot. New Phytol. 47 177.

Ashby, E., Wangermann, E. and Winter, E.J. (1949). Studies in the morphogenesis of leaves. III. Preliminary Observations on Vegetative Growth in *Lemna minor*. New Phytol. 48 374.

Atchley, W.R. (1971). A Comparative Study of the Cause and Significance of Morphological Variation in the Adults and Pupae of Culicoides: A Factor Analysis and Multiple Regression Study. Evoln. 25 563-583.

Atchley, W.R. and Hensleigh, D.A. (1974). The Congruence of Morphometric Shape in Relation to Genetic Divergence in Four Races of Morabine Grasshoppers. Evoln. 28 416-427.

Baker, H.G. (1965). Characteristics and Modes of Origin of Weeds. In "The Genetics of Colonizing Species" ed. Baker. H.C. and Stebbins, G.L. 147-168.

Banks, M.R. (1972a). General Geology in "The Lake Country of Tasmania" ed. M.R. Banks, Symp. Roy. Soc. Tas. 25-33.

Banks, M.R. (1972b). Geomorphology in "The Lake Country of Tasmania" ed. M.R. Banks, Symp. Roy. Soc. Tas. 55-60.

Barber, H.N. (1965). Selection in Natural Populations. Heredity 20 551.

Barber, H.N. and Jackson, W.D. (1957). Natural Selection in Action in *Eucalyptus*. Nature 179 1267-1269.

Barnéoud, F.M. (1845). Monogr. Génér. de la Famille des Plantaginées.

Bassett, H.I.S. (1966). Taxonomy of North American *Plantago* L., section *Micropsyllium* Decne. Can. J. Bot. 44 467-479.

Bassett, H.I.S. (1967). Taxonomy of *Plantago* L. in North America, sections *Holopsyllium* Pilger, *Palaeopsyllium* Pilger and *Lamprosantha* Decne. Can. J. Bot. 45 565.

Bassett, H.I.S., and Crompton, C.W. (1968). Pollen Morphology and Chromosome Numbers of the Family Plantaginaceae in North America. Can. J. Bot. 46 349-361.

Beals, E.W. (1973). Ordination: Mathematical Elegance and Ecological Naiveté. J. Ecol. 61 23.

Benson, L. (1957). Plant Classification. D.C. Heath & Co.

Bentham, G. (1870). Flora Australiensis. V. 137-142.

Bentham, G., and Hooker, J.D. (1862-1883). Genera Plantarum. London.

Bessey, C.E. (1897). Phylogeny and Taxonomy of the Angiosperms. Bot. Gaz. 24 145-178.

Blackith, R.E. and Reyment, R.A. (1971). Multivariate Morphometrics. Academic Press, London and New York.

Böcher, T.W. (1967). Continuous Variation and Taxonomy. Taxon. 16 255-258.

Böcher, T.W., Larson, K. and Rahn, K. (1953). Experimental and Cytological Studies on Plant Species. I. Hereditas 39 289-302.

Böcher, T.W., Larson, K. and Rahn, K. (1955). Experimental and Cytological Studies on Plant Species. III. Hereditas 41 423-453.

Bouwkamp, J.C. and Honma, S. (1970). Vernalization Reponse, Pinnae Number, and Leaf Shape in Celery. J. Hered. 61 115-118.

Boyer, J.S. (1966). Leaf Water Potential Measured with a Pressure Chamber. Pl. Physiol. 42 133-137.

Bradshaw, A.D. (1952). Populations of *Agrostis tenuis* Resistant to Lead and Zinc Poisoning. Nature 169 1098.

Bradshaw, A.D. (1965). The Evolutionary Significance of Phenotypic Plasticity in Plants. Adv. Genet. 13 115.

Bradshaw, A.D. (1974). The Evolutionary Lessons of Heavy Metal Tolerance. Heredity 33 450.

Briggs, B.G. (1973). Chromosomal Studies on *Plantago* in Australia. Cont. N.S.W. Nat. Herb. 4 (7) 399-405.

Briggs, B.G. (1977). Research on *Plantago*. Aus. Plants 9 84.

Briggs, B.G., Carolin, R.C. and Pulley, J.M. (1973). New Species and Leptotypification in Australian *Plantago*. Cont. N.S.W. Nat. Herb. 4 (7) 395-398.

Briggs, B.G., Carolin, R.C. and Pulley, J.M. (1977). Plantaginaceae. (M.D. Tindale, Ed.). No. 181, in "Flora of New South Wales". (L.A.S. Johnson, Dir.) National Herbarium of New South Wales. 35 pp.

Brown, R. (1810). Prodromus Florae Novae Hollandiae et Insulae Van Dieman. 279-281.

Buckney, R.T., and Tyler, P.A. (1973). Chemistry of Tasmanian Inland Waters. Int. Rev. Ges. Hydrobiol. 58 61-78.

Bureau of Meteorology (1976). Climate of Tasmania, Rep. from Tasmanian Year Book, no. 10.

Cain, A.J. and Sheppard, P.M. (1954). The Theory of Adaptive Polymorphism. Am. Nat. 88 321-326.

Clausen, J., Keck, D.D. and Hiesey, W.M. (1939). The Concept of Species, based on Experiment. Am. J. Bot. 26 103-106.

Clausen, J., Keck, D.D., and Hiesey, W.M. (1940). Experimental Studies on the Nature of Species. I. The Effect of Varied Environments on Western North American Plants. Publ. Carnegie Inst. 520 452 pp.

Clausen, J. and Hiesey, W.M. (1958). Experimental Studies on the Nature of Species. IV. Genetic Structure of Ecological Races. Carneg. Inst. Wash. Publ. 615 312 pp.

Clausen, J. and Hiesey, W.M. (1960). The Balance Between Coherence and Variation in Evolution. Proc. Nat. Ac. Sci. 46 494-506.

Clifford-Evans, G. (1972). The Quantitative Analysis of Plant Growth.

Cline, M.G. and Agatep, A.O. (1970a). Temperature and Photoperiodic Control of Developmental Responses in Climatic Races of *Achillea*. Plant and Cell Phys. 11 599-608.

Cline, M.G. and Agatep, A.O. (1970b). Temperature and Photoperiodic Control of Developmental Responses in Climatic Races of *Mimulus*. Plant and Cell Phys. 11 609-619.

Cook, C.D.K. (1968). Phenotypic Plasticity with Particular Reference to Three Amphibious Plant Species. In "Modern Methods in Plant Taxonomy" ed. V. Heywood. Academic Press.

Cook, C.D.K. (1969). On the Determination of Leaf Form in *Ranunculus aquatilis*. New Phytol. 68 469-480.

Cooley, W.W. and Lohnes, P.R. (1971). Multivariate Data Analysis. Wiley and Sons.

Costin, A.B. (1972). Characteristics and use of Australian High Country, in "The Lake Country of Tasmania" ed. M.R. Banks, Symp. Roy. Soc. Tas. 1-23.

Cronquist, A. (1968). The Evolution and Classification of Flowering Plants. T. Nelson and Sons Ltd.

Curtis, W.M. (1967). A Students' Flora of Tasmania. III. 557-565.

Dale, M.B., Groves, R.H., Hall, V.J. and O'Callaghan, J.F. (1971). A New Method for Describing Leaf Shape. New Phytol. 70 437-442.

Dancik, B.P. and Barnes, B.V. (1973). Leaf Diversity in Yellow Birch. Can. J. Bot. 52 2407-2414.

Daubenmire, R.F. (1974). Plants and Environment. Third Ed. Wiley and Sons.

Davies, M.S. and Snaydon, R.W. (1973). Physiological Differences Among Populations of *Anthoxanthum odoratum* L. Collected from the Park Grass Experiment, Rothamsted. I. Response to Calcium. J. Appl. Ecol. 10 33-55.

Davies, M.S. and Snaydon, R.W. (1974). Physiological Differences Among Populations of *Anthoxanthum odoratum* L. Collected from the Park Grass Experiment, Rothamsted. II. Response to Phosphate. J. Appl. Ecol. 11 669-707.

Davis, P.H. and Heywood, V.H. (1963). Principles of Angiosperm Taxonomy. Oliver and Boyd, London.

Day, B. and Fisher, R.A. (1937). The Comparison of Variation in Populations having Unequal Means. Ann. Eng. 7 33.

de Wit, H.C.D. (1965). Plants of the World. The Higher Plants. II. Thames and Hudson, London.

Decaisne, (1852). Plantaginaceae in D.C. Prod. XIII. 693-737.

Dobzhansky, T. (1951). Genetics and the Origin of Species. Third Ed., Revised. Columbia University Press.

Edwards, I.J. (1973). Interactions between Vegetation and Water Yield in Tasmanian Highland Catchments. Ph.D. Thesis, Uni. of Tas.

Engler, A. (1954). Quoted in Willis (1973). q.v.

Engler, A. and Prantl, K. (1897). Die Natürliche Pflanzenfamilien. IV. 3b. 363-373.

Falconer, D.S. (1970). Introduction to Quantitative Genetics. Oliver and Boyd, London.

Fisher, F.J.F. (1960a). Leaf Shape in *Ranunculus insignis*. N.Z. J. of Sci. 3 676-684.

Fisher, F.J.F. (1960b). A Discussion of Leaf Morphogenesis in *Ranunculus hirtus*. N.Z. J. of Sci. 3 685-693.

Fisher, R.A. (1936). The Use of Multiple Measurements in Taxonomic Problems. Ann. Eug. 7 179-188.

Fisher, R.A. (1950). Gene Frequencies in a Cline Determined by Selection and Diffusion. Biometrics. 353-361.

Ford, E.B. (1975). Ecological Genetics. 5th ed. Methuen.

Good, R. (1965). The Geography of Flowering Plants. Longmans.

Gower, J.C. and Ross, G.J.S. (1969). Minimum Spanning Trees and Single Linkage Cluster Analysis. Appl. Stat. 18 54.

Gregor, J.W. (1930). Experiments on the Genetics of Wild Populations. I. *Plantago maritima*. Journ. Genet. 22 15.

Gregor, J.W. (1939). Experimental Taxonomy. IV. Population Differentiation in N. American and S. American Sea Plantains allied to *Plantago maritima*. New Phytol. 38 293.

Gregor, J.W. and Watson, P.J. (1961). Ecotypic Differentiation: Observations and Reflections. Evoln. 15 166-173.

Greig-Smith, P. (1964). Quantitative Plant Ecology. Butterworth, London.

Groot, J. and Böschhuizen, R. (1970). A Preliminary Investigation into the Genecology of *Plantago major*. J. Expl. Bot. 21 (68) 835.

Groves, B.E. and Hair, J.B. (1971). Contributions to a Chromosome Atlas of the New Zealand Flora - 15, Miscellaneous Families. N.Z. J. of Bot. IV. 569.

Haccius, B. and Garrecht, M. (1963). Durch Phenylbörsaure induzierte "Lanze blattrigheit" bei *Solanum lycopersicum*. Naturwissenschaften 50 133-134

Haldane, J.B.S. (1948). The Theory of a Cline. J. of Genetics. 48 277-284.

Harberd, D.J. (1957). The Within Population Variance in Genecological Trials. New Phytol. 56 269-280.

Heslop-Harrison, J. (1953). New Concepts in Flowering Plant Taxonomy. Heinemann.

Heslop-Harrison, J. and J. (1958). Studies on Flowering Plant Growth and Organogenesis. III. Leaf Shape Changes Associated with Flowering and Sex Differentiation in *Cannabis sativa*. Proc. Roy. Irish Acad. Sect. B. 59 257.

Heslop-Harrison, J. (1964). Forty Years of Genecology. Adv. Ecol. Res. 2 159-247.

Hiesey, W.M. and Milner, H.W. (1965). The Physiology of Ecological Races and Species. Ann. Rev. Pl. Physiol. 16 203-216.

Hiesey, W.M. and Nobs, M.A. (1970). Genetic and Transplant Studies on Contrasting Species and Ecological Races of the *Achillea millifolium* Complex. Bot. Gaz. 131 245-259.

Hiesey, W.M., Nobs, M.A. and Bjorkman, O. (1971). Experimental Studies on the Nature of Species. V. Biosystematics, Genetics and Physiological Ecology of the *Erythranthe* section of *Mimulus*. Carn. Inst. of Wash. Publ. 628.

Holmgren, P. (1968). Leaf Factors Affecting Light Saturated Photosynthesis in Ecotypes of *Solidago virgaurea* from Exposed and Shaded Habitats. Physiol. Pl. 21 676.

Holmgren, P., Jarvis, P.G. and Jarvis, M.S. (1965). Resistance to Carbon Dioxide and Water Vapour Transfer in Leaves of Different Plant Species. Physiol. Pl. 18 557.

Hooker, J.D. (1847). In Lond. J. Botany. VI. 276.

Hooker, J.D. (1860). Botany of the Antarctic Voyage. Flora Tasmaniense pt. III. Vol. I. 301-305.

Hopkins, J.W. (1966). Some Considerations in Multivariate Allometry. Biometrics 22 747-760.

Humphrey, A.B., Matzinger, D.F. and Mann, T.J. (1964). Inheritance of Leaf Shape in Flue Cured Tobacco (*N. tabacum* L.). Heredity 19 615.

Hutchinson, J. (1959). Families of Flowering Plants, 2nd ed. Vol. I. Dicotyledons. Clarendon Press.

Hutchinson, J. (1969). Evolution and Phylogeny of Flowering Plants. Academic Press.

Huxley, J.S. (1938). Clines: an Auxiliary Taxonomic Principle. Nature 142 219.

Jackson, W.D. (1960). Studies in Chromosome Breakage and Natural Selection. Ph.D. Thesis, Uni. of Tas.

Jackson, W.D. (1965). Vegetation in "The Atlas of Tasmania" ed J.L. Davies, Lands and Surveys Dept. Hobart 30-35.

Jackson, W.D. (1968). Fire, Air, Water and Earth - an Elemental Ecology of Tasmania. Proc. Ecol. Soc. Aus. 3 9-16.

Jackson, W.D. (1972). Vegetation of the Central Plateau. In "The Lake Country of Tasmania" Symp. Roy. Soc. Tas. ed. M.R. Banks 61-85.

Jackson, W.D. (1974). Conservation in Tasmania. In "Conservation of Major Plant Communities in Australia and Papua New Guinea". Aus. J. Bot. Supp. Ser. 7.

Jackson, W.D. (Unpubl.). Chromosome Numbers in some Tasmanian Plant Species.

Jain, S.K. (1969). Comparative Ecogenetics of two *Avena* Species Occurring in Central California. Evol. Biol. 3 78.

Jain, S.K. and Bradshaw, A.D. (1966). Evolutionary Divergence Among Adjacent Plant Populations. I. The Evidence and its Theoretical Analysis. Heredity 21 407-441.

Jindla, L.N. and Singh, K.B. (1970). The Inheritance of Flower Colour, Leaf Shape and Pod Length in the Cowpea (*Vigna sinensis* L.). Ind. J. Hered. 2 45-49.

Jones, D.A. and Wilkins, D.A. (1971). Variation and Adaptation in Plant Species. Heinemann.

Joliceur, P. (1963). The Multivariate Generalization of the Allometry Equation. Biometrics 19 497.

Kimball, S.L. and Salisbury, F.B. (1974). Plant Development Under Snow. Bot. Gaz. 135 147-149.

Ladiges, P.Y. (1974). Variation in Drought Tolerance in *Eucalyptus viminalis* Labill. Aust. J. Bot. 22 489-500.

Ladiges, P.Y. and Ashton, D.M. (1974). Variation in Some Central Victorian Populations of *Eucalyptus viminalis*. Aust. J. Bot. 22 81-102.

Lawrence, G.H.M. (1951). Taxonomy of Vascular Plants. Macmillan and Co. N.Y.

Leeper, G.W. (1964). An Introduction to Soil Science. Melb. Univ. Press.

Lewis, M.C. (1969). Genecological Differentiation of Leaf Morphology in *Geranium sanguineum*. New Phytol. 68 481.

Lewontin, R.C. (1957). The Adaptations of Populations to Varying Environments. Cold Spring Harbor Symp. Quart. Biol. 22 395-408.

Linhart, Y.B. (1974). Intra-population Differentiation in Annual Plants. I. *Veronica peregrina* L. Raised Under Non-Competitive Conditions. Evoln. 28 232.

McCullagh, D. (1934). Chromosomes and Chromosome Morphology in Plantaginaceae. I. Genetica 16 1-44.

McCully, M.E. and Dale, H.M. (1961). Heterophylly in *Hippuris* - a Problem in Identification. Can. J. Bot. 39 1099.

Marriott, F.H.C. (1974). The Interpretation of Multiple Observations. Academic Press, London.

Marsden-Jones, E.M. and Turrill, W.B. (1930-1945). Reports on the Transplant Experiments of the British Ecological Society at Poterne Wilts. J. Ecol. 18 352 (1930); 21 268 (1933); 23 443 (1935); 25 189 (1937); 26 359 (1938); 33 57 (1945).

Maude, P.F. (1939). The Merton Catalogue. New Phytol. 38 1-31.

Mayr, E. (1965). In "The Genetics of Colonizing Species" ed Baker, H.G. and Stebbins, G.L. p. 268.

Melville, R. (1937). The Accurate Definition of Leaf Shapes by Rectangular Coordinates. Ann. Bot. N.S. 1 673-679.

Meltzer, B. Searle, N.H. and Brown R. (1967). Numerical Specification of Biological Form. Nature 216 32.

Mulligan, G.A. (1965). Recent Colonization by Herbaceous Plants in Canada. In "The Genetics of Colonizing Species" ed. Baker, H.G. and Stebbins, G.L. 127-143.

Murfet, I.C. (1977). Environmental Interaction and the Genetics of Flowering. Am. Rev. Plant Physiol. 28 253-78.

Namkoong,G. (1966). Statistical Analysis of Introgression. Biometrics 22 488.

Nicolls, K.D. (1965). Soils in "The Atlas of Tasmania" ed. J.L. Davies, Lands and Surveys Department, Hobart, 27-29.

Njoku, E. (1956a). Studies in the Morphogenesis of Leaves. XI. The Effect of Light Intensity on Leaf Shape in *Ipomoea caerula*. New Phytol. 55 91.

Njoku, E. (1956b). The Effect of Defoliation on Leaf Shape in *Ipomoea caerula*. New Phytol. 55 213.

Njoku, E. (1957). The Effect of Mineral Nutrition and Temperature on Leaf Shape in *Ipomoea caerula*. New Phytol. 56 154.

Paliwal, R.P. and Hyde, G.H. (1959). The Association of a Single B-chromosome with Male Sterility in *Plantago coronopus*. Am. J. Bot. 14 460-466.

Pearsall, W.H. (1927). Growth Studies. VI. On the Relative Sizes of Growing Plant Organs. Ann. Bot. 41 549.

Phillips, B.F., Campbell, N.A. and Wilson, B.R. (1973). A Multivariate Study of Geographical Variation in the Whelk *Dicthais*. J. Exp. Mar. Biol. Ecol. 11 27-69.

Pilger, R. (1937). In 'das Pflanzenreich'. IV. 269 H.102.

Quinn, J.A. and Fairbrothers, D.E. (1971). Habitat Ecology and Chromosome Numbers of Natural Populations of the *Danthonia sericea* Complex. Am. Mid. Nat. 85 531.

Rahn, K. (1957). Chromosome Numbers in *Plantago*. Bot. Tidsskr. 53 369-378.

Rahn, K. (1974). *Plantago* section *Virginica*. Dansk. Bot. Arkiv. 30 (2) 1-180.

Rao, C.R. (1952). Advanced Statistical Methods in Biometric Research. J. Wiley and Sons, London.

Raschke, K. (1960). Heat Transfer between the Plant and the Environment. Ann. Rev. Pl. Physiol. 11 111.

Ratkowsky, D.A. and Martin, D. (1974). The Use of Multivariate Statistics in Identifying Relationships Among Disorder and Mineral Element Content in Apples. Aust. J. Agric. Res. 25 783-790.

Rempe, U. and Weber, E.E. (1972). An Illustration of the Principal Ideas of MANOVA. Biometrics 28 235-238.

Riggs, T.J. (1973). The Use of Canonical Analysis for Selection Within a Population of Spring Barley. Ann. Appl. Biol. 74 249.

Rodway, L. (1903). The Tasmanian Flora. 152-153. Govt. Printer, Hobart.

Rose, C.W. (1966). Agricultural Physics. Pergamon Press.

Ross, M.D. (1970). Breeding Systems in *Plantago*. Heredity 25 129-133.

Salisbury, E. (1952). Downs and Dunes. G. Bell and Sons, London.

Sanchez, R.A. (1967). Some Observations about the Effect of Light on the Leaf Shape in *Taraxacum officinale* L. Meded. Landbr. Wageningen. 67 1-11.

Sarruge, J.R. and Malavolta, E. (1970). Studies on the Mineral Nutrition of the Coffee Plant. XXIII. On the Relationships Ca/B and K/B. Ann. Acad. Brasil. Cience. 42 323.

Schmidt, B.L. and Millington, W.F. (1968). Regulation of Leaf Shape in *Proserpinaca palustris*. Bull. Torr. Bot. Club 95 264.

Seal, H. (1964). Multivariate Statistical Analysis for Biologists. Methuen.

Siddiqui, J.A. and Swaminathan, M.S. (1971). Intraspecific Differentiatio in *Gossypium arboreum*. Ind. J. Genet. and Pl. Breeding. 31 55.

Silow, R.A. (1939). The Genetics of Leaf Shape in Diploid Cottons and the Theory of Gene Interaction. J. Genetics. 38 229-275.

Sinnott, E.W. (1958). The Genetic Basis of Organic Form. Ann. N.Y. Acad. Sci. 71 (6) 1223-1233.

Slatyer, R.O. (1967). Plant-Water Relationships. Academic Press, London.

Snaydon, R.W. (1970). Rapid Population Differentiation in a Mosaic Environment. I. The Response of *Anthoxanthum odoratum* Populations to Soils. Evoln. 24 257-269.

Snaydon, R.W. (1973). Ecological Factors, Genetic Variation and Speciation in Plants. In "Taxonomy and Ecology" ed. V. Heywood. 1-29.

Snaydon, R.W. and Davies, M.S. (1976). Rapid Population Differentiation in a Mosaic Environment. IV. Populations of *Anthoxanthum odoratum* at Sharp Boundaries. Heredity 37 9-25.

Sneath, P.H.A. and Sokal, R.R. (1972). Numerical Taxonomy. Freeman and Co.

Snedecor, G.W. and Cochran, W.G. (1967). Statistical Methods. 6th Ed. Iowa State University Press.

Sprent, P. (1972). The Mathematics of Size and Shape. Biometrics 28 23-28.

Stebbins, G.L. (1974). Flowering Plants. Evolution above the Species Level. The Belknap Press of Harvard University Press.

Stebbins, G.L. and Day, A. (1967). Cytogenetic Evidence for Long Continued Stability in the Genus *Plantago*. Evoln. 21 409-428.

Systematics Association Committee (1962). Standardized Terms for Simple Symmetrical Plane Shapes. Taxo n 11 145-156, 245-247.

Takhatajan, A. (1969). Flowering Plants: Origin and Dispersal. Oliver and Boyd.

Thoday, J.M. (1959). Effects of Disruptive Selection. I. Genetic Flexibility. Heredity 13 187-203.

Thomas, D.A. and Barber, H.N. (1974). Studies on Leaf Characteristics of a Cline of *Eucalyptus urnigera* from Mt. Wellington, Tasmania. I. Water Repellency and the Freezing of Leaves. Aust. J. Bot. 22 501.

II. Reflection, Transmission and Absorption of Radiation. Aus. J. Bot. 22 701.

Thorne, R.F. (1968). Synopsis of a Putatively Phylogenetic Classification of the Flowering Plants. Aliso 6 57-66.

Thorpe, T.A. and Hield, H.Z. (1970). Gibberellin and Rejuvenation in *Citrus*. Phyton. Rev. Int. Bot. Exp. 27 63-68.

Turesson, G. (1922a). The Species and the Variety as Ecological Units. Hereditas 3 100-113.

Turesson, G. (1922b). The Genotypic Responses of the Plant Species to the Habitat. Hereditas 3 211-350.

Tyree, M.T., Dainty, J., and Benis, M. (1973). The Water Relations of Hemlock (*Tsuga canadensis*). I. Some Equilibrium Water Relations as Measured by the Pressure Bomb Technique. Can. J. Bot. 51 1471-1480.

Vasek, F.C. (1964). The Evolution of *Clarkia unguiculata* Derivatives Adapted to Relatively Xeric Environments. Evoln. 18 26.

Vasek, F.C. (1968). The Relationships of Two Ecologically Marginal, Sympatric *Clarkia* Populations. Am. Nat. 103 25.

Waddington, C.H. (1953). Genetic Assimilation of an Acquired Character. Evoln. 7 118-126.

Waddington, C.H. (1957). The Strategy of Genes. Allen and Unwin, London.

Waisel, J. (1959). Ecotypic Variation in *Nigella arvensis* L. Evoln. 13 469.

Westerman, J.M. and Lawrence, M.J. (1970). Genotype-Environment Interaction and Developmental Regulation in *Arabidopsis thaliana*. I. Inbred lines; Description. Heredity 25 609-627.

Wilcock, C.C. (1974). Population Variation, Differentiation and Reproductive Biology of *Stachys palustris* L., *S. sylvatica* L., and *S. x ambigua* S.M. New Phytol. 73 1233.

Wilkins, D.A. (1960). The Measurement and Genetical Analysis of Lead Tolerance in *Festuca ovina*. Scottish Plant Breeding Station Report. pp. 85-98 quoted in Jones and Wilkins (1971) q.v.

Wilkins, D.A. (1968). The Scale of Genecological Differentiation, p. 227. In "Modern Methods in Plant Taxonomy" ed. V.H. Heywood. Academic Press.

Wilkins, D.A. and Lewis, M.C. (1969). An Application or Ordination to Genecology. New Phytol. 68 861.

Willis, J.C. (1973). A Dictionary of the Flowering Plants and Ferns. 8th ed. Camb. Univ. Press.

Willis, J.H. (1973). A Handbook of Plants in Victoria. II. Dicotyledons. Melb. Univ. Press.

Woodell, S.R.J. and Kootin-Sanwu, M. (1971). Intraspecific Variation in *Caltha palustris*. New Phytol. 70 173.

Woodson, R.E. (1947). Some Dynamics of Leaf Variation in *Asclepias tuberosa*. Ann. Miss. Bot. Gard. 34 353.

Wu, L. and Antonovics, J. (1975). Experimental Ecological Genetics in *Plantago*. I. Induction of Roots and Shoots on Leaves for Large Scale Vegetative Propagation and Metal Tolerance Testing in *P. lanceolata*. New Phytol. 75 277-282.

Wu, L. and Antonovics, J. (1976). Experimental Ecological Genetics in *Plantago*. II. Lead Tolerance in *P. lanceolata* and *Cynodon dactylon* from a Roadside. Ecology 57 205.

Appendix A.1.

The Morphometric Analysis of Differences in the Size and Shape of Objects

There are three ways in which entire leaves of plants can differ in respect of the planar form. Firstly, the leaves can have the same shape but have different sizes (leaf areas). Secondly, they can have the same size but have different shapes (e.g. ovate and obovate). Thirdly, the leaves can differ simultaneously in size and shape. The value of multivariate techniques,such as principal components analysis, lies in their ability to partition these aspects of variation. Three examples are given below. The examples have been chosen to:-

1. show the correspondence between leaf area ("size") and a vector of equal relative proportions obtained from multivariate analysis;
2. demonstrate that when the size and shape of leaves vary independently, then the independence is detected by the multivariate technique;
3. show that when variation in the size and shape of leaves is inextricably linked (as may occur during the development of a heteroblastic species), this too can be quantified by multivariate analysis.

In each example, three linear dimensions (length [*l*], length to widest part [*lwp*] and width at widest part [*w*]) of rhomboids of known area are analysed by principal components.

In the first example (Fig. 1), rhomboids having the same relative proportions but falling into two different area classes were chosen. To simulate random biological variation, 6 'samples' of each area class were made. The 'samples' consisted of small fluctuations about arbitrarily selected working-means of the three dimensions

Fig. 1.

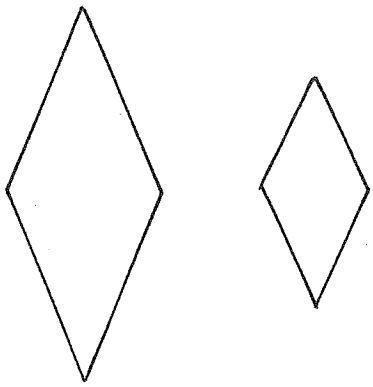


Fig. 2.

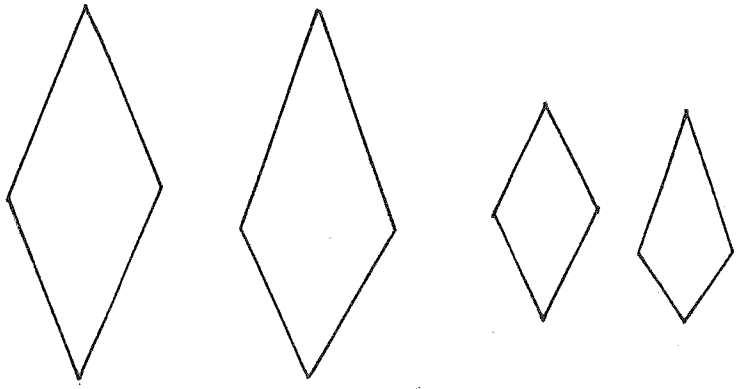
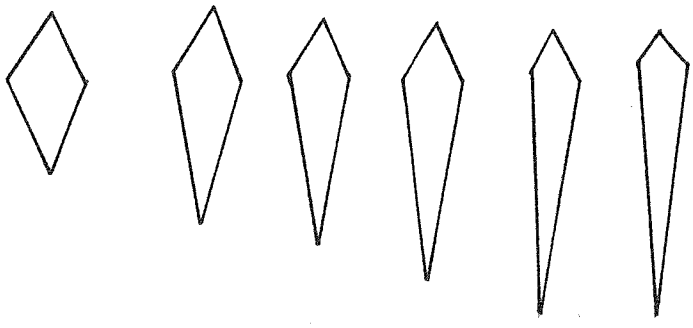


Fig. 3.



Rhomboids used to simulate leaf-shapes.

in each area class. The values obtained for the 12 'measured' individuals were incorporated in a PCA. The first principal component of this analysis takes up 97.6% of the total variation and corresponds to the eigenvector (0.581, 0.571, 0.580), which is very close to the value (0.577, 0.577, 0.577) expected for equal relative increase among the dimensions (Joliceur 1963). The correlation between the individual principal component scores and the areas of the corresponding rhomboids is r = 1.00**, so that it appears valid to regard the first component as a descriptor of rhomboid 'size' or area. The remaining components then represent random (biological) noise.

In the second example (Fig. 2), 4 rhomboids were chosen to represent two different 'sizes' i.e. areas. Within each area class, the widest part of the rhomboids was chosen to occur at two different positions relative to the apex. The rhomboids were each 'sampled' 3 times to give 12 individuals for analysis. The first and second components respectively account for 94.1 and 4.4% of the total variation. The first vector (0.581, 0.582, 0.568) again represents equal relative increase among the dimensions and is highly correlated with area (r = 0.99***). The second (independent) vector has values (-0.42, -0.383, 0.823). This represents a shape vector in which the length and width are contrasted with *lwp*. The vector is most heavily weighted by the *lwp* dimension. Thus the analysis agrees well with the model, in that it provides for two independent aspects of variation. The major aspect is the difference in size of the rhomboids. There is also a 'shape' difference arising from the differences in the position of the widest point of the rhomboids. The analysis has faithfully reproduced the two aspects of variation and maintained their independence.

The third sample (Fig. 3) consists of a set of 6 rhomboids which might represent the heteroblastic development of leaves on a single plant. The variation exhibited by these rhomboids is complex, involving the simultaneous change of size, the position of the widest part of the rhomboid, and the width of the rhomboids relative to their length. The first principal component contains 97.4% of the total variation. This vector succinctly represents the variation in rhomboid form, contrasting the length of the rhomboids with their width and position of widest part (-0.58, 0.58, 0.57). At the same time, the vector is highly correlated with rhomboid 'size' or area ($r = 0.98^{***}$).

These examples have been given to clarify the concepts of'size', which relates to proportional increase of the leaf dimensions and which is clearly related to leaf-area, and 'shape' which represents contrasting variation between the linear dimensions. At the same time, the examples demonstrate the efficiency of the principal components analytic technique.

The results of these examples have a direct bearing on the interpretation of the experimental results reported in this thesis. If the coefficients of one of the vectors (usually the first) from a principal components analysis are all positive, it can be assumed that the remaining vectors represent differences of shape which are not covariant with size. Alternatively, if none of the principal components have coefficients which are all positive, then it can be concluded that:-

1. size and shape are covariants;
2. the effect of differences of size have been removed from the analysis (i.e. the initial variables are not size-related);

or 3. differences of size were not present initially.

Appendix A.2

Theoretical Assumptions and Background to the Multivariate Methods

1. Mahalanobis' Generalized Distance (D^2)

D^2 is evaluated as

$$D^2_{jk} = d'_{jk} W^{-1} d_{jk}$$

where W^{-1} is the inverse of the variance-covariance matrix pooled within samples, and d_{jk} represents a vector of means differences of the samples j and k for all characters (Sneath and Sokal, 1972). D^2 assumes homogeneity of covariance matrices, and multivariate normality. The method is a discriminatory technique, and maximizes the differences between individuals of previously constructed groups. Thus the original distances may be distorted. It has been shown (Rao, 1952) that D^2 may also be evaluated as $\Sigma\lambda_i d_i$ where λ_i is the discriminant coefficient of the i^{th} character. Thus, Fisher's (1936) discriminant function can also be obtained between any pair of groups.

There is a problem with the use of the D^2 model in the analysis of differences of shape in a number of predesignated groups: if the original linear dimensions or their logarithms are used as the initial characters, then the D^2 values will reflect size effects as well as contrasts of form. In many cases size effects may be irrelevant, yet contain a major share of the total variation. A second problem is that whilst the technique may effectively cluster groups of similar shape (or size), it reveals little of the underlying nature of the variation on which the clusters are made manifest.

However, the technique has several advantages in the study of the distance between two populations. Firstly, by calculating the discriminant function, individuals can be placed with respect

to the two populations. Secondly, the statistic

$$\frac{n_1 n_2 (n_1 + n_2 - p - 1) D^2}{p(n_1 + n_2)(n_1 + n_2 - 2)}$$

can be used as a variance ratio with p and $(n_1 + n_2 - 1 - p)$ degrees of freedom (Rao, 1952), so that the significance of the difference between populations can be established.

2. Principal Components Analysis

Principal components analysis is an ordination procedure which attempts to elucidate the underlying structure of a multivariate sample in terms of uncorrelated linear combinations of the original variables. These linear combinations are determined as the eigen vectors associated with the p eigen values, λ_i, obtained from the solution of the determinantal equation

$$|R - \lambda I| = 0$$

where R is the correlation or covariance matrix of the original variables, and I is the identity matrix (Seal, 1964; Blackith and Reyment, 1971).

The axes obtained by PCA are orthogonal, so that the variates considered along each are independent. Thus if suitable biological interpretations can be ascribed to the axes, then the technique will provide a means of partitioning independent aspects of the total variation. It is generally accepted that when the coefficients associated with a particular axis are all positive, then that axis can be considered as a 'size' component (see Appendix A.1). A combination of positive and negative coefficients will comprise a contrast of characters, i.e. a shape measure, whilst coefficients close to zero will have correspondingly small effects in determining the contrast of form obtained by a particular component.

There has been some controversy in the literature over the mathematical validity of the use of eigen values which account for statistically non-significant portions of the total variation.

However, the situation frequently arises where size dependent factors of variability tend to swamp the variation ascribable to shape, the very parameter required for study. The fact that the numerically small eigen values are often capable of structuring samples in a way that has biological import, and independently of size, provides sufficient reason for at least a preliminary examination of all eigen values extracted in the analysis (Blackith and Reyment, 1971).

A further problem that has arisen in the past is that of the validity of using PCA in situations where *a priori* groups have been defined. The technique is strictly applicable only in the case of a single sample, for the elucidation of the relationships among the variables. Thus the eigenvectors obtained in a multiple group situation may confound aspects of the within and between group variation. However, Seal (1964) and more recently Ratkowsky and Martin (1974) have shown that valid deductions about the interrelationships among the variables may be drawn from the PCA of a grouped data set, provided it is conducted on the within group matrix, i.e. is freed of 'between groups' comparisons.

The major problem associated with PCA is that the technique is not scale invariant, i.e. different results will obtain if the scale of one of the variables is altered. This is usually overcome by suitable *a priori* scaling of the variables, so that their variances become equivalent, e.g. by using log values, or by manipulating the correlation matrix.

3. Canonical Variates Analysis

Canonical variates analysis is a technique which determines the axes of variation underlying the variation between h groups subject to the variation within groups (Seal, 1964). Linear combinations of the p original characters are obtained, weighted by the p eigenvectors associated with the eigenvalues ϕ_i obtained from the solution of the determinantal equation

$$|B - \phi W| = 0$$

where B is the between groups sums of squares and products matrix (SSP matrix) and W is the SSP matrix obtained within groups. The technique can therefore be seen as the multivariate analog of the univariate one way analysis of variance.

If the number of groups (h) under study is less than the number of variables (p), then only (h - 1) linear combinations of the original variables can be obtained. Thus in the case of two groups, only a single linear combination is obtained, and this is equivalent to the discriminant function. The D^2 value between any two groups in the analysis is obtained by calculating the sums of squares of the differences between their mean vectors scored along the respective canonical axes.

The reification of vectors obtained on CVA is similar to that described for PCA. However, the coefficients must first be weighted by the standard deviations of the original characters, obtained from the within groups matrix of covariance (Phillips, Campbell and Wilson, 1973). Interpretation is simplified further if the resulting coefficients are normalized so that their sums of squares total unity.

From the above discussion, it will be obvious that analysis of the same data set by the techniques of canonical variates and

principal components will often yield different sets of descriptions. This is a direct result of the different models underlying each approach. In PCA the resulting axes are oriented orthogonally along the directions of greatest to least variability, as obtained from the covariance matrix pooled within groups. Thus the axes are not confounded by between treatment effects. The canonical axes are obtained by the comparison of the between to within groups matrices. Rempe and Weber (1972), have shown that in general, the orientations of canonical axes will not coincide with those obtained from either the within or between groups matrix. However, in the situation where the *a priori* groups have been excised from what is essentially a continuum or single universe, as might be the case for example in a plant species varying eco-clinally, the two techniques may well give equivalent results.

4. Canonical Correlations

Canonical correlation is a technique which maximizes the correlation of transformed variate pairs, and eliminates correlations among terms (Namkoong 1966). The method involves the partitioning of the original correlation matrix according to the alternate sets, x and y say, thus

$$\begin{bmatrix} Rxx & Rxy \\ Rxy & Ryy \end{bmatrix}$$

and finding the transformation vectors $\underline{a}$ for the x variates, and $\underline{b}$ for the y variates, which correspond to the roots of

$$|R^{-1}yy\ R'xy\ R^{-1}xx\ Rxy - \lambda I| = 0$$

The correlation coefficients between members of the paired sets are given as $\sqrt{\lambda_i}$,

Independent variate pairs are produced with minimum residual variance. The significance of the canonical correlations can be tested by a procedure derived by Bartlett (Cooley and Lohnes, 1971).

Like principal components, the method is strictly for use in the case of a single population, to examine the correlation between two sets of variables of interest to the user. Thus its use between populations would require the assumption of homogeneity of the covariance matrices of the population. The technique is most appropriate in situations where some account is required of the interrelationships occurring between two internally correlated sets of variables, e.g. in relating the vegetative performance of a species to its yield or reproductive potential.

Appendix B.1.

A key to the Tasmanian species of *Plantago*.

1.a.	Flowers cleistogamous (corolla lobes erect, appressed, persistent, anthers not exserted)	*P. australis*
b.	Flowers chasmogamous (corolla lobes spreading, anthers exserted)	2
2.a.	Plants with scapes furrowed and anterior sepals fused for at least ½ their length	*P. lanceolata*
b.	Scapes not furrowed, anterior sepals free ± to base	3
3.a.	Plants with keels of posterior sepals winged	*P. coronopus*
b.	Keels of posterior sepals not winged	4
4.a.	Leaves broadly ovate, with 5-7 ± equal veins and narrowed abruptly to a long winged petiole. Capsules with 8-16 seeds	*P. major*
b.	Plants with leaves either not 5-7 nerved, or having capsules with 2-4 seeds	5
5.a.	Plants with short rootstocks and spreading lateral roots	6
b.	Plants with long (sometimes branched) taproots	12
6.a.	Plants with thick leaves which are densely tomentose, shortly petiolate; spikes cylindrical, seeds pigmented a deep, matte, purple-brown	7
b.	Plants with leaves glabrous or sparingly hispid, or if tomentose, then spikes capitate, and seeds pale brown, glistening	8

7.a.	Fruiting scapes approximately twice leaf length	*P. tasmanica* var. *tasmanica*
b.	Fruiting scapes scarcely as long as the leaves	*P. tasmanica* var. *archeri*
8.a.	Small plants growing in alpine cushion plants, leaves spathulate with an indumentum of long pilose hairs spread evenly over the upper surface, axillary tuft of hairs silvery-white	*P. gunnii*
b.	Plants with leaves ± glabrous, or with hairs confined to bands on the upper surface, axillary hairs (if present) golden brown	9
9.a.	Spikes (sub-)sessile at anthesis	10
b.	Spikes elongate at anthesis	11
10.a.	Plants with an axillary tuft of golden brown hairs, leaves glabrous, seeds (1-)4	*P. muelleri*
b.	Plants with an axillary tuft of silvery-white hairs (if present), leaves with bands of hairs ± across the blade, seeds (4-)8	*P. paradoxa*
11.a.	Flowers crowded in a cylindrical (but sometimes reduced) spike, petioles about as long as the blade, axillary hairs silvery-white and with a tuft of hairs in the axil of the bract	*P. daltonii*
b.	Flowers (1-)2-3(-5) on a capitate spike, petioles shorter than the blade, axillary hairs golden brown, tuft of hairs absent from the axil of the bract	*P. glacialis*

12.a. Plants with leaves ± glabrous, fleshy, 3-5 cuspid, bracts and sepals glabrous, plants of coastal rocks — *P. triantha*

b. Plants with leaves, bracts and/or sepals pilose — 13

13.a. Plants with the keels of the bracts and sepals pilose — 14

b. Plants with keels of sepals ± glabrous, bracts glabrous or with fimbriate-ciliate margins — 17

14.a. Bracts minute, or if long, then less than half the calyx length, hairs in leaf axils short, whitish or pale yellow brown — *P. hispida*

b. Bracts greater than half sepal length, hairs in leaf axils long, tufted, deep golden brown — 15

15.a. Plants with leaves broadly obovate, anterior sepals, 1.8-2 mm long, narrowly elliptic, bracts ± $^{2}/_{3}$ sepals — *P. bellidiodes*

b. Plants with leaves linear to lanceolate, anterior sepals 2.8-3.5 mm long, broadly elliptic-obovate, bracts ± equal sepals — 16

16.a. Leaves narrow-elliptic to lanceolate, pilose, length 5-10 times the breadth — *P. varia*

b. Leaves linear to narrow elliptic ± glabrous, length more than 15 times the breadth — *P. gaudichaudii*

17.a. Bracts fimbriate-ciliate, as long or longer than the calyces — *P. glabrata*

b. Bracts entire, shorter than the calyx — 18

18.a. Plants with slender scapes bearing interrupted spikes of small, distant flowers — *P. debilis*

b. Plants with dense spikes of closely imbricate flowers — *P. antarctica*

Appendix B.2.

A Summary of the *Plantago* Species in Tasmania

This appendix gives a descriptive summary of the species of *Plantago* which occur (or possibly are present) in Tasmania. The species are listed according to Pilger's (1937) classification, with the exception of *P. australis* which has been placed in section *Virginica* by Rahn (1974).

The information about each species is in four parts:- authority for the species description accepted for use in the present study; geographic status; brief notes on the distribution and ecology of the species within Tasmania; chromosome number.

Authorities for the chromosome numbers have been given on p. 42. Unless otherwise stated, the chromosome numbers given in this appendix have been determined on Tasmanian material. The distributions of the species within Tasmania are shown in Figs. 1-16.

Section *Polyneuron*

1. *P. major* L.

   Curtis (1967), Briggs *et al.* (1977); introduced;
   A widespread weed which is confined largely to fertilizer enriched lawns and pastures in lowland areas (Fig. 1); 2n = 12.

Section *Oliganthos*

2. *P. tasmanica* Hook.f. var. *tasmanica* Curtis

   Curtis (1967);Tas., Vic.;
   Occurs in a wide range of habitats (*Astelia alpina*

wet bogs, open shrublands, bare areas) in alpine Tasmania (Fig. 12); 2n = 12.

3. *P. tasmanica* Hook.f. var. *archeri* Curtis

Curtis (1967); endemic;

Occurrence as *P. tasmanica* var. *tasmanica*; 2n = ?

4. *P. daltonii* Dcne.

Curtis (1967); but see p. 40; endemic;

Edges of streams, lakes and sites of frequent flooding in sub-alpine and alpine Tasmania. Also on river banks near sea level on the west coast (Fig. 13); 2n = 12.

5. *P. paradoxa* Hook.f.

Curtis (1967), but see Chapter 8; endemic;

Grasslands, herbfields in depressions and margins of lakes and streams of sub-alpine - alpine areas, and at sea level on the west coast. Also found in bolster moor communities (Fig. 10); 2n = 48.

6. *P. gunnii* Hook.f.

Curtis (1967); endemic;

Found only in bolster moor communities on the dolerite mountains of Tasmania (Fig. 11); 2n = 36.

7. *P. triantha* Spreng.

Curtis (1967); Tas., N.Z.;

A halophyte which colonizes salt-spray zones of coastal rocks in NW-W-SE Tasmania (Fig. 9); 2n = 12.

8. *P. muelleri* Pilger

Briggs *et al.* (1977); not confirmed for Tasmania, N.S.W.;

Not known for Tasmania; 2n = ? Briggs (1973) reports 2n = 36 for N.S.W. plants. Jackson (unpubl.) found

2n = 12 in plants from the A.C.T., but his voucher specimens are *P. glacialis*.

9. *P. glacialis* Briggs, Carolin *et* Pulley

Briggs *et al.* (1977); not confirmed for Tasmania, N.S.W., Vic.; Not known for Tasmania; 2n = ? Briggs (1973) and Jackson (see 8. above) report 2n = 12 for mainland material.

Section *Coronopus*

10. *P. coronopus* L.

Curtis (1967), Briggs *et al.* (1977); introduced; A widespread weed, naturalized in dunes, swales and saltmarshes (Fig. 3); 2n = ? Briggs (1973) reports 2n = 10 for mainland plants.

Section *Virginica*

11. *P. australis* Lamk.

Rahn (1974); introduced;
Established in pastures, dune slacks and ruderal areas of lowland western Tasmania (Fig. 4); 2n = ? Rahn (1974) reports 2n = 24, 48 for S. American plants.

Section *Mesembrynia*

12. *P. varia* R. Br.

Briggs *et al.* (1977); Tas., temperate Australia; Widespread on the dry plains and rocky slopes of the eastern half of the state in grassland, savannah woodland and dry sclerophyll communities (Fig. 6); 2n = 24, Jackson (unpubl.) and Brown and Jackson

(this study). Curtis (1967) reports 2n = 12, 24. All of the Australian mainland plants studied by Briggs (1973) were 2n = 24.

13. *P. debilis* R. Br.

Curtis (1967), Briggs *et al.* (1977); Tas., Vic., N.S.W., Queensland, S.A., and southern parts of the Northern Territory;
Localized in dry sclerophyll forests in N.E. Tasmania (Fig. 8); 2n = 12.

14. *P. bellidiodes* Dcne.

Curtis (1967); endemic;
Common in dune herbfields and grasslands of N.E. and NW-W Tasmania (Fig. 5); 2n = 12.

15. *P. hispida* R. Br.

Briggs *et al.* (1977); Tas., temperate Australia;
Widespread in grasslands and *Casuarina stricta* low open-forests along the coastal cliffs and rocky headlands of N-E-SE Tasmania (Fig. 7); 2n = 12.

16. *P. antarctica* Dcne.

Curtis (1967), Briggs *et al.* (1977); Tas., Vic., N.S.W.;
Local in sub-alpine and alpine grasslands of the Central Plateau and of the N.E. (Fig. 15); 2n = 12.

17. *P. gaudichaudii* Dcne.

Briggs *et al.* (1977); Not confirmed for Tasmania, temperate Australia;
Recorded once (1928) from Blackman's Bay (Fig. 16); 2n = ? Briggs (1973) reports 2n = 12, 24, 36 for mainland plants.

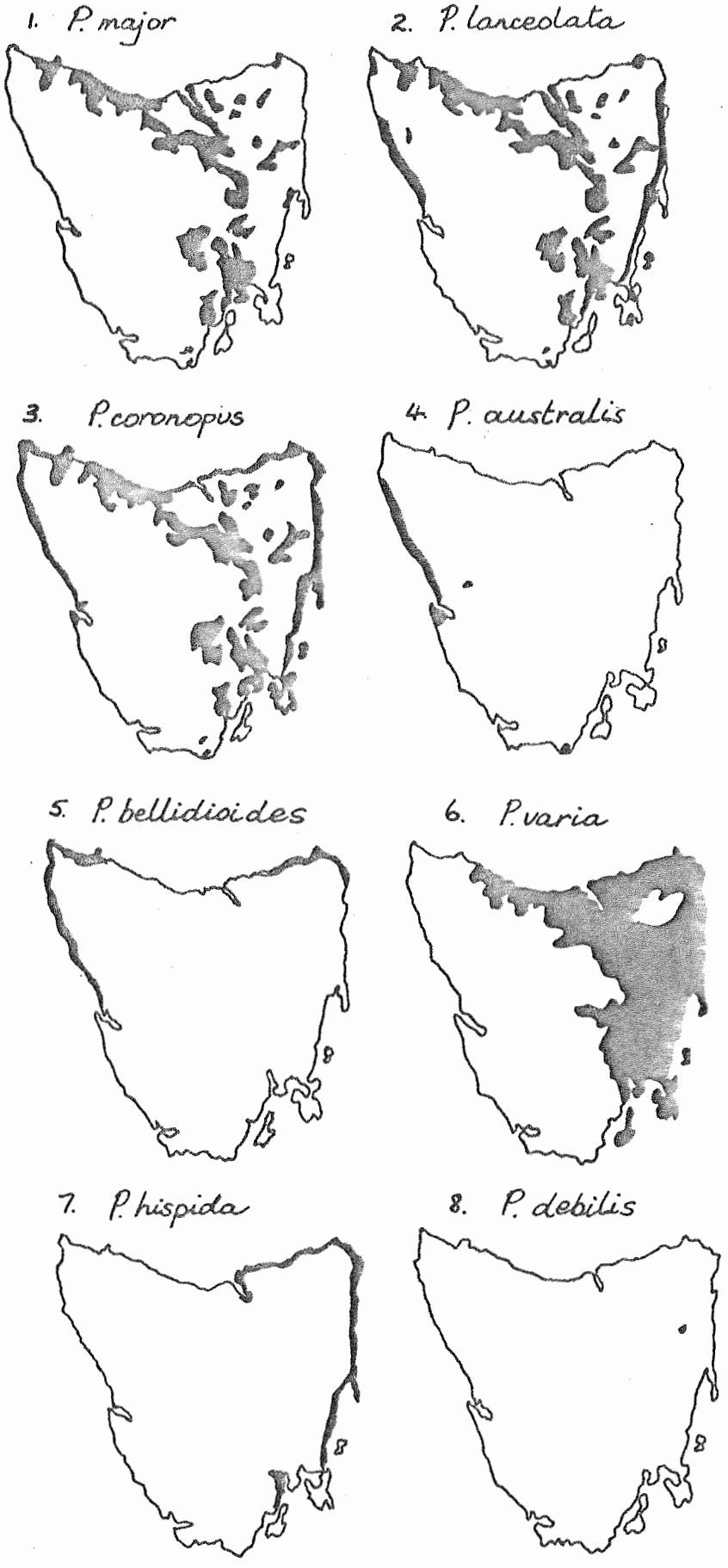
18. *P. glabrata* Hook.f.

Curtis (1967), but see p. 38; endemic; Sub-alpine grasslands and shrublands of the Central Plateau (Fig. 14); n = 12, 2n = 24; found in both the narrow- and broad-leaf variants of this species.

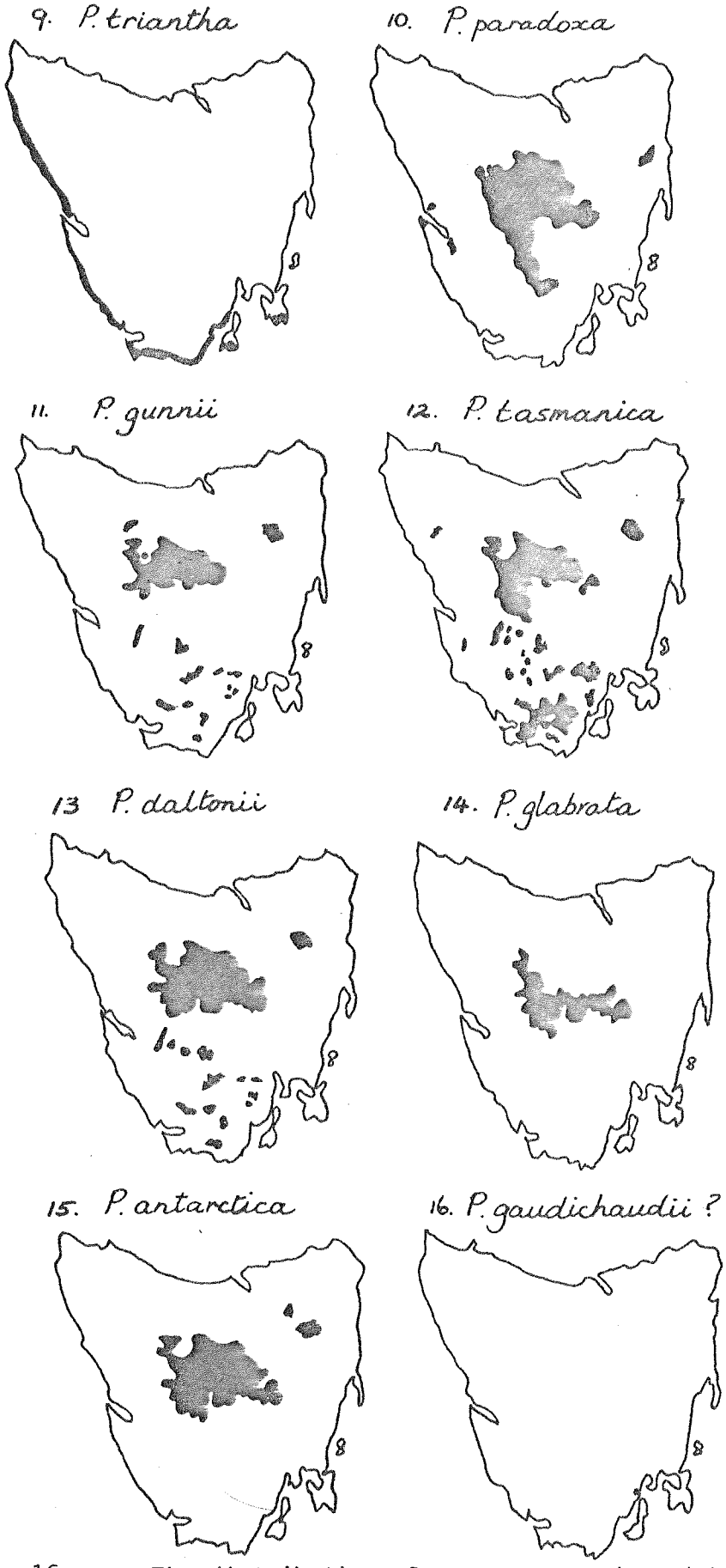
Section *Arnoglossum*

19. *P. lanceolata* L.

Curtis (1967); Briggs *et al.* (1977); introduced; Widespread in lawns, pastures, roadsides and rough grazing areas. In some areas, e.g. Mt. Nelson, the species is invading woodlands occupied by *P. varia* (Fig. 2); 2n = 12.



Figs. 1 - 8. The distribution of *Plantago* species within Tasmania.



Figs. 9 - 16. The distribution of *Plantago* species within Tasmania.

Appendix C

Data Tables from the Study of Seasonal Variation in Leaf Shape of *P. paradoxa* and *P. glabrata* (Chapter 5)

Appendix C.1. The Within Plant Variation of Leaf Shape in *P. paradoxa* and *P. glabrata*.

Table 1

The Within Plant Correlation Matrices. The unities of the leading diagonals are omitted. n = number of leaves per plant.

P. paradoxa

plant 1, n = 10

l	*lwp*	*ll*	*w*
	0.9798	0.9886	0.986
		0.9915	0.9878
			0.9966

plant 2, n = 16

l	*lwp*	*ll*	*w*
	0.9498	0.9846	0.9463
		0.9822	0.9468
			0.9516

plant 3, n = 11

l	*lwp*	*ll*	*w*
	0.9795	0.9903	0.9893
		0.9752	0.9644
			0.9853

plant 4, n = 23

l	*lwp*	*ll*	*w*
	0.9241	0.9807	0.9812
		0.9665	0.8832
			0.9533

P. glabrata

plant 1, n = 20

l	*lwp*	*ll*	*w*
	0.8824	0.9814	0.924
		0.8885	0.8624
			0.9295

plant 2, n = 22

l	*lwp*	*ll*	*w*
	0.9226	0.9716	0.9574
		0.9756	0.9127
			0.9384

plant 3, n = 21

l	*lwp*	*ll*	*w*
	0.8911	0.9852	0.8997
		0.9045	0.7954
			0.9374

plant 4, n = 29

l	*lwp*	*ll*	*w*
	0.8623	0.9879	0.881
		0.8868	0.8171
			0.8979

Table 2 . The variation of leaf-form in *P. glabrata* and *P. paradoxa* during leaf-expansion.
The percentage of variation, and the coefficients associated with the first principal component are shown.

P. glabrata	Eigenvectors associated with PC1 (by rows)			
% of variation	*l*	*lwp*	*w*	*ll*
93.4	0.5074	0.4858	0.4975	0.5089
96.0	0.5016	0.4963	0.4959	0.5061
92.7	0.5095	0.4837	0.4898	0.5162
91.7	0.5092	0.4856	0.4899	0.5147
97.3	0.5009	0.493	0.5035	0.5026
97.4	0.5038	0.4976	0.4959	0.5028
98.0	0.4981	0.498	0.5016	0.5023
96.2	0.492	0.4971	0.5066	0.5042
96.0	0.5059	0.4805	0.5068	0.5062
98.6	0.5031	0.4994	0.4994	0.503
98.3	0.4988	0.5004	0.4987	0.502
99.3	0.4983	0.4988	0.5013	0.5017
96.0	0.5003	0.4853	0.5076	0.5064
94.4	0.5103	0.5033	0.4783	0.5074
97.5	0.503	0.4944	0.4971	0.5054
98.7	0.4996	0.4994	0.4997	0.5012
98.7	0.5	0.4991	0.4983	0.5026
96.8	0.5038	0.492	0.4997	0.5044
97.2	0.5014	0.4936	0.5009	0.5041
98.6	0.5014	0.4979	0.499	0.5017
98.4	0.4988	0.4999	0.4994	0.502
98.0	0.5014	0.4982	0.4976	0.5028
99.0	0.5004	0.4978	0.5003	0.5015
99.0	0.4999	0.5014	0.4966	0.502
98.7	0.5021	0.4987	0.4985	0.5008
98.4	0.4984	0.4981	0.503	0.5005
92.1	0.5079	0.5051	0.4724	0.5136

Table 2 (Continued).

P. paradoxa	Eigenvectors associated with PC1 (by rows)			
% of variation	*l*	*lwp*	*w*	*ll*
99.1	0.4986	0.4992	0.5007	0.5015
97.0	0.5	0.4998	0.4953	0.5049
98.6	0.5022	0.4971	0.4996	0.5011
96.1	0.5054	0.4906	0.4966	0.5072
97.8	0.5013	0.5012	0.4939	0.5035
96.3	0.4977	0.4905	0.5041	0.5075
98.2	0.4974	0.501	0.4982	0.5035
99.2	0.4994	0.4998	0.5003	0.5005
98.7	0.4967	0.5	0.501	0.5023
98.4	0.5001	0.4981	0.4997	0.5021
97.6	0.4974	0.4983	0.5037	0.5007
98.6	0.4989	0.5001	0.4997	0.5014
96.7	0.4951	0.4902	0.5069	0.5076
97.4	0.5025	0.4967	0.4976	0.5031
95.6	0.498	0.5033	0.4891	0.5094
97.7	0.3748	0.3409	0.5141	0.6921
95.6	0.4978	0.4886	0.5032	0.5102
90.7	0.4465	0.516	0.5153	0.5184
98.5	0.4986	0.498	0.5013	0.5021
96.4	0.4994	0.5028	0.4914	0.5062
96.1	0.4993	0.4898	0.5034	0.5073
96.0	0.5032	0.499	0.4892	0.5083
94.6	0.5	0.4861	0.5036	0.5101
97.9	0.4928	0.4996	0.5033	0.5043
98.7	0.4986	0.5012	0.4992	0.5011
96.9	0.5015	0.5007	0.4906	0.5071
92.9	0.498	0.483	0.5012	0.5168

Table 3. PCA of the Individual Plants

P. glabrata

Plant 1:

eigenvalues		3.7353	0.1552	0.0911	0.0184
% of variation		93.4	3.9	2.3	0.5
eigen vectors (by column)	*l*	0.5074	0.268	-0.4525	0.6826
	lwp	0.4858	-0.872	0.0567	0.0188
	ll	0.5089	0.2426	-0.3867	-0.7298
	w	0.4975	0.33	0.8016	0.032

Plant 2:

eigenvalues		3.8394	0.1047	0.0481	0.0078
% of variation		96.0	2.6	1.2	0.2
eigenvectors	*l*	0.5016	0.3462	-0.6375	-0.4712
	lwp	0.4963	-0.6717	0.3433	-0.4297
	ll	0.5061	-0.2695	-0.309	0.7588
	w	0.4959	0.5969	0.6166	0.1324

Plant 3:

eigenvalues		3.7092	0.2066	0.0762	0.008
% of variation		92.7	5.2	1.9	0.2
eigenvectors	*l*	0.5095	0.0079	-0.6766	0.5315
	lwp	0.4837	-0.7551	0.4318	0.0972
	ll	0.5162	0.0827	-0.2504	-0.8148
	w	0.4898	0.6503	0.5413	0.2099

Plant 4:

eigenvalues		3.6686	0.1853	0.1358	0.0102
% of variation		91.7	4.6	3.4	0.3
eigenvectors	*l*	0.5092	0.1332	-0.5513	0.6474
	lwp	0.4856	-0.8022	0.3397	0.0725
	ll	0.5147	0.0758	-0.3953	-0.757
	w	0.4899	0.577	0.6515	0.0507

Table 3 (Contd.)

P. paradoxa

Plant 1:

eigen values		3.9651	0.0205	0.0116	0.0028
% of variation		99.12	0.51	0.30	0.07
eigen vectors (by column)	*l*	0.4986	-0.7835	0.3594	0.0914
	lwp	0.4992	0.6092	-0.5914	0.1727
	ll	0.5015	0.0944	0.2833	-0.812
	w	0.5007	0.0783	0.6639	0.55

Plant 2:

eigenvalues		3.8808	0.064	0.0502	0.005
% of variation		97.02	1.6	1.26	0.12
eigen vectors	*l*	0.5000	-0.3015	0.6754	-0.4505
	lwp	0.4998	-0.2074	-0.7351	-0.4085
	ll	0.5049	-0.3442	0.0005	0.7916
	w	0.4953	0.8646	0.0594	0.0601

Plant 3:

eigenvalues		3.942	0.0376	0.013	0.0074
% of variation		98.55	0.94	0.33	0.18
eigen vectors	*l*	0.5022	0.1111	0.09	-0.8529
	lwp	0.4971	-0.818	0.2021	0.2074
	ll	0.5011	0.1604	-0.8188	0.2295
	w	0.4996	0.5412	0.5297	0.4206

Plant 4:

eigenvalues		3.8453	0.1304	0.0164	0.0079
% of variation		96.13	3.26	0.41	0.20
eigen vectors	*l*	0.5054	0.2847	-0.4767	-0.6605
	lwp	0.4906	-0.7402	0.3977	-0.2307
	ll	0.5072	-0.1465	-0.5005	0.6861
	w	0.4966	0.5912	0.6035	0.1993

Table 4. PCA of the Eight Plants

(a) The 'within groups' correlation matrix

l	*lwp*	*ll*	*w*
	0.889	0.9789	0.8262
		0.925	0.8208
			0.865

(b) PCA

eigen value		3.655	0.2091	0.1209	0.015
% of variation		91.37	5.23	3.02	0.38
eigen vectors	*l*	0.5061	-0.3416	0.5322	0.5865
	lwp	0.4975	-0.2727	-0.8102	0.147
	ll	0.5162	-0.2137	0.2451	0.7924
	w	0.4795	0.8736	0.0152	0.0814

(c) Mean values of the principal components for each plant

Species	Plant No.	PC1	PC2	PC3	PC4
P. glabrata	1	4.94	0.12	-0.07	-0.29
	2	6.33	1.11	-0.43	-0.25
	3	8.73	-0.66	0.03	-0.43
	4	7.15	0.78	-0.17	-0.21
P. paradoxa	1	3.44	0.32	-0.54	-0.05
	2	5.53	1.652	-0.89	-0.176
	3	2.68	0.36	-0.42	-0.07
	4	5.92	1.24	-0.74	-0.21

Table 5. CVA of the Eight Plants

(a) SSP Matrices

B	1420.5282	241.4437	753.881	70.6778
		49.6529	135.5168	20.9995
			408.343	46.2063
				16.7963
W	1411.0248	302.682	714.3282	145.3345
		82.1589	162.8944	34.8392
			377.4226	78.6925
				21.9289

(b) CVA

latent roots	3.4879	0.9779	0.1781	0.0991
% of variation	75.54	20.62	3.75	2.09
eigen values (by column) *l*	0.1055	-0.837	0.9474	1.0158
lwp	1.1903	-0.8239	-2.2261	2.394
ll	-1.843	2.0339	-1.2766	-2.663
w	4.2724	1.7278	2.4508	0.2126

eigen values weighted by the within groups standard deviations

l	0.3304	-2.62	2.9655	3.1796
lwp	0.8991	-0.6223	-1.6814	1.8083
ll	-2.9838	3.2927	-2.0667	-4.3112
w	1.6672	0.6742	0.9564	0.083

Table 5 (Contd.)

(c) Canonical variate loadings at the mean of each plant

species	Plant No.	CV1	CV2	CV3	CV4
P. glabrata	1	-1.15	3.26	-0.75	-0.04
	2	0.96	4.58	-0.703	0.37
	3	-3.50	4.55	-1.28	0.79
	4	0.07	4.40	-0.14	0.83
P. paradoxa	1	0.66	1.87	-1.04	1.11
	2	2.79	4.51	-1.23	0.58
	3	0.62	1.69	-0.822	0.63
	4	1.74	4.41	-1.25	0.62

Table 6. Mean values of leaf characters measured on fresh and pressed leaves of *P. glabrata* and *P. paradoxa* (n = 15). Paired *t* tests are given.

Species	Variable	n	$\bar{x}$ fresh leaves	$\bar{x}$ pressed leaves	*t* paired
P. glabrata	*l*	15	6.20	6.13	1.85
	lwp	15	2.06	2.03	1.47
	w	15	0.70	0.70	<1
	ll	15	4.57	4.55	<1
P. paradoxa	*l*	15	3.71	3.66	2.78*
	lwp	15	1.53	1.50	1.10
	w	15	0.92	0.92	<1
	ll	15	2.58	2.51	3.67**

Table 7. The correlation matrices obtained from leaf measurements made on fresh and pressed leaves of *P. glabrata* and *P. paradoxa*. The unities of the leading diagonals are omitted. The sample size is 15 leaves in each case.

P. glabrata, fresh leaves

l	*lwp*	*w*	*ll*
	0.7170	0.6536	0.9402
		0.3384	0.7257
			0.7080

P. glabrata, pressed leaves

l	*lwp*	*w*	*ll*
	0.7556	0.6643	0.9373
		0.4003	0.7369
			0.7829

P. paradoxa, fresh leaves

l	*lwp*	*w*	*ll*
	0.8570	0.5461	0.9800
		0.4977	0.8972
			0.5684

P. paradoxa, pressed leaves

l	*lwp*	*w*	*ll*
	0.8761	0.5254	0.9834
		0.4324	0.8952
			0.5648

Table 8. PCA of fresh and pressed leaves of *P. glabrata* and *P. paradoxa*.

1. *P. glabrata*, fresh leaves					
eigenvalues		3.0739	0.6636	0.2075	0.0550
% of variation		76.85	16.59	5.19	1.37
eigenvectors	*l*	0.5453	-0.0473	0.5482	-0.6323
(by column)	*lwp*	0.4536	-0.6679	-0.5862	-0.0671
	w	0.4352	0.7427	-0.4968	-0.1109
	ll	0.5545	0.01	0.3302	0.7638
2. *P. glabrata*, pressed leaves					
eigenvalues		3.1634	0.6091	0.1839	0.0436
% of variation		79.09	15.23	4.60	1.08
eigenvectors	*l*	0.5358	-0.1076	0.6215	-0.5613
(by column)	*lwp*	0.4575	-0.6742	-0.5754	-0.0712
	w	0.4483	0.727	-0.4671	-0.2288
	ll	0.5502	0.0731	0.2537	0.7922
3. *P. paradoxa*, fresh leaves					
eigenvalues		3.2155	0.6103	0.1586	0.0156
% of variation		80.39	15.26	3.96	0.39
eigenvectors	*l*	0.5359	0.2093	0.5244	-0.6277
(by column)	*lwp*	0.5151	0.2703	-0.8013	-0.1395
	w	0.3877	-0.9197	-0.057	-0.0233
	ll	0.5451	0.1929	0.2823	0.7655
4. *P. paradoxa*, pressed leaves					
eigenvalues		3.1915	0.6569	0.1375	0.0142
% of variation		79.79	16.42	3.44	0.35
eigenvectors	*l*	0.5419	0.1853	0.4992	-0.6502
(by column)	*lwp*	0.5148	0.3226	-0.7897	-0.0854
	w	0.3731	-0.9179	-0.1265	-0.0477
	ll	0.5496	0.1383	0.3334	0.7534

Table 9. Discriminant analyses of *P. glabrata* and *P. paradoxa* using fresh and pressed leaves.

Leaf treatment	Discriminant Function	$\bar{x}$ *P. glabrata*	$\bar{x}$ *P. paradoxa*	D^2
Fresh	-0.808 *l* -1.579 *lwp* -4.222 *w* +3.549 *ll*	2.56	-2.56	5.12***
Pressed	-0.944 *l* -1.605 *lwp* -4.84 *w* +3.706 *ll*	2.70	-2.70	5.40***

ANOVA of linear regression between discriminant scores of the two species using fresh (x) and pressed (y) leaves.

Treatment	DF	SS	MS	F
regression	1	240.0537	240.0537	999.54***
residual	28	6.7793	0.2421	
total	29	246.833		

$y = 1.0339\ x, \quad r = 0.986$

Appendix C.2. The Seasonal Convergence of Leaf Shape in a Common Environment.

Table 1 : Seasonal Variation in mean values and the covariance matrices for leaf characters of *P. glabrata* and *P. paradoxa* growing in admixture at Arthur's Lake.

Season		Pooled Covariance Matrix			Mean (cm)	
					P. paradoxa	*P. glabrata*
Summer	*l*	2.3711	1.1318	0.1893	3.78	6.63
	lwp		0.5264	0.0511	1.28	2.11
	w			0.0211	0.65	0.75
Autumn	*l*	1.4379	0.4369	0.0796	3.73	4.50
	lwp		0.1991	0.0227	1.47	1.62
	w			0.0117	0.61	0.67
Winter	*l*	1.9181	0.8133	0.2836	3.70	3.90
	lwp		0.4307	0.123	1.44	1.51
	w			0.056	0.63	0.59
Spring	*l*	1.0106	0.2637	0.0483	2.34	3.60
	lwp		0.1731	0.024	0.95	1.24
	w			0.0117	0.60	0.59

Table 2 : PCA of the seasonal variation in leaf characters of the two species growing in admixture at Arthur's Lake.

(a) Covariance matrix, pooled within groups.

log *l*	0.0198	0.0163	0.0118
log *lwp*		0.0255	0.0127
log *w*			0.0133

eigenvalues	0.0479	0.0064	0.0043
% of variation	81.7	10.9	7.4
eigenvectors *l*	0.5814	0.5875	-0.5628
lwp	0.679	-0.7315	-0.0621
w	0.4482	0.346	0.8242

(b) Mean values of the principal components at the group means.

PC1	Season			
Species	Summer	Autumn	Winter	Spring
P. glabrata	0.6154	0.4253	0.2841	0.2543
P. paradoxa	0.2912	0.32	0.3058	0.0755
PC2				
P. glabrata	0.2136	0.1727	0.1421	0.1888
P. paradoxa	0.1787	0.1365	0.1515	0.1593
PC3				
P. glabrata	-0.5886	-0.5251	-0.5492	-0.5072
P. paradoxa	-0.4726	-0.5013	-0.4951	-0.3824

Appendix C.3.

The Seasonal Variation in Leaf-Shape of *P. paradoxa* and *P. glabrata* from Different Habitats

Table 1 : Seasonal Changes in Mean Values for the Characters *lwp/l*, *w/l* and *ll/l* of the Ten Field Plots of *P. glabrata* and *P. paradoxa*.

Plot No.	Species	Site	Summer	Autumn	Winter	Spring
1	*P. paradoxa*	A.L.	0.3767	0.3671	0.3943	0.4697
			0.1697	0.1812	0.1838	0.3039
			0.6163	0.6291	0.6267	0.7515
2		Kan.	0.3872	0.37	0.4024	0.4686
			0.1613	0.1625	0.1545	0.309
			0.6197	0.637	0.655	0.7704
3		P.C.	0.3771	0.3623	0.4159	0.4726
			0.1534	0.173	0.1654	0.2885
			0.6155	0.6362	0.6427	0.7569
4	*P. glabrata*	A.L.	0.3447	0.3002	0.3019	0.3064
			0.0907	0.0795	0.0797	0.1066
			0.6907	0.6584	0.6686	0.5793
5		A.L.	0.3674	0.3603	0.3736	0.322
			0.1001	0.1141	0.1261	0.1227
			0.7274	0.722	0.7347	0.6608
6		A.L.	0.341	0.3636	0.346	0.3144
			0.116	0.1129	0.1269	0.1116
			0.6895	0.742	0.7295	0.641
7		Kan.	0.3623	0.3171	0.3459	0.3578
			0.1386	0.1405	0.1394	0.1415
			0.7511	0.7515	0.7245	0.646
8		Kan.	0.3359	0.3229	0.309	0.3714
			0.1283	0.1334	0.1441	0.1508
			0.7587	0.7717	0.7013	0.6668
9		P.C.	0.4193	0.3583	0.3594	0.3011
			0.188	0.1731	0.1734	0.1818
			0.7661	0.7675	0.7075	0.589
10		P.C.	0.4111	0.3554	0.452	0.3812
			0.1958	0.1641	0.1761	0.167
			0.7994	0.7232	0.7747	0.6502

Table 2a: Seasonal Change in Mean Values* of *lwp/l*, *ll/l* and *w/l* in *P. paradoxa*.

Ratio/Season	Summer	Autumn	Winter	Spring
lwp/l	38.04	36.65	40.42	47.03
ll/l	61.72	63.41	64.15	75.96
w/l	16.15	17.22	16.79	30.05

Table 2b: Seasonal Change in Mean Values* of *lwp/l*, *ll/l* and *w/l* in *P. glabrata*.

Ratio/Season	Summer	Autumn	Winter	Spring
lwp/l	36.88	33.97	35.54	33.63
ll/l	74.04	73.38	72.01	63.33
w/l	13.68	13.11	13.8	14.03

Table 2c: Site Differences in Mean Values* of *lwp/l*, *ll/l* and *w/l* in *P. glabrata*.

Site	Arthur's Lake			Kannaleena		Pine Creek	
Ratio/Plot	4	5	6	7	8	9	10
lwp/l	31.33	35.58	34.12	35.58	33.48	35.95	39.99
ll/l	64.93	71.12	70.05	71.88	72.46	70.75	73.69
w/l	8.91	11.57	11.69	14.00	13.92	17.91	17.58

*Mean values which are not significantly different at P = 0.05 are underlined. In each case, the means have been converted to percentages for ease of comparison.

Table 3 : Two way ANOVA of Leaf Shape Variation in *P. paradoxa* by Site and by Season. Model I is assumed.

a. *lwp/l*

Source	DF	SS	MS	F
Site	2	0.0010	0.0005	<1
Seasons	3	0.2859	0.0953	46.15***
Site/Seasons	6	0.0042	0.0007	<1
Error	168	0.3469	0.0021	
Total	179	0.6379		

b. *ll/l*

Source	DF	SS	MS	F
Site	2	0.0064	0.0032	1.03
Seasons	3	0.5726	0.1909	61.21***
Site/Seasons	6	0.0032	0.0005	<1
Error	168	0.5239	0.0031	
Total	179	1.1062		

c. *w/l*

Source	DF	SS	MS	F
Site	2	0.0076	0.0038	2.26
Seasons	3	0.6021	0.2007	119.63***
Site/Seasons	6	0.0070	0.0012	<1
Error	168	0.2819	0.0017	
Total	179	0.8986		

Table 4 : Two way ANOVA of Leaf Shape Variation in *P.glabrata* by Site and by Season. Model I is assumed.

a. *lwp/l*

Source	DF	SS	MS	F
Site	6	0.2575	0.0429	6.97***
Seasons	3	0.0711	0.0237	3.85***
Site/Seasons	18	0.225	0.0125	2.03*
Error	383(9)	2.3595	0.0062	
Total	410	2.9132		

b. *ll/l*

Source	DF	SS	MS	F
Site	6	0.283	0.0472	10.19***
Seasons	3	0.781	0.2603	56.22***
Site/Seasons	18	0.2029	0.0113	2.44**
Error	383(9)	1.7734	0.0046	
Total	410	3.04		

c. *w/l*

Source	DF	SS	MS	F
Site	6	0.3861	0.0644	69.39***
Seasons	3	0.0048	0.0016	1.74*
Site/Seasons	18	0.027	0.0015	1.62*
Error	383(9)	0.3552	0.0009	
Total	410	0.7731		

Table 5 : Two way ANOVA of Leaf Shape Variation in the Ten Plots by Site and by Season. Model I is assumed

a. *lwp/l*

Source	DF	SS	MS	F
Site	9	0.6437	0.0715	14.56***
Seasons	3	0.0748	0.0249	5.07***
Site/Seasons	27	0.5114	0.0189	3.86***
Error	551(9)	2.7064	0.0049	
Total	590	3.9363		

b. *ll/l*

Source	DF	SS	MS	F
Site	9	0.5314	0.059	14.16***
Seasons	3	0.107	0.0357	8.56***
Site/Seasons	27	1.4527	0.0538	12.91***
Error	551(9)	2.2973	0.0042	
Total	590	4.3884		

c. *w/l*

Source	DF	SS	MS	F
Site	9	0.9094	0.101	87.40***
Seasons	3	0.2138	0.0713	61.65***
Site/Seasons	27	0.4272	0.0158	13.68***
Error	551(9)	0.6370	0.0012	
Total	590	2.1874		

Table 6 : CVA of Seasonal Variation in Leaf Shape of *P. paradoxa* and *P. glabrata*, based on the characters log *l*, log *lwp*, log *ll*, and log *w*.

B	62.911	52.272	62.042	19.48	
		50.758	53.253	26.455	
			65.635	21.96	
				31.064	
W	35.499	30.782	32.01	21.773	
		48.937	32.123	24.481	
			33.64	22.729	
				32.859	
eigen values		2.8754	1.0278	0.8684	0.1461
% of variation		58.47	20.9	17.66	2.97
eigenvectors	*l*	-0.3111	6.1575	-7.6693	3.5456
	lwp	0.9405	0.2164	-2.0572	-5.0761
	ll	-5.9665	-6.0695	8.7484	0.0356
	w	3.6426	-3.3463	-1.725	2.1961
standardized eigenvectors	*l*	-0.045	0.708	-0.649	0.489
	lwp	0.16	0.029	-0.204	-0.822
	ll	-0.844	-0.679	0.72	0.005
	w	0.509	-0.37	-0.14	0.291

roots excluded	D.F.	X^2
0	156	1603.5***
1	114	834.0***
2	74	432.5***
3	36	77.4**

Table 7: Mean Values (x 100) of the Canonical Variates for the species in Each Season.

Plot No.	Species	Site	Summer	Autumn	Winter	Spring
1	*P. paradoxa*	A.L.	84.91	96.71	148.96	292.42
			-41.01	-58.04	-3.60	-184.16
			-194.88	-165.77	-138.65	-12.7
			20.69	44.97	0.38	-2.11
2		Kan.	79.95	90.62	120.09	296.68
			2.13	25.46	130.27	-177.57
			-159.81	-87.49	19.81	30.16
			-12.48	4.00	-71.56	-1.38
3		P.C.	55.18	93.3	122.91	287.94
			-1.32	-28.71	51.63	-142.86
			-171.67	-125.73	-72.78	24.97
			-3.13	38.21	-57.60	-22.8
4	*P. glabrata*	A.L.	-316.32	-343.07	-335.99	-28.96
			-62.48	14.69	41.25	230.8
			-106.26	-98.04	-48.73	-64.75
			-33.28	11.27	5.85	18.18
5		A.L.	-236.11	-157.87	-129.03	-27.08
			-7.81	1.46	-40.81	136.24
			14.39	33.61	20.12	53.23
			-56.92	-37.97	-35.04	7.55
6		A.L.	-172.73	-199.65	-103.2	-62.48
			-18.0	-44.23	-0.75	153.80
			-32.53	30.34	69.39	28.19
			9.36	-35.-5	-3.99	13.76
7		Kan.	-109.09	-87.17	-45.64	53.44
			-87.11	-56.79	5.72	114.12
			35.56	88.34	79.78	0.09
			5.04	59.97	23.34	-16.24
8		Kan.	-163.46	-130.68	-55.11	57.55
			-86.39	-66.49	-11.91	72.0
			61.76	114.79	40.77	8.83
			36.85	46.29	75.96	-20.04
9		P.C.	90.23	46.9	101.51	286.51
			-43.57	-11.46	36.36	279.38
			104.65	163.18	89.41	80.13
			-29.58	19.51	14.93	76.77
10		P.C.	37.75	15.02	68.67	166.29
			-145.85	-50.73	-35.2	170.49
			82.73	40.81	105.03	67.54
			4.35	38.83	-90.29	-37.8

Table 8 : Values of D^2 used in the Construction of Minimum Spanning Trees between the Ten Plots in Each Season.

Summer		Autumn		Winter		Spring	
D^2	Plots joined	D^2	Plots joined	D^2	Plots joined	D^2	Plots joined
0.29	(2, 3)	0.50	(1, 3)	0.65	(6, 7)	0.42	(2, 3)
0.60	(1, 3)	0.54	(7, 8)	0.69	(7, 8)	0.43	(7, 8)
0.68	(7, 8)	0.62	(5, 6)	0.75	(6, 5)	0.49	(1, 2)
1.04	(5, 6)	0.75	(2, 3)	1.07	(1, 3)	0.47	(5, 6)
1.16	(6, 9)	1.15	(7, 10)	1.22	(2, 3)	1.02	(5, 9)
1.22	(9, 10)	1.34	(9, 10)	1.32	(9, 10)	1.25	(6, 4)
1.57	(4, 5)	1.38	(6, 8)	1.47	(2, 9)	1.45	(7, 10)
1.65	(8, 9)	1.71	(2, 10)	1.51	(7, 9)	1.99	(9, 10)
2.68	(3, 6)	2.07	(4, 6)	2.37	(4, 5)	3.15	(3, 8)

Appendix C.4. Experimental Garden Studies.

Table 1 : Summer and Winter Mean Values (x 100) of the characters *lwp/l*, *ll/l* and *w/l* for the plants of *P. glabrata* and *P. paradoxa* grown in the experimental garden.

			Site:	Interlaken			Lake Augusta	
Species	Character	n	Season:	Summer	Winter	n	Summer	Winter
P. glabrata	*lwp/l*	15		33.21	30.21	15	40.49	32.92
	ll/l			64.37	50.20		71.12	53.92
	w/l			11.98	8.70		20.27	13.67
P. paradoxa	*lwp/l*	12		44.88	29.49	15	42.13	30.47
	ll/l			71.21	47.01		69.13	50.88
	w/l			20.2	10.63		26.98	16.15

Table 2 : Three way ANOVA for Species, Site and Season for Leaf Shape characters of plants grown in the experimental garden. Model I is assumed.

a. *lwp/l*

Source	DF	SS	MS	F
Species	1	0.0176	0.0176	1.53
Season	1	0.2356	0.2356	20.49***
Site	1	0.0157	0.0157	1.37
Species/Season	1	0.046	0.046	4.00
Species/Site	1	0.0227	0.0227	1.97
Season/Site	1	0.0007	0.0007	<1
Species/Season/Site	1	0.0018	0.0018	<1
Error	49	0.5641	0.0115	
Total	56	0.9142		

b. *ll/l*

Source	DF	SS	MS	F
Species	1	0.0002	0.0002	<1
Season	1	0.9384	0.9384	67.94***
Site	1	0.0286	0.0286	2.07
Species/Season	1	0.0194	0.0194	1.40
Species/Site	1	0.0137	0.0137	<1
Season/Site	1	0.0005	0.0005	<1
Species/Season/Site	1	0.0147	0.0147	1.07
Error	49	0.6768	0.0138	
Total	56	1.6923		

Table 2 (Contd.)

c. *w/l*

Source	DF	SS	MS	F
Species	1	0.0763	0.0763	19.08***
Season	1	0.1588	0.1588	39.70***
Site	1	0.1263	0.1263	31.58***
Species/Season	1	0.0204	0.0204	5.10*
Species/Site	1	0.0101	0.0101	2.53
Season/Site	1	0.0051	0.0051	1.28
Species/Season/Site	1	0.0005	0.0005	<1
Error	49	0.1969	0.004	
Total	56	0.5944		

Table 3 : CVA of Seasonal Variation in Leaf Shape of *P. paradoxa* and *P. glabrata* plants grown in an experimental garden.

B	3498.9361	5486.4932	3068.6371	*lwp/l*
		10159.4172	4621.8184	*ll/l*
			3761.227	*w/l*
W	5630.7685	4985.1212	1476.8033	
		6788.293	1868.6387	
			2193.3987	

eigenvalues		2.0304	0.9854	0.1279
% of variation		64.59	31.35	4.06
eigenvectors	*lwp/l*	-0.0858	0.1702	-0.1957
	ll/l	0.1146	-0.1767	0.0634
	w/l	0.1304	0.1991	0.0821
standardized	*lwp/l*	-0.402	0.422	-0.915
eigenvectors	*ll/l*	0.769	-0.763	0.325
	w/l	0.497	0.489	0.239

roots excluded	DF	X^2
0	21	205.85***
1	12	86.67***
2	5	12.94*

Mean values of the canonical variates for each species-site-season

Species	Canonical Variate	Site:	Interlaken		Lake Augusta	
		Season:	Summer	Winter	Summer	Winter
P. glabrata	CV1		6.75	4.90	8.13	5.79
	CV2		-5.43	-3.90	-4.19	-3.28
	CV3		-1.43	-2.02	-1.75	-1.90
P.paradoxa	CV1		7.84	4.83	8.67	5.93
	CV2		-3.75	-3.03	-2.33	-2.51
	CV3		-2.61	-1.92	-1.65	-1.41

Appendix D. Data Tables from the Glasshouse Studies (Chapter 6).

Table 1 : The Effect of Inundation on Leaf Shape in *P. paradoxa* and *P. glabrata*.

(a) Mean values, standard errors and sample size for the variables *lwp/l*, *ll/l* and *w/l*.

		Treatment					
		+ water			- water		
Species	Variable	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$
P. paradoxa	*lwp/l*	10	33.18	0.953	11	41.39	0.722
	ll/l		57.66	1.004		71.85	0.688
	w/l		11.64	0.274		20.55	0.441
P. glabrata	*lwp/l*	12	24.45	0.803	30	27.48	0.785
	ll/l		51.17	0.999		57.71	1.023
	w/l		9.11	0.471		11.52	0.674

(b) ANOVA Model I is assumed.

	Source	DF	SS	MS	F
lwp/l	Water	1	0.1265	0.1265	19.17***
	Species	1	0.4325	0.4325	65.53***
	Water/Species	1	0.0000	0.0000	<1
	Error	39	0.2586	0.0066	
	Total	42	0.8176		
ll/l	Water	1	0.3839	0.3839	43.13***
	Species	1	0.3806	0.3806	42.76***
	Water/Species	1	0.0016	0.0016	<1
	Error	39	0.3487	0.0089	
	Total	42	1.1148		
w/l	Water	1	0.1143	0.1143	47.63***
	Species	1	0.1187	0.1178	49.08***
	Water/Species	1	0.02	0.02	8.33**
	Error	39	0.0941	0.0024	
	Total	42	0.3462		

(c) C.V.A.

B	5536.9144	6208.2859	3530.7719
		7657.8654	4362.67
			2524.3852
W	2585.6153	2027.0652	303.682
		3486.9683	938.8789
			940.9435

Eigenvalues	3.9307	0.3024	0.0392
% of variation	92.0	7.08	0.92
Eigenvectors	0.1313	-0.2703	0.0048
	-0.0087	0.2198	0.2065
	0.2662	0.0148	0.3522
Standardized	0.639	-0.731	0.015
eigenvectors	-0.048	0.682	-0.75
	0.768	0.024	0.662

Table 1 (Contd.)

roots excluded	DF	X^2
0	9	234.22***
1	4	37.38***
2	1	4.74*

(d) Mean values of canonical variates, evaluated at group means.

Species	Treatment	CV_1	CV_2	CV_3
P. glabrata	+ water	5.19	4.77	-7.24
	- water	6.17	5.42	-7.72
P. paradoxa	+ water	6.95	3.87	-7.65
	- water	10.28	4.91	-7.40

Table 2 : The Effect of Temperature on Leaf Shape in the Species.

(a)(i) Mean values, standard errors, and sample size for the variables *lwp/l*, *ll/l* and *w/l*, at 10, 15, 20 and 25°C.

	Temperature (°C)	*P. glabrata* n	$\bar{x}$	$S\bar{x}$	*P. paradoxa* n	$\bar{x}$	$S\bar{x}$
lwp/l	25	8	27.53	1.451	22	36.69	1.011
	20	15	27.31	1.316	19	38.11	0.978
	15	16	29.10	1.188	18	35.89	1.278
	10	16	29.88	1.792	17	36.29	1.376
ll/l	25	8	55.15	1.675	22	63.22	1.669
	20	15	59.59	1.232	19	64.88	1.438
	15	16	56.63	1.305	18	63.93	1.864
	10	16	56.28	1.98	17	65.18	1.932
w/l	25	8	11.39	0.612	22	21.78	1.157
	20	15	12.24	0.452	19	20.45	1.232
	15	16	13.00	0.778	18	17.52	0.704
	10	16	13.57	0.881	17	18.06	0.84

(ii) ANOVA Model I is assumed.

	Source	DF	SS	MS	F
lwp/l	Species	1	0.2121	0.2121	75.75***
	Temperature	3	0.0039	0.0013	1.07
	Species/Temperature	3	0.0079	0.0026	<1
	Error	123	0.3424	0.0028	
	Total	130	0.5662		
ll/l	Species	1	0.1615	0.1615	33.65***
	Temperature	3	0.0084	0.0028	<1
	Species/Temperature	3	0.0102	0.0034	<1
	Error	123	0.5958	0.0048	
	Total	130	0.7759		
w/l	Species	1	0.1519	0.1519	94.9***
	Temperature	3	0.0241	0.008	5.00**
	Species/Temperature	3	0.0029	0.001	<1
	Error	123	0.1921	0.0016	
	Total	130	0.371		

(iii) C.V.A.

B	2260.8985	1833.9744	1912.0402
		1816.2687	1515.7527
			1792.276
W	3423.9478	2893.7897	963.3916
		5958.2431	1914.1737
			1921.4113
Eigenvalues	1.3144	0.1296	0.0465
% of variation	88.19	8.69	3.12
Eigenvectors	0.1344	0.0072	-0.2066
	-0.081	-0.1761	0.0811
	0.224	0.1364	0.1587
Standardized	0.708	0.038	-1.089
eigenvectors	-0.562	-1.222	0.563
	0.885	0.539	0.627

Table 2 (Contd.)

roots excluded	DF	X^2
0	21	126.31***
1	12	20.99
2	5	5.71

(iv) Mean values of the canonical variates evaluated at the group means.

Canonical Variate	Temperature (°C)			
CV_1	25	20	15	10
P. glabrata	1.59	1.79	2.24	2.50
P. paradoxa	4.69	4.45	3.58	3.65
CV_2				
P. glabrata	-8.62	-7.96	-7.99	-7.84
P. paradoxa	-7.89	-8.36	-8.61	-8.75
CV_3				
P. glabrata	1.13	0.59	0.64	0.54
P. paradoxa	1.00	0.63	0.55	0.65

(b)(i) Mean values, standard errors, and sample size for the variables *lwp/l*, *ll/l* and *w/l* at 6, 9 and 12°C.

lwp/l	*P. glabrata*			*P. paradoxa*		
Temperature (°C)	n	$\bar{x}$	$S\bar{x}$	n	$\bar{x}$	$S\bar{x}$
6	10	27.57	1.692	10	32.68	1.909
9	10	25.69	1.922	10	34.37	2.358
12	10	27.13	1.412	10	39.68	1.941
ll/l						
6	10	64.07	1.757	10	56.63	1.937
9	10	63.93	1.554	10	65.69	5.136
12	10	62.45	2.78	10	69.36	3.396
w/l						
6	10	15.19	1.961	10	12.45	1.426
9	10	13.59	1.322	10	16.05	1.683
12	10	11.43	1.478	10	17.9	1.781

Table 2 (Contd.)

(ii) ANOVA Model I is assumed.

	Source	DF	SS	MS	F
lwp/l	Temperature	2	147.7203	73.8602	1.89
	Species	1	1156.326	1156.326	29.55***
	Temperature/Species	2	138.459	69.2295	1.77
	Error	54	2113.204	39.1334	
	Total	59	3555.7093		
ll/l	Temperature	2	346.3243	173.1622	2.52
	Species	1	2.5215	2.5215	<1
	Temperature/Species	2	528.475	264.2375	3.85*
	Error	54	3707.181	68.6515	
	Total	59	4584.5018		
w/l	Temperature	2	11.587	5.7935	<1
	Species	1	63.8602	63.8602	2.42
	Temperature/Species	2	213.2403	106.6202	4.04*
	Error	54	1423.689	26.3646	
	Total	59	1712.3765		

(iii) C.V.A.

B	1583.1695	415.324	486.0145	
		832.362	378.101	
			288.6875	
W	1937.247	1230.667	883.427	
		4940.312	1632.76	
			1423.689	
Eigenvalues		0.8917	0.1707	0.066
% of variation		79.02	15.13	5.85
Eigenvectors		-0.1882	-0.0352	-0.0515
		0.0276	0.0811	-0.1022
		0.0275	0.0817	0.2551
Standardized		-1.1273	-0.2107	-0.3085
eigenvectors		0.2642	0.7762	-0.9779
		0.1415	0.4197	1.3098

roots excluded	DF	X^2
0	15	46.82***
1	8	12.08
2	3	3.49

(iv) Mean values of the canonical variates at the group means.

Temperature		6	9	12
CV_1	*P. paradoxa*	-4.24	-4.60	-5.06
	P. glabrata	-3.00	-2.69	-3.07
CV_2	*P. paradoxa*	4.46	5.23	5.70
	P. glabrata	5.47	5.40	5.05
CV_3	*P. paradoxa*	-4.29	-4.3	-4.57
	P. glabrata	-4.10	-4.39	-4.87

Table 3 : The Effect of Light Intensity on Leaf Shape in *P. paradoxa* and *P. glabrata*.

(a) Mean values, standard errors and sample sizes for the variables *lwp/l*, *ll/l* and *w/l*.

% Shade	Variable	*P. glabrata* n	$\bar{x}$	$S\bar{x}$	*P. paradoxa* n	$\bar{x}$	$S\bar{x}$
0	*lwp/l*	10	29.98	1.671	11	39.82	1.064
	ll/l		65.87	1.534		70.36	1.096
	w/l		14.36	0.812		24.54	1.385
28	*lwp/l*	10	30.53	1.401	11	38.44	1.580
	ll/l		61.18	1.428		68.16	1.30
	w/l		12.73	1.029		24.21	0.955
52	*lwp/l*	9	29.13	1.582	11	39.60	1.692
	ll/l		60.91	1.462		65.73	1.792
	w/l		12.37	1.077		21.58	0.841
72	*lwp/l*	10	29.49	1.289	11	33.1	0.91
	ll/l		56.96	1.149		59.5	0.997
	w/l		9.47	0.445		20.31	0.831
81	*lwp/l*	10	26.95	1.444	10	34.18	1.571
	ll/l		54.79	1.247		60.74	2.017
	w/l		9.49	0.680		21.13	1.349

(b) ANOVA (Model I is assumed.)

	Source	DF	SS	MS	F
lwp/l	Species	1	0.0159	0.0159	7.57**
	Shade	4	0.0386	0.0097	4.62**
	Species/Shade	4	0.1551	0.0388	18.48***
	Error	93	0.1962	0.0021	
	Total	102	0.4058		
ll/l	Species	1	0.0678	0.0678	32.29***
	Shade	4	0.1604	0.0401	19.10***
	Species/Shade	4	0.002	0.0005	<1
	Error	93	0.1955	0.0021	
	Total	102	0.4257		
w/l	Species	1	0.2943	0.2943	294.3***
	Shade	4	0.034	0.0085	8.5***
	Species/Shade	4	0.0001	0.0000	<1
	Error	93	0.0938	0.001	
	Total	102			

(c) C.V.A.

B	2093.758	1782.0089	2436.5516
		2303.4443	2124.6662
			3286.4125
W	1962.4288	1267.9675	2.8101
		1954.8029	222.7405
			938.496

Eigenvalues	4.5018	0.7189	0.1147
% of variation	84.38	13.47	2.1499
Eigenvectors	0.1125	0.1756	-0.1974
	-0.0189	-0.2918	0.0081
	0.2844	0.0556	0.1459
Standardized	0.5181	0.8089	-0.909
eigenvectors	0.0866	-1.3377	0.0372
	0.8976	0.1754	0.4604

roots excluded	DF	X^2
0	27	224.94***
1	16	62.1***
2	7	10.37

Mean values of the canonical variates evaluated at the group means.

	P. glabrata			*P. paradoxa*		
% Shade	CV_1	CV_2	CV_3	CV_1	CV_2	CV_3
0	6.21	-13.1	-3.29	10.13	-12.17	-3.71
28	5.9	-11.78	-3.67	9.92	-11.79	-3.50
52	5.64	-11.97	-3.45	9.35	-11.02	-4.14
72	4.94	-10.91	-3.98	8.37	-10.55	-3.09
81	4.70	-10.73	-3.49	8.71	-10.55	-3.17

Table 3(d): The Effect of Light Intensity on Leaf Shape in *P.glabrata*.

B	75.2625	169.4121	77.3326	
		729.5264	356.5793	
			183.8891	
W	944.891	432.8077	149.785	
		807.8589	315.6243	
			297.475	
Eigenvalues		1.0394	0.0574	0.0385
% of variation		91.55	5.06	3.39
Eigenvectors *lwp/l*		-0.0763	-0.1189	0.2048
ll/l		0.2194	-0.1698	-0.1919
w/l		0.0976	0.3935	0.2983
Standardized *lwp/l*		-0.3534	-0.551	0.949
eigenvectors *ll/l*		0.9399	-0.7276	-0.8234
w/l		0.2537	1.0232	0.7758

root excluded	DF	X^2
0	12	35.48***
1	6	4.12
2	2	1.66

% Shade	0	28	52	72	81
CV_1	13.56	12.33	12.35	11.17	10.89
CV_2	-9.1	-9.01	-8.94	-9.45	-8.77
CV_3	-2.22	-1.69	-2.03	-2.07	-2.16

Table 3(e): The Effect of Light Intensity on Leaf Shape in *P. paradoxa*.

B	430.9098	573.3456	197.0284
		893.6125	352.6929
			157.7577
W	1027.5378	835.1598	-146.9749
		1146.944	-92.8838
			629.021

Eigenvalues		1.1184	0.1481	0.0058
% of variation		87.9	11.64	0.46
Eigenvectors	*lwp/l*	-0.-245	-0.3263	0.1124
	ll/l	0.2017	0.1906	-0.1671
	w/l	0.1525	0.0239	0.2387
Standardized	*lwp/l*	-0.1124	-1.5943	0.5148
eigenvectors	*ll/l*	0.9756	0.9222	-0.8085
	w/l	0.5462	0.0857	0.8552

% Shade	0	28	52	72	81
CV_1	16.95	16.49	15.57	14.37	14.63
CV_2	1.01	1.03	0.12	1.11	0.93
CV_3	-1.43	-1.29	-1.38	-1.45	01.26

root excluded	DF	X^2
0	12	43.83***
1	6	7.05
2	2	0.28

Table 4 : The Effect of Photoperiod on Leaf Shape in *P. paradoxa* and *P. glabrata*.

(a) : Mean values (x100)of the ratios *lwp/l*, *ll/l* and *w/l* for *P. paradoxa* and *P. glabrata* from Arthur's Lake (AL), Kannaleena (K), and Pine Creek (PC), grown under long days (LD) and short days (SD).

Species	Daylength	Site	n	*lwp/l*	*ll/l*	*w/l*
P. glabrata	SD	PC	20	27.81	56.39	12.75
			22	27.96	57.62	13.31
		K	19	26.75	53.17	8.85
			22	26.52	58.10	10.17
		AL	22	26.10	58.35	10.29
			21	26.00	54.01	7.80
	LD	PC	19	21.92	50.93	10.35
			15	20.09	50.43	9.17
		K	18	21.33	51.74	8.87
			15	20.19	46.82	7.06
		AL	17	21.12	51.13	7.47
			13	19.36	49.36	7.23
P. paradoxa	SD	PC	24	34.27	60.94	18.33
			24	36.40	63.66	18.36
		K	23	35.66	64.60	19.13
			23	37.43	65.07	19.47
		AL	24	35.11	62.15	18.53
			24	34.83	63.48	19.04
	LD	PC	24	36.46	64.15	19.58
			6	33.35	60.63	15.42
		K	24	38.79	70.24	22.23
			23	36.30	64.89	18.28
		AL	22	33.36	60.88	17.95
			19	32.71	59.04	14.82

(b) : Three way ANOVA of species, site and daylength for the characters *lwp/l*, *ll/l*, and *w/l*. Model I is assumed

(a) *lwp/l*

Source	DF	SS	MS	F
Species	1	811.0763	811.0763	610.7***
Site	2	13.4366	6.7183	5.06*
Daylength	1	66.2008	66.2008	49.85***
Species/Site	2	8.917	4.4585	3.36
Species/Daylength	1	49.3067	49.3067	37.13***
Site/Daylength	2	2.3925	1.1963	<1
Species/Site/Daylength	2	2.6357	1.3179	<1
Error	12	15.9368	1.3281	
Total	23	969.9024		

(b) *ll/l*

Source	DF	SS	MS	F
Species	1	616.9176	616.9176	117.48***
Site	2	16.7229	8.3615	1.59
Daylength	1	57.9704	57.9704	11.04**
Species/Site	2	39.0412	19.5206	3.72
Species/Daylength	1	57.5362	57.5362	10.96**
Site/Daylength	2	6.6695	3.3348	<1
Species/Site/Daylength	2	9.0522	4.5261	<1
Error	12	63.0144	5.2512	<1
Total	23	866.9244		

(c) *w/l*

Source	DF	SS	MS	F
Species	1	484.3813	484.3813	207.36***
Site	2	13.7379	6.869	2.94
Daylength	1	12.9067	12.9067	5.53*
Species/Site	2	20.8486	10.4243	4.46
Species/Daylength	1	2.9681	2.9681	1.27
Site/Daylength	2	4.1184	2.0592	<1
Species/Site/Daylength	2	3.3462	1.6731	
Error	12	28.0316	2.336	
Total	23	570.3388		

(c) : C.V.A. of *P. paradoxa* and *P. glabrata* from AL. K and PC grown under long days and short days for the leaf characters *lwp/l, ll/l* and *w/l*.

B	17472.5789	15828.3769	13366.4383	*lwp/l*
		15724.8792	12267.2878	*ll/l*
			11191.4178	*w/l*
W	7792.3331	6171.0004	2353.7843	
		11003.6152	4014.1686	
			3590.7476	

Eigenvalues	3.5459	0.3498	0.1643
% of variation	88.47	7.84	3.68
Eigenvectors	0.1523	0.0111	-0.2873
	-0.0873	-0.2708	0.1434
	0.3133	0.2821	0.1947
Standardized	0.6281	0.0458	-1.1851
eigenvectors	-0.4278	-1.3274	0.703
	0.8772	0.7898	0.5452

roots excluded	DF	X^2
0	69	958.7***
1	44	211.3***
2	21	71.1***

Mean values of the three canonical variates.

		P. paradoxa			*P. glabrata*		
		AL	K	PC	AL	K	PC
SD	CV_1	5.73	6.0	5.69	1.9	2.18	3.36
	CV_2	11.32	11.71	11.31	12.38	12.09	11.46
	CV_3	2.62	2.56	2.36	2.34	2.18	2.7
LD	CV_1	4.93	6.17	5.36	1.0	1.36	1.84
	CV_2	11.25	12.17	11.58	11.31	10.89	10.74
	CV_3	2.3	2.85	2.33	2.82	2.67	3.14

Table 5 : The Effect of Light-Temperature Interaction on Leaf Shape in *P. glabrata* and *P. paradoxa*.

(a) : Mean values of the ratios *lwp/l*, *ll/l* and *w/l* (x 100) for *P. paradoxa* and *P. glabrata* from Interlaken (I) and Lake Augusta (LA), grown under two temperatures (10, 17.5°C) and three light regimes. The light regimes were long days, low light intensity (LL); short days, high intensity (SH), and short days, low intensity (SL).

Species	Site	Temperature	Light	n	*lwp/l*	*ll/l*	*w/l*
P. glabrata	I	10	SL	20	31.22	57.15	10.37
					31.26	58.20	10.36
					30.76	57.69	10.23
			SH	18	29.44	60.68	14.44
					31.77	62.40	14.84
					31.76	61.96	14.85
			LL	17	39.88	71.23	14.74
					39.10	71.84	15.42
					38.05	69.18	15.86
		17.5	SL	20	30.48	61.81	9.57
					31.26	62.53	9.45
					30.30	57.64	9.27
			SH	18	27.50	61.01	9.61
					26.03	59.72	9.48
					26.27	60.71	9.74
			LL	17	27.06	55.28	7.62
					25.05	52.99	7.89
					26.22	54.99	8.25
	LA	10	SL	12	31.93	62.55	21.64
					31.92	63.71	22.06
					32.00	63.52	21.85
			SH	8	32.01	62.63	22.29
					34.33	66.08	22.79
					32.90	65.59	23.77
			LL	17	37.27	71.93	24.74
					35.57	70.61	23.46
					35.52	70.34	24.46
		17.5	SL	18	31.81	63.52	22.87
					30.63	63.14	22.46
					30.59	62.61	22.74
			SH	18	32.69	67.71	19.26
					32.47	66.12	19.34
					30.81	66.09	18.79
			LL	15	30.75	61.43	17.51
					33.03	62.36	17.77
					32.76	63.17	18.39

Table 5(a) (Contd.)

Species	Site	Temperature	Light	n	*lwp/l*	*ll/l*	*w/l*
P. paradoxa	I	10	SL	17	37.39	67.51	26.73
					36.66	66.06	26.35
					36.47	68.39	26.39
			SH	19	36.82	65.17	23.44
					37.50	66.39	23.86
					36.86	66.41	23.41
			LL	16	40.24	66.26	24.94
					41.02	69.26	25.51
					40.54	70.97	27.28
		17.5	SL	14	34.36	59.80	22.22
					35.20	60.91	21.84
					34.81	60.88	21.73
			SH	19	37.57	66.71	24.27
					36.06	66.36	23.70
					37.13	66.31	24.09
			LL	19	37.27	64.99	21.51
					36.27	65.92	21.98
					36.86	65.95	21.58
	LA	10	SL	16	34.19	59.26	19.02
					35.55	60.44	18.54
					33.49	58.14	18.24
			SH	19	36.03	65.15	21.24
					35.79	65.14	21.64
					35.97	65.58	21.71
			LL	17	37.31	63.35	22.14
					38.94	64.69	21.62
					40.00	67.05	22.78
		17.5	SL	19	32.82	63.67	20.76
					32.96	62.31	20.98
					33.75	64.04	21.20
			SH	20	34.68	63.16	23.00
					34.39	62.30	22.14
					34.70	62.43	22.30
			LL	19	36.88	67.02	21.13
					34.35	64.00	20.70
					34.82	64.4	20.42

(b) : Four way ANOVA of species (Sp), site (S), temperature (T) and light regime (L) for the leaf characters *lwp/l*, *ll/l*, and *w/l*. Model I is assumed.

(a) *lwp/l*

Source	DF	SS	MS	F
Sp	1	370.192	370.192	532.6***
S	1	0.1404	0.1404	<1
T	1	158.598	158.598	228.17***
L	2	95.7433	47.8717	68.87***
Sp/S	1	64.1847	64.1847	92.34***
Sp/T	1	17.1115	17.1115	24.62***
Sp/L	2	10.1037	5.0519	7.27**
S/T	1	16.8465	16.8465	24.24***
S/L	2	8.9498	4.4749	6.44**
T/L	2	84.2923	42.1462	60.63***
Sp/T/S	1	17.4928	17.4928	25.17***
Sp/T/L	2	25.1491	12.5746	18.09***
Sp/S/L	2	6.7398	3.3699	4.85*
S/T/L	2	17.6906	8.8453	12.73***
Sp/S/T/L	2	18.271	9.1355	13.14***
Error	48	33.3668	0.6951	
Total	71	944.8723		

(b) *ll/l*

Source	DF	SS	MS	F
Sp	1	43.8048	43.8048	29.65***
S	1	16.1312	16.1312	10.92**
T	1	109.0764	109.0764	73.84***
L	2	151.9492	75.9746	51.43***
Sp/S	1	194.4394	194.4394	131.63***
Sp/T	1	22.7814	22.7814	15.42***
Sp/L	2	0.1867	0.0934	<1
S/T	1	43.3382	43.3382	29.34***
S/L	2	0.7289	0.3645	0.25
T/L	2	187.2989	93.6495	63.40***
Sp/S/T	1	1.496	1.496	1.01
Sp/S/L	2	0.3828	0.1914	<1
Sp/T/L	2	171.0926	85.5463	57.91***
S/T/L	2	24.3562	12.1781	8.24***
Sp/S/T/L	2	93.4407	46.7204	31.63***
Error	48	70.9042	1.4772	
Total	71	1131.4076		

(c) *w/l*

Source	DF	SS	MS	F
Sp	1	685.7956	685.7956	3522.32***
S	1	245.422	245.422	1260.51***
T	1	106.2153	106.2153	545.53***
L	2	6.2558	3.1279	16.07***
Sp/S	1	770.0852	770.0852	3955.24***
Sp/T	1	33.1707	33.1707	170.37***
Sp/L	2	0.5406	0.2703	1.39
S/T	1	25.6687	25.6687	131.84***
S/L	2	0.7833	0.3917	2.01
T/L	2	57.991	28.9955	148.92***
Sp/S/T	1	4.6967	4.6967	24.12***
Sp/S/L	2	32.251	16.1255	82.82***
Sp/T/L	2	33.0729	16.5365	84.93***
S/T/L	2	9.3534	4.6767	24.02***
Sp/S/T/L	2	6.7936	3.3968	17.45***
Error	48	9.3448	0.1947	
Total	71	2027.4406		

(c) : C.V.A. of the leaf characters *lwp/l*, *ll/l* and *w/l* (x100) for *P. paradoxa* and *P. glabrata* from Interlaken and Lake Augusta grown under two temperatures and three light regimes.

B	911.5056	753.2477	986.6748
		1060.5034	982.0144
			2018.0958
W	33.3668	32.2726	4.7427
		70.9042	12.8563
			9.3448

Eigenvalues	266.96	15.22	9.47
% of variation	91.53	5.22	3.25
Eigenvectors	-0.4497	-1.5341	-0.1968
	0.5481	0.5343	0.9609
	-2.5541	0.5239	-0.3324
Standardized	-0.3749	-1.279	-0.1641
eigenvectors	0.667	0.6494	1.1679
	-1.127	0.2312	-0.1467

roots excluded	DF	X^2
0	69	616.7***
1	44	295.2***
2	21	135.0***

(d) : Canonical Correlation Analysis of Leaf Shape Variation in *P. glabrata* and *P. paradoxa* from Interlaken and Lake Augusta, grown under two temperature levels and three light regimes.

(i) Correlation Matrices

P. glabrata, Lake Augusta

lwp/l	1	0.8425	0.4918	0.5496	-0.5579	0.0729
ll/l		1	0.4857	0.3794	-0.3375	-0.1251
w/l			1	0.687	0.1252	0.1299
temperature				1	0	0
daylength					1	-0.5
light intensity						1

P. glabrata, Interlaken

lwp/l	1	0.8747	0.7854	0.6879	-0.2994	0.3217
ll/l		1	0.7973	0.4772	-0.2258	-0.0186
w/l			1	0.7986	-0.1032	-0.2371
temperature				1	0	0
daylength					1	-0.5
light intensity						1

P. paradoxa, Lake Augusta

lwp/l	1	0.5798	0.4133	0.5266	-0.6293	0.0405
ll/l		1	0.6751	-0.4957	-0.1543	-0.1543
w/l			1	-0.242	-0.2045	-0.4963
temperature				1	0	0
daylength					1	-0.5
light intensity						1

P. paradoxa, Interlaken

lwp/l	1	0.7813	0.5926	0.5641	-0.6119	0.0712
ll/l		1	0.7369	0.5780	-0.3688	-0.1115
w/l			1	0.745	0.0512	0.0531
temperature				1	0	0
daylength					1	-0.5
light intensity						1

(ii) The canonical correlation between leaf shape and environments.

P. glabrata, Lake Augusta

canonical correlations:		0.8549***	0.7949**	0.2944
coefficients:	temperature	0.0915	-0.202	-0.0798
	daylength	-0.265	-0.1133	-0.0618
	light intensity	-0.1118	-0.14	0.2264
	lwp/l	0.1736	-0.0586	0.1633
	ll/l	-0.0193	0.0587	-0.1307
	w/l	-0.0464	-0.1098	-0.022

P. glabrata, Interlaken

canonical correlations:		0.9554***	0.9241***	0.2286
coefficients:	temperature	0.1122	0.2047	-0.0329
	daylength	0.0413	-0.0673	-0.2777
	light intensity	0.2376	-0.1431	0.0000
	lwp/l	0.1171	-0.0116	-0.0177
	ll/l	-0.0588	-0.0209	0.0805
	w/l	-0.0427	0.1226	0.0674

(ii) (Contd.)

P.paradoxa, Lake Augusta			
canonical correlations:	0.9149***	0.7718**	0.3246
coefficients: temperature	-0.1867	-0.0636	-0.1291
daylength	0.1464	-0.228	-0.0995
light intensity	-0.0116	-0.2517	0.1408
lwp/l	-0.1403	0.0022	-0.0615
ll/l	0.0097	0.0301	0.1501
w/l	0.0908	0.1379	-0.1802
P. paradoxa, Interlaken			
canonical correlations:	0.9625***	0.7677**	0.3094
coefficients: temperature	0.0275	-0.2314	-0.0353
daylength	-0.2859	-0.0299	-0.0268
light intensity	-0.1618	-0.0547	0.2327
lwp/l	0.1054	-0.0412	0.1811
ll/l	0.0591	0.0330	-0.1492
w/l	-0.1244	-0.1361	0.0327

Appendix E. Data Tables from the Progeny Tests. (Chapter 7.).

Table 1 . PCA of the progeny plants used in the estimation of leaf shape inheritance in *P. glabrata* and *P. paradoxa*.

(a) *P. glabrata*

Variance-Covariance matrix of the logs of *l*, *lwp*, *ll* and *w*

0.0167	0.0118	0.0145	0.0092
	0.0127	0.0124	0.0088
		0.0148	0.0108
			0.0147

eigenvalues		0.0489	0.0069	0.0026	0.0006
% of variation		82.9	11.7	4.4	1.0
eigenvectors	*l*	0.5428	-0.4407	0.5814	0.4161
	lwp	0.4702	-0.1653	-0.8012	0.3311
	ll	0.5401	-0.1231	-0.0018	-0.8326
	w	0.4388	0.8737	0.1414	0.1552

(b) *P. paradoxa*

Variance-Covariance matrix of the logs of *l*. *lwp*, *ll* and *w*

0.0192	0.0172	0.0176	0.0157
	0.0177	0.0165	0.0157
		0.0173	0.0161
			0.0195

eigenvalues		0.0679	0.0042	0.0013	0.0006
% of variation		91.7	5.6	1.8	0.8
eigenvectors	*l*	0.5162	-0.4694	0.5054	0.5076
	lwp	0.4935	-0.213	-0.8331	0.1309
	ll	0.4972	-0.1293	0.1994	-0.8329
	w	0.4927	0.8456	0.1037	0.1776

Table 2 . Mean values of parent and progeny plants used in the estimation of the heritability of the leaf characters *lwp/l*, *ll/l*, and *w/l*. (x100)

(a) *P. glabrata*

lwp/l		*ll/l*		*w/l*		*n*
parent	progeny	parent	progeny	parent	progeny	(progeny)
19.3	27.4	51.3	59.2	7.4	12.1	27
23.9	30.1	57.7	60.5	10.6	15.6	24
25.9	27.0	56.9	56.8	10.7	14.0	35
26.2	32.3	61.7	61.2	10.5	16.4	24
23.7	24.6	55.4	54.3	5.7	12.6	27
24.1	26.8	56.1	55.0	9.5	13.9	32
27.0	28.5	61.4	55.3	11.6	13.6	20
19.1	27.9	44.9	54.2	6.5	13.2	24
30.2	32.1	67.1	62.9	16.9	19.4	30
31.4	32.7	68.4	66.4	17.8	19.1	16
25.1	27.1	62.4	56.5	17.8	16.9	31
27.7	27.7	62.4	58.5	14.9	16.6	35

Table 2 . (Contd.)

(b) *P. paradoxa*

lwp/l		*ll/l*		*w/l*		*n*
parent	progeny	parent	progeny	parent	progeny	(progeny)
36.9	39.7	69.4	74.4	20.6	30.3	35
37.1	41.9	73.5	75.8	21.6	30.2	31
41.8	43.8	71.0	71.7	24.8	33.1	25
38.5	41.6	65.5	71.2	16.1	24.3	40
34.5	38.4	65.2	68.5	21.0	28.2	28
31.5	35.6	60.1	64.9	16.6	22.9	32
34.6	39.5	65.8	65.3	15.0	22.9	42
30.0	35.4	62.8	65.4	20.0	26.3	24
33.5	40.9	61.6	71.7	17.2	26.8	24
29.1	40.2	54.2	69.2	12.4	21.2	39
31.4	38.6	60.8	68.3	16.3	26.2	39
40.1	43.2	72.8	73.5	22.3	32.0	39

Table 3. The multivariate estimation of leaf shape inheritance in *P. glabrata* and *P. paradoxa*.

(a) The matrix of correlation coefficients within and between parent and progeny for the characters *lwp /l*, *ll/l* and *w/l*.

P. glabrata

a	b	c	d	e	f
1	0.9483	0.7959	0.5805	0.6232	0.8165
	1	0.8575	0.5894	0.6693	0.8403
		1	0.528	0.6012	0.8906
			1	0.8635	0.7567
				1	0.7765
					1

P. paradoxa

a	b	c	d	e	f
1	0.8615	0.6863	0.7898	0.6498	0.7461
	1	0.8136	0.5697	0.6385	0.8329
		1	0.3601	0.486	0.9428
			1	0.7637	0.5503
				1	0.6823
					1

a-c and d-f are the variables *lwp/l*, *ll/l* and *w/l* for parent and progeny respectively in each case.

Table 3. (Contd.)

(b) Canonical correlations and coefficients for the three characters among parents and progeny in the two species.

P. glabrata				
canonical correlations		0.96***	0.345	0.185
coefficients: progeny				
	lwp/l	-0.039	0.0113	-0.2398
	ll/l	0.0008	-0.1299	0.1089
	w/l	0.1509	0.132	0.0378
	parent			
	lwp/l	0.026	0.1199	-0.2262
	ll/l	-0.0054	-0.1467	0.0875
	w/l	0.0588	0.1135	0.0506
P. paradoxa				
canonical correlations		0.97***	0.83*	0.328
coefficients: progeny				
	lwp/l	-0.0214	0.1485	0.0938
	ll/l	-0.0172	-0.0226	-0.1431
	w/l	0.0952	-0.0233	0.0459
	parent			
	lwp/l	-0.0082	0.1205	0.0838
	ll/l	0.0014	-0.0248	-0.127
	w/l	0.0883	-0.0551	0.1005

Appendix F. Data Tables from the Study of the Correlation of Leaf Shape and Habitat in *P. paradoxa* (Chapter 8).

Table 1 : Mean values of the logarithms of the four leaf characters, *l*, *lwp*, *ll* and *w* for *P. paradoxa* plants from three habitats at Canal Bay and Lake Augusta.

(a) Field Values

Character/Site	Canal Bay			Lake Augusta		
	Soak	Mudflat	Cushion	Soak	Mudflat	Cushion
l	1.6964	1.7343	1.1878	1.6055	1.7656	1.2472
lwp	1.3021	1.06	0.8532	1.2144	1.1653	0.8748
ll	1.5025	1.3453	1.0216	1.4216	1.3891	1.0438
w	1.0406	0.6903	0.7125	1.0315	0.6642	0.6686

(b) Progeny Values

Character/Site	Canal Bay			Lake Augusta		
	Soak	Mudflat	Cushion	Soak	Mudflat	Cushion
l	1.8166	1.846	1.8166	1.5473	1.5293	1.5609
lwp	1.3682	1.4243	1.382	1.1792	1.1603	1.1022
ll	1.6326	1.6637	1.6497	1.4113	1.3929	1.3477
w	1.1151	1.1321	1.0893	1.0395	1.0352	0.885

Table 2: CVA of Field Variation in Leaf Shape of *P. paradoxa* in three habitats at Canal Bay and Lake Augusta.

B	6.5441	3.9773	4.8312	1.4031	
		3.3719	3.6717	2.4396	
			4.1587	2.3586	
				3.3376	
W	0.7249	0.5945	0.6058	0.4347	
		1.0288	0.8706	0.5895	
			0.9738	0.5264	
				0.8255	
eigenvalues		12.2355	4.1681	0.346	0.0012
% of variation		73.04	24.88	2.07	0.01
eigenvectors	*l*	-15.6011	-0.8903	3.927	9.6628
	lwp	2.1304	-1.4583	-21.3441	7.2031
	ll	-1.0088	4.1444	10.2516	-20.5214
	w	8.1383	10.3592	6.713	5.1749
Standardized eigenvectors	*l*	-1.2441	-0.071	0.3132	0.7706
	lwp	0.2024	-0.1385	-2.0277	0.6843
	ll	-0.0932	0.383	0.9475	-1.8967
	w	0.6925	0.8815	0.5713	0.4404

roots excluded	DF	X^2
0	20	515.71***
1	12	221.26***
2	6	34.01***
3	2	0.14

	CV_1	CV_2	CV_3	CV_4
Lake Augusta				
soak	-15.5	13.38	1.88	0.43
mudflat	-21.06	9.37	0.76	0.39
cushion	-13.21	8.87	1.41	0.39
Canal Bay				
soak	-16.74	13.60	1.26	0.32
mudflat	-20.54	9.64	2.61	0.36
cushion	-11.94	9.31	1.71	0.35

Table 3 : CVA of Leaf Shapes of progeny plants of *P. paradoxa* from three habitats at Canal Bay and Lake Augusta.

B	2.7016	2.2387	2.4059	1.2012	
		1.8886	2.0096	1.0744	
			2.1550	1.1134	
				0.8013	
W	1.0979	0.8644	0.9466	0.7634	
		0.9872	0.883	0.7716	
			0.9413	0.7882	
				1.0198	
eigen values		3.2025	0.4823	0.0764	0.0371
% variation		84.31	12.70	2.01	0.98
eigen vectors	*l*	-5.8766	-1.1796	20.3606	18.928
	lwp	-1.783	4.3466	20.8364	-16.9912
	ll	-9.1306	-8.803	-42.3738	-8.6001
	w	9.202	14.1698	0.2744	6.4087
Standardized eigenvectors	*l*	-0.5767	-0.1158	1.9981	1.8575
	lwp	-0.1659	0.4045	1.9390	-1.5812
	ll	-0.8297	-0.7999	-3.8505	-0.7815
	w	0.8704	1.3402	0.026	0.6062

roots excluded	DF	X^2
0	20	221.08***
1	12	57.42***
2	6	12.55*
3	2	4.15

	CV_1	CV_2	CV_3	CV_4
Lake Augusta				
soak	-14.52	5.61	-3.44	3.78
mudflat	-14.25	5.65	-3.42	3.89
cushion	-14.98	3.69	-3.22	3.88
Canal Bay				
soak	-17.76	5.23	-3.38	4.24
mudflat	-18.16	5.41	-2.92	3.69
cushion	-18.18	4.78	-3.82	3.70

Table 4 : The Effect of GA_3 on leaf form in *P. paradoxa*.

(a) Control (untreated) plants

	Week 0	Week 5
dentate, pilose	66	67
entire, glabrous	30	29

$X_1^2 = (1)^2/66 + (1)^2/30 = 0.048,\ 0.9 > P > 0.8$

(b) GA_3 (treated) plants

	Week 0	Week 5
dentate, pilose	59	17
entire, glabrous	37	79

$X_1^2 = (42)^2/59 + (42)^2/37 = 77.57^{***}$

Table 5 : Stomatal number in leaves from plants of *P. paradoxa* taken from the soak, mudflat and cushion communities at Canal Bay.

The stomatal counts for each leaf are averages of readings taken in the centre of the widest part of the lamina, one each side of the midrib, for 2 leaves of each of 7 plants at magnification x300.

1a. Mean Number of Stomata per Unit Area (± standard error). 1 ocular unit = 0.126 mm^2.

Soak	Mudflat	Cushion
20.29 (± 2.20)	14.68 (± 2.84)	33.75 (± 3.90)

b. ANOVA.

Source	D.F.	S.S.	M.S.	F
between sites	2	1345.0417	672.52	71.85***
within	18	168.5179	9.36	
total	20	1513.5595		

2a. Mean Number (x 10^3) of Stomata per Leaf (± standard error).

Soak	Mudflat	Cushion
39.22 (± 4.19)	5.05 (± 0.92)	7.74 (± 0.90)

b. ANOVA.

Source	D.F.	S.S.	M.S.	F
between sites	2	5055.70	2527.85	56.46***
within	18	805.91	44.77	
total	20	5860.61		

Appendix G: Data Tables from the Study of Variation in *P. glabrata* (Chapter 9).

Table 1: (a) Mean Rates of Germination of Seed from Four Populations of *P. glabrata* at Five Temperatures (± Standard Errors).

Population Temperature (°C)	Interlaken	Kannaleena	Pine Creek	Lake Augusta
5	0	0	0	0
10	0.07 (0)	0.07 (0)	0.07 (0)	0.11 (0.006)
15	0.08 (0.005)	0.09 (0.008)	0.10 (0.004)	0.13 (0.009)
20	0.18 (0.009)	0.15 (0.009)	0.15 (0.005)	0.16 (0.008)
25	0.16 (0.005)	0.14 (0.005)	0.14 (0.008)	0.14 (0.013)

(b) Analysis of Variance.

Treatment	D.F.	S.S.	M.S.	F.
Temperature	3	0.1369	0.0456	127.8 ***
Population	3	0.0091	0.003	8.53 ***
Interaction	9	0.0187	0.0021	5.83 ***
Error	112	0.04	0.0004	
Total	117	0.2048		

Table 2: Sampling Structure Used in the Analysis of Variation in the *P. antarctica* - *P. glabrata* Complex.

Sample No.	Location	Leaf Type	*n*
1	Clarence Plains	broad	11
2	Clarence Plains	narrow	10
3	Interlaken	broad	9
4	Interlaken	narrow	5
5	Arthur's Lake	broad	9
6	Arthur's Lake	narrow	10
7	Shannon	broad	6
8	Shannon	narrow	6
9	Pine Creek	broad	12
10	Pine Creek	narrow	11
11	Lake Augusta	broad	2
12	Lake Augusta	narrow	6

Table 3: Characters Scored in the Analysis of the *P. antarctica* - *P. glabrata* Complex.

1.	leaf length	*l*
2.	length to widest part of leaf, from the apex	*lwp*
3.	leaf width	*w*
4.	lamina length	*ll*
5.	number of veins (scored on 1-5 scale)	*v*
6.	number of lobes on leaf	*tn*
7.	length of longest lobe	*tl*
8.	leaf indumentum (scored on 0-4 scale)	*li*
9.	length of longest scape	*sc*
10.	length of spike on longest scape	*sp*
11.	bract length (0-3 scale in relation to calyx)	*bl*
12.	bract indumentum (0-4 scale)	*bi*
13.	width of heel of bract	*bk*
14.	width of bract margin	*bm*
15.	anterior sepal length	*sl*
16.	anterior sepal width	*sw*
17.	anterior sepal, length to widest part	*slwp*
18.	anterior petal lobe length	*pl*
29.	anterior petal lobe width	*pw*
20.	anterior petal lobe length to widest part	*plwp*
21.	presence of petal midrib (1-3 scale)	*pm*

Table 4: Mean Values for the Twenty One Characters Measured for the Twelve Samples of the *P. antarctica* - *P. glabrata* Complex.

Character	Sample Number											
	1	2	3	4	5	6	7	8	9	10	11	12
l	9.65	5.74	6.27	6.14	13.38	9.51	11.42	5.95	8.11	4.56	11.42	5.60
lwp	2.75	1.73	2.53	1.86	3.84	2.89	3.83	1.92	2.46	1.47	3.83	1.95
w	1.58	1.02	1.51	0.86	1.60	0.90	1.38	0.76	1.31	0.80	1.38	1.21
ll	6.55	3.98	4.82	4.00	8.66	6.53	7.95	4.17	5.53	3.11	7.95	3.95
v	3.91	2.40	3.67	2.60	4.33	3.00	4.17	2.67	3.75	2.27	4.17	3.50
tn	4.64	5.50	4.67	6.80	7.78	6.80	7.17	7.33	4.50	4.18	7.17	5.67
tl	3.70	4.44	0.24	1.68	2.77	3.16	2.78	2.72	1.00	1.28	2.78	3.97
li	3.45	3.27	3.00	2.30	3.39	2.60	4.00	2.58	3.28	3.23	4.00	2.83
sc	19.56	13.17	9.46	13.74	24.67	15.03	20.83	12.00	19.19	10.52	20.83	10.98
sp	2.23	1.51	1.71	1.40	3.06	1.42	1.92	1.12	2.20	1.27	1.92	1.77
bl	1.82	1.20	0.22	1.00	1.28	1.20	1.25	1.17	1.92	1.27	1.25	1.67
bk	3.64	3.40	0.67	2.60	2.78	2.75	3.25	2.17	2.88	2.91	3.25	2.58
bm	0.76	0.72	0.79	0.82	0.92	0.67	0.68	0.80	0.75	0.85	0.68	0.72
sl	5.66	5.35	4.49	5.12	5.52	5.13	5.05	5.22	5.42	5.18	5.05	5.25
sw	2.83	2.69	2.70	3.04	2.97	2.77	2.75	2.83	2.83	2.80	2.75	2.83
slwp	2.25	1.75	1.80	2.22	2.29	2.11	2.02	1.93	2.43	2.01	2.02	2.55
pl	4.96	4.54	3.74	4.06	4.44	4.26	4.05	4.13	4.15	4.21	4.05	4.15
pw	3.54	3.20	3.18	3.56	3.56	3.30	3.18	3.43	3.46	3.41	3.18	3.18
plwp	2.84	2.53	2.20	2.34	2.40	2.57	2.30	2.75	2.43	2.45	2.30	2.50
pm	1.00	1.00	1.33	1.40	1.44	1.45	1.67	1.00	1.17	1.05	1.67	1.33

Table 5: Within Groups Covariance Matrix Obtained for the 21 Characters Used in Analysis of the *P. antarctica* - *P. glabrata* Complex

Variable	*l*	*lwp*	*w*	*ll*	*v*	*tn*	*tl*	*li*	*sc*	*sp*	*bb*	*bi*	*bk*	*bm*	*sl*	*sw*	*slwp*	*pl*	*pw*	*plwp*	*pm*
l	7.438																				
lwp	1.857	0.677																			
w	0.458	0.114	0.098																		
ll	4.836	1.259	0.319	3.289																	
v	1.456	0.427	0.149	1.013	0.689																
tn	-1.175	-0.258	-0.052	-0.705	-0.229	3.542															
tl	-1.422	-0.421	-0.029	-0.953	-0.638	1.234	5.952														
li	0.202	0.052	0.026	0.157	0.037	0.079	-0.053	0.468													
sc	11.172	2.971	0.942	7.565	2.477	-2.416	-0.865	0.438	27.367												
sp	1.116	0.346	0.097	0.743	0.236	-0.259	0.016	-0.025	2.605	0.543											
bb	-0.255	-0.054	0.008	-0.170	-0.009	0.262	-0.165	-0.173	-0.725	0.054	0.542										
bi	0.590	0.091	0.055	0.321	0.035	-0.128	-0.017	0.045	1.161	0.148	0.047	0.588									
bk	0.089	0.017	0.018	0.056	0.045	0.011	-0.055	-0.008	0.242	0.041	0.045	0.008	0.030								
bm	-0.086	-0.022	-0.000	-0.060	-0.019	0.053	0.057	-0.022	-0.051	-0.011	-0.024	-0.212	-0.004	0.037							
sl	0.613	0.177	0.052	0.388	0.150	-0.107	-0.096	-0.011	1.167	0.162	-0.010	0.046	0.029	-0.004	0.242						
sw	0.197	0.057	0.029	0.137	0.078	-0.076	-0.072	-0.008	0.361	0.061	0.003	0.048	0.024	0.011	0.092	0.153					
slwp	0.325	0.092	0.039	0.216	0.109	-0.103	-0.105	-0.007	0.372	0.076	0.068	-0.004	0.027	-0.012	0.109	0.049	0.184				
pl	0.465	0.140	0.038	0.322	0.161	-0.086	-0.162	0.021	1.096	0.111	0.020	0.058	0.026	-0.016	0.120	0.046	0.040	0.201			
pw	0.201	0.066	0.047	0.166	0.090	0.024	0.052	0.033	0.532	0.085	0.027	0.023	0.018	0.009	0.058	0.027	0.017	0.074	0.166		
plwp	0.146	0.047	0.020	0.084	0.067	-0.001	0.061	-0.011	0.488	0.066	0.037	0.022	0.025	-0.006	0.079	0.026	0.032	0.087	0.042	0.133	
pm	0.056	-0.002	0.007	0.025	-0.016	0.042	0.167	-0.045	0.053	0.041	0.018	0.014	-0.011	-0.001	-0.043	-0.029	-0.048	-0.032	-0.038	-0.015	0.161

Table 6: Canonical Variates Analysis of the *P. antarctica* - *P. glabrata* Complex.

Eigen value	3.334	2.652	1.807
% variation	31.25	24.86	16.94

	Canonical Coefficients			Standardized Coefficients		
Variable	CV1	CV2	CV3	CV1	CV2	CV3
l	-0.028	-0.605	-0.475	-0.045	-0.525	-0.652
lwp	-0.375	-0.229	-0.222	-0.183	-0.060	-0.092
w	-1.956	2.229	-1.094	-0.362	0.222	-0.173
ll	0.018	0.928	0.605	0.019	0.541	0.552
v	0.158	0.172	-0.384	0.077	0.045	-0.160
tn	-0.085	-0.340	-0.116	-0.095	-0.204	-0.110
tl	0.122	0.128	-0.114	0.176	0.099	-0.140
li	0.483	0.510	-0.598	0.195	0.111	-0.206
sc	0.149	-0.218	-0.015	0.46	-0.363	-0.040
sp	-0.678	0.603	-0.373	-0.295	0.141	-0.138
bl	0.977	0.229	-0.501	0.425	0.054	-0.186
bi	0.706	0.182	0.192	0.32	0.045	0.074
bk	-0.824	0.948	0.067	-0.084	0.052	0.006
bm	1.091	1.462	-1.080	0.124	0.090	-0.105
sl	1.233	0.841	0.148	0.359	0.132	0.037
sw	-0.471	-1.137	0.156	-0.109	-0.141	0.031
slwp	0.082	-1.279	0.350	0.021	-0.175	0.076
pl	0.100	0.979	-0.170	0.027	0.140	-0.038
pw	-0.003	-1.566	0.876	-0.001	-0.203	0.180
plwp	-0.293	-0.024	0.711	-0.063	-0.003	0.131
pm	0.002	-1.028	0.161	0.001	-0.131	0.033

Table 7: Canonical Variables Evaluated at the Means of the Twelve Sample Groups of the *P. antarctica* - *P. glabrata* Complex .

Canonical Variable	Sample Number 1	2	3	4	5	6	7	8	9	10	11	12
CV1	1.47	1.17	-5.01	-0.40	-0.30	0.02	0.31	0.04	0.95	0.83	-1.20	-0.12
CV2	2.13	1.87	1.44	-2.89	-1.52	-1.88	-1.03	-1.46	-0.17	0.46	3.11	0.63
CV3	-0.98	0.59	0.12	1.65	-2.72	0.69	-1.86	1.52	-0.37	1.69	-1.38	0.21

Table 8: Mean Values of CV2, and Physical Parameters for Each Location.

Location	Altitude (m)	km West of Interlaken	Annual Rainfall (mm)	CV2	*n*
Interlaken	800	0	708	-2.89	5
Arthur's Lake	951	20.5	775	-1.71	19
Kannaleena	970	30.0	791	-1.25	12
Pine Creek	1034	41.0	988	0.15	23
Lake Augusta	1160	55.5	*c.* 1400	1.25	8
Clarence Plains	800	67.0	*c.* 1500	2.01	21

Appendix H. Data Tables from the Study of Within Population Variation of Leaf-Shape in *P. glabrata* (Chapter 10).

Table 1: The mean values and common variance-covariance matrix obtained for the narrow and broad leaf forms of *P. glabrata*, grown in a common environment.

Mean values

	l	lwp	w	ll
broad	15.60	3.86	2.43	9.43
narrow	13.48	4.43	1.25	9.06

Variance-covariance matrix

l	lwp	w	ll
2.004	-0.0267	0.0814	0.6995
	0.1581	0.0023	-0.0411
		0.0258	0.0579
			0.4653

Discriminant function: 2.30 l - 7.04 lwp + 64.27 w - 11.30 ll

$D^2 = 80.27^{***}$

Appendix I. Data Tables from the Analysis of the *P. tasmanica* Complex (Chapter 11).

Table 1: Mean values of the six leaf characters measured in the analysis of the *P. tasmanica-P. daltonii* complex.

(a) Field Populations

Taxon	Population	No.	n	Mean Value					
				l	*w*	*ll*	*pw*	*t*	*lwp*
archeri	Projection Bluff	1	12	3.20	1.06	2.10	0.25	17.75	1.08
	Mt. Field	2	18	2.90	1.08	1.82	0.29	23.98	0.96
	Mt. Anne	3	5	2.20	0.90	1.40	0.22	20.10	0.70
P. tasmanica	Crystal Waters	4	25	3.2	1.06	2.15	0.25	19.38	1.08
	Scenic Point	5	25	4.94	1.15	2.97	0.26	17.82	1.45
	Mt. Wellington	6	25	3.86	1.17	2.36	0.40	19.71	1.15
	Hartz Mountains	7	25	2.91	0.90	1.73	0.18	16.37	0.92
Intermediates	Projection Bluff	8	43	5.19	1.05	2.98	0.20	14.37	1.63
P.daltonii	Pine Creek	9	25	6.54	1.06	3.53	0.16	12.06	1.92
	Hartz Mountains	10	35	4.04	0.69	1.91	0.10	10.70	1.01
	Gordon River	11	4	2.50	0.40	1.50	0.09	11.00	1.00
(b) Controlled Environment: Hartz Mountain Populations									
P. tasmanica	Long days		15	5.64	1.47	3.51	0.35	18.09	1.71
	Short days		18	5.61	1.38	3.46	0.44	16.99	1.65
P. daltonii	Long days		16	6.66	1.29	3.49	0.20	9.55	1.63
	Short days		17	4.95	1.14	2.58	0.20	8.56	1.17
(c) Controlled Environment: Central Plateau Populations									
P. tasmanica	Crystal Waters		11	5.14	1.35	2.92	0.24	16.74	1.3
Intermediates	Projection Bluff		16	5.95	1.28	2.95	0.15	14.53	1.45
P. daltonii	Pine Creek		15	5.61	1.11	2.58	0.13	13.15	1.37
Progeny	Projection Bluff		21	5.4	1.25	3.0	0.22	15.3	1.5

Table 2: Canonical Variate Analysis of the Field Populations of the *P. tasmanica-P. daltonii* Complex.

	W					
l	636.05					
w	70.38	15.34				
ll	283.38	35.28	151.92			
pw	19.19	3.15	9.90	8.27		
t	-23.66	42.48	17.07	11.50	1878.5	
lwp	130.10	17.61	65.90	5.18	19.84	36.11

	B					
l	305.79					
w	8.05	5.24				
ll	149.33	10.52	82.67			
pw	-6.15	2.23	-0.30	1.56		
t	-574.55	85.75	-157.26	60.05	3376.8	
lwp	85.22	4.81	45.71	-0.93	-112.87	26.12

Latent Roots

592.77	155.33	26.15	18.91	6.69	0.51

% of Variation

74.1	19.4	3.3	2.4	0.8	0.1

Canonical Variate Loadings (by row)

0.209	-2.254	-0.475	0.1285	-0.235	2.293
-0.687	-0.431	1.167	-0.472	0.094	2.623
-0.882	3.637	1.683	1.533	-0.263	-1.835
-0.846	0.825	-0.528	-2.423	-0.04	3.182
-0.231	-1.11	2.342	-3.445	0.058	-3.038

Table 2: (Contd.)

Canonical Variate Means

Population	CV1	CV2	95% Confidence Limit
1	-1.04	-0.11	0.57
2	-2.80	0.01	0.46
3	-1.93	-0.93	0.88
4	-1.43	0.11	0.39
5	-0.46	0.66	0.39
6	-1.76	-0.01	0.39
7	-0.51	-0.78	0.38
8	1.12	0.72	0.30
9	2.35	0.98	0.39
10	1.74	-1.52	0.33
11	2.20	-0.80	0.98

Table 3: C.V.A. of the Hartz Mountain Populations

	W					
l	62.70					
w	8.89	4.16				
ll	35.72	6.79	25.95			
pw	1.59	5.00	1.14	0.25		
t	8.13	6.95	13.13	3.25	494.23	
lwp	16.86	2.81	11.76	0.72	1.86	8.02

	B					
l	24.31					
w	1.55	0.91				
ll	11.71	2.68	10.50			
pw	-0.54	0.58	1.46	0.70		
t	-7.45	30.16	72.50	26.44	119.57	
lwp	5.77	1.54	5.66	0.86	43.6	3.09

Latent Roots

295.73	45.58	20.88

% of Variation

81.7	12.6	5.8

Canonical Variate Loadings (by row)

-0.793	-1.029	1.067	12.792	0.155	-0.278
-0.204	-1.28	0.449	13.484	-0.273	-2.273
1.117	-2.476	0.318	7.18	-0.074	-1.104

Table 3: (Contd.)

Canonical Variate Means

		CV1	CV2	CV3	95% Confidence Limit
P. tasmanica	LD	1.47	-1.14	-0.57	0.51
	SD	2.57	0.65	0.42	0.46
P. daltonii	LD	-2.34	-0.58	0.67	0.49
	SD	-1.83	0.87	-0.58	0.48

Table 4: C.V.A. of the Central Plateau Populations

	W					
l	316.74					
w	39.32	10.27				
ll	159.24	21.02	97.77			
pw	3.42	0.93	1.99	0.26		
t	-2.29	20.21	-2.80	5.39	573.51	
lwp	73.41	10.52	40.57	1.04	4.57	23.25

	B					
l	156.16					
w	2.81	1.37				
ll	56.93	0.22	25.55			
pw	-4.68	-0.03	-1.23	0.21		
t	-334.90	2.93	-121.34	11.10	799.86	
lwp	33.71	-0.95	15.46	-0.82	-80.16	10.34

Latent Roots

326.55	134.67	16.27	3.63	1.36	

% of Variation

67.7	27.9	3.4	0.8	0.2

Canonical Variate Loadings (by row)

-0.604	-0.099	0.855	12.157	0.243	-0.909
-0.681	-3.56	0.511	11.90	-0.112	3.216
-0.042	1.587	0.962	14.70	-0.284	-1.918
-0.426	0.306	1.477	-16.657	0.341	0.613
-1.077	4.159	-0.139	-6.854	-0.158	2.338

Table 4 : (Contd.)

Canonical Variate Means

Sample	CV1	CV2	95% Confidence Limit
P. tasmanica field	2.78	0.02	0.39
P. daltonii field	-1.64	0.95	0.39
Intermediate field	-0.10	0.80	0.30
P. tasmanica c.g.	1.25	-1.09	0.59
P. daltonii c.g.	-1.53	-1.37	0.51
Intermediate c.g.	-0.94	-1.64	0.49
Progeny c.g.	0.42	-0.27	0.43

Appendix J. Data Tables from the Distance Analysis of Section *Mesembrynia* (Chapter 12).

Table 1: Mean Values of the 21 characters measured on the 13 taxa of *Plantago* section *Mesembrynia*. The values are the means of the transformed characters, yi = log(1 + xi).

Character	Taxon 1	2	3	4	5	6	7	8	9	10	11	12	13
l	2.24	2.51	2.23	1.98	2.14	2.17	2.61	2.44	2.35	1.85	1.74	1.94	2.68
lwp	1.17	1.37	1.24	1.26	1.43	1.23	1.56	1.34	1.26	0.94	0.75	0.95	1.54
w	0.78	0.75	0.61	0.92	1.05	0.83	1.13	0.72	0.77	0.96	0.44	0.54	0.46
ll	1.78	2.12	1.89	1.76	1.92	1.85	2.20	1.94	1.88	1.49	1.29	1.52	2.28
v	1.37	1.45	1.33	1.54	1.73	1.54	1.72	1.30	1.27	1.41	0.78	1.05	0.97
tn	1.78	1.74	2.02	1.49	0.98	1.60	1.45	1.31	2.39	1.64	1.36	1.23	1.58
tl	1.18	1.11	1.22	0.21	0.42	0.60	0.34	0.97	0.57	0.98	1.05	0.81	1.18
li	1.49	1.60	1.30	1.39	1.61	1.52	1.40	1.47	1.43	1.61	1.55	1.54	0.70
sc	2.88	3.04	2.65	2.30	3.12	2.98	3.46	2.81	2.63	2.30	2.33	2.33	3.06
sp	1.90	1.76	0.85	0.93	1.95	1.15	2.05	1.93	1.61	1.42	1.21	1.29	1.80
bl	0.48	0.92	0.70	0.12	0.79	1.05	0.91	0.61	0.58	0.32	0.69	0.08	0.75
bi	0.66	1.57	1.32	0.43	0.94	1.34	0.89	0.93	1.09	1.29	0.48	1.60	1.33
bk	0.31	0.57	0.53	0.58	0.66	0.64	0.37	0.57	0.88	0.74	0.41	0.37	0.49
bm	0.45	0.50	0.54	0.58	0.52	0.56	0.72	0.41	0.58	0.51	0.71	0.59	0.65
sl	1.58	1.93	1.81	1.70	1.75	1.86	1.62	1.70	1.92	1.72	1.63	1.82	1.95
sw	1.10	1.27	1.34	1.31	1.25	1.33	1.19	1.23	1.63	1.34	1.22	1.32	1.28
slwp	1.02	1.11	1.15	1.03	0.93	1.22	1.03	1.03	1.11	0.87	0.98	1.11	1.13
pl	1.22	1.65	1.65	1.55	1.58	1.63	1.42	1.34	1.61	1.52	1.01	1.59	1.65
pw	0.99	1.54	1.46	1.43	1.47	1.49	1.22	1.15	1.43	1.50	0.92	1.50	1.43
plwp	0.90	1.26	1.26	1.16	1.19	1.23	1.06	0.98	1.22	1.15	0.69	1.18	1.24
pm	0.86	0.74	0.85	0.83	0.75	0.76	0.69	1.09	1.24	1.10	1.23	0.69	0.69

Table 2 : PCA of petal and sepal characters of species of *Plantago* sect. *Mesembrynia*.

(a) Sepal Characters

Correlation matrix:		1	0.2859	0.3931 *sl*
			1	0.2793 *sw*
				1 *slwp*
Eigenvalues		1.6419	0.7513	0.6068
% of variation		54.7	25.1	20.2
Eigenvectors	*sl*	0.6019	0.3542	-0.7157
	sw	0.5286	-0.8485	0.0245
	slwp	0.5986	0.393	0.698

(b) Petal Characters

Correlation matrix:		1	0.5742	0.7167 *pl*
			1	0.5475 *pw*
				1 *plwp*
Eigenvalues		2.2288	0.4892	0.282
% of variation		74.3	16.3	9.4
Eigenvectors	*pl*	0.5978	0.3282	-0.7314
	pw	0.5424	-0.8374	0.0675
	plwp	0.5903	0.437	0.6786

(c) Mean values of the principal components of sepal and petal characters obtained for each taxon.

Taxon	Principal Components:Sepals			Principal Components:Petals		
	PC1	PC2	PC3	PC1	PC2	PC3
P. debilis	12.60	1.17	-7.55	7.43	0.47	-3.65
P. varia	16.59	1.18	-10.03	14.91	-0.97	-4.68
P. glabrata (SE)	16.30	0.11	-8.80	14.60	-0.33	-4.76
P. antarctica (Tas.)	14.78	-0.33	-8.50	13.36	-0.80	-4.54
P. antarctica (Aus.)	14.30	0.02	-9.47	13.89	-0.91	-4.57
P. glabrata (NW)	16.86	0.61	-8.86	14.45	-0.70	-4.74
P. cladarophylla	13.53	0.40	-7.83	10.93	-0.10	-4.21
P. cunninghamii	14.29	0.38	-8.47	9.66	-0.15	-4.08
P. drummondii	18.44	-2.06	-9.83	14.30	-0.40	-4.64
P. bellidioides	14.36	-1.10	-9.53	13.45	-1.44	-4.33
P. turrifera	13.59	-0.01	-8.17	4.22	-0.80	-3.24
P. hispida	16.08	0.22	-9.08	14.02	-1.09	-4.69
P. gaudichaudii	16.83	1.24	-10.13	14.33	-0.25	-4.83

Table 3: Within Groups SSP Matrix Obtained for the 21 Characters used in the Analysis of *Plantago* section *Mesembrynia*.

Variable	*l*	*lwp*	*w*	*bl*	*v*	*tn*	*tl*	*li*	*sc*	*sp*	*bl*	*bi*	*bk*	*bm*	*sl*	*sw*	*slwp*	*pl*	*pw*	*plwp*	*pm*
l	15.398																				
lwp	9.524	10.053																			
w	5.134	4.353	5.683																		
ll	13.384	9.140	5.087	12.590																	
v	3.733	3.207	3.111	3.617	5.094																
tn	1.770	2.213	4.641	2.271	3.513	59.576															
tl	3.985	2.992	5.728	4.438	1.423	26.469	52.338														
li	0.308	0.025	-0.539	0.018	-0.856	-1.602	-2.081	6.054													
sc	12.864	8.181	4.895	11.127	4.022	-1.086	3.150	1.250	18.673												
sp	12.597	8.251	5.283	11.205	4.949	-0.560	2.614	0.469	18.388	25.592											
bl	2.936	2.633	2.776	3.449	1.036	1.116	1.627	-0.019	1.863	1.116	11.565										
bi	4.979	1.905	1.941	4.017	0.985	1.230	5.211	1.968	3.938	4.016	2.850	22.619									
bk	1.091	0.516	0.729	0.851	0.531	1.008	1.461	-0.113	1.062	0.855	1.153	1.260	1.288								
bm	0.014	-0.252	0.011	0.007	-0.623	-0.377	1.590	0.065	0.640	0.498	-0.132	1.097	0.109	1.764							
sl	0.868	0.480	0.089	0.791	0.136	-0.130	0.494	0.135	0.818	0.461	0.351	0.882	0.324	0.349	0.942						
swp	-0.311	-0.448	-0.116	-0.395	-0.107	0.034	-0.727	0.138	-0.569	-0.483	-0.300	0.381	0.144	0.225	0.277	1.084					
slwp	0.574	0.517	-0.060	0.611	0.007	-0.528	-0.952	0.033	0.753	0.482	0.291	0.691	0.368	0.265	0.604	0.452	2.430				
pl	0.223	-0.052	-0.205	-0.019	0.185	0.736	-1.028	0.289	0.451	-0.146	0.357	0.413	0.194	0.064	0.384	0.149	0.385	1.125			
pw	0.213	-0.074	0.006	0.151	0.268	1.642	0.220	0.056	0.029	-0.235	0.301	0.960	0.301	0.019	0.381	0.324	0.294	0.743	1.491		
plwp	0.229	0.094	-0.187	0.203	0.223	0.093	-0.893	0.385	0.916	-0.106	0.651	1.096	0.261	0.072	0.461	0.071	0.510	1.042	0.881	1.821	
pm	-0.370	-0.519	0.104	-0.265	0.083	-2.279	2.280	-0.740	-0.705	-0.291	-0.257	01.753	-0.302	0.007	-0.008	0.214	-0.282	-0.400	-0.417	-0.267	3.261

Table 4 : Coefficients of Canonical Variates 1-8.

Character	Canonical Coefficient							
	1	2	3	4	5	6	7	8
l	6.48	-1.91	-0.71	-1.51	1.85	1.46	-2.09	5.23
lwp	-0.54	-0.33	-0.88	0.41	-0.76	-0.95	0.38	-0.38
w	-0.21	2.51	0.20	02.58	-0.72	1.08	1.31	2.20
ll	-7.05	1.00	0.26	0.36	-3.25	-0.72	0.10	-5.73
v	0.07	2.44	3.13	0.99	0.67	-0.28	-2.08	0.22
tn	0.35	-0.17	-0.30	0.53	-0.14	0.19	-0.36	0.56
tl	0.07	-0.38	0.13	-0.30	0.54	0.02	-0.40	-0.69
li	0.74	2.97	-0.04	-0.16	2.35	0.73	-0.21	0.15
sc	-0.71	-0.36	2.36	4.19	0.19	-0.04	0.50	-0.56
sp	0.71	-0.19	-1.67	-2.84	0.59	-1.84	0.47	0.98
bl	1.03	-0.83	0.90	1.91	1.20	-1.30	0.46	0.58
bi	-0.56	-0.51	-0.10	0.02	0.87	0.55	-0.05	0.14
bk	-2.93	4.66	-6.88	2.62	-1.71	-2.89	-0.49	-3.38
bm	0.88	1.36	1.42	1.52	-4.26	1.58	3.76	3.17
sl	-0.21	-3.88	-1.24	-0.63	5.27	-1.58	4.44	0.74
sw	-1.72	-0.79	-2.62	2.84	-2.39	0.09	-1.98	4.90
slwp	2.06	-0.39	1.69	0.65	1.03	2.59	-2.40	-0.33
pl	-10.56	-1.51	-0.26	-0.90	-0.44	-0.23	-3.90	-1.02
pw	-2.52	1.43	1.10	0.20	3.37	2.05	5.85	-0.66
plwp	3.44	-0.00	-1.32	-3.35	-2.57	-0.62	0.283	2.52
pm	0.24	1.15	-3.35	2.32	0.99	0.61	-0.86	-0.84

Table 5 : Standardized Coefficients of Canonical Variates 1-8.

Character	Standardized Canonical Coefficient							
l	0.66	-0.47	-0.14	-0.22	0.37	0.40	-0.51	0.65
lwp	-0.05	-0.06	-0.14	0.05	-0.12	-0.21	0.07	-0.04
w	-0.01	0.36	0.02	-0.22	-0.08	0.17	0.19	0.16
ll	-0.64	0.14	0.05	0.05	-0.58	-0.17	0.02	-0.63
v	0.00	0.33	0.36	0.08	0.08	-0.04	-0.28	0.01
tn	0.07	-0.08	-0.12	0.15	-0.05	-0.10	-0.17	0.14
tl	0.01	0.17	0.05	-0.08	0.20	0.01	-0.18	-0.16
li	0.05	0.45	-0.01	-0.01	0.29	0.12	0.03	0.01
sc	-0.08	-0.09	0.52	0.67	0.04	-0.01	0.14	-0.08
sp	0.09	-0.06	-0.43	-0.52	0.15	-0.63	0.14	0.15
bl	0.09	-0.17	0.16	0.24	0.21	-0.30	0.09	0.06
bi	-0.07	-0.15	-0.02	0.00	0.21	0.18	-0.01	0.02
bk	-0.09	0.34	-0.41	0.11	-0.10	-0.23	-0.04	-0.12
bm	0.03	0.11	0.10	0.07	-0.28	0.14	0.29	0.13
sl	-0.01	-0.22	-0.06	-0.02	0.25	-0.10	0.26	0.02
sw	-0.05	-0.05	-0.14	0.11	-0.13	0.01	-0.13	0.16
slwp	0.08	-0.04	0.13	0.04	0.08	0.27	-0.22	-0.01
pl	-0.29	-0.10	-0.01	-0.03	-0.02	-0.02	-0.25	-0.03
pw	-0.08	0.10	0.07	0.01	-0.20	0.17	0.41	-0.03
plwp	0.12	-0.00	-0.10	-0.17	-0.18	-0.06	-0.24	0.11
pm	0.01	0.12	-0.30	0.15	0.09	0.07	-0.09	-0.05

Table 6 : Significance Tests for Equality of Canonical Roots

Root No.	D.F.	X^2
1	252	1418.81***
2	220	1097.51***
3	190	838.05***
4	162	629.40***
5	136	470.12***
6	112	340.86***
7	90	231.26***
8	70	136.66***
9	52	84.41**
10	36	53.81*
11	22	31.85
12	10	14.27

Table 7 : Values of the Taxa on Canonical Variates 1-8.

Taxon	Canonical Variate							
	1	2	3	4	5	6	7	8
1. *P. debilis*	4.74	0.07	1.63	-1.32	-0.08	-0.25	-1.15	0.02
2. *P. varia*	-1.75	-0.83	0.52	-0.12	2.29	-0.31	0.05	0.18
3. *P. glabrata* (SE)	-2.20	-1.22	0.63	1.61	-0.27	1.45	-1.36	-0.65
4. *P. antarctica* (Tas.)	-2.41	3.04	-0.04	-1.01	-2.81	1.30	-0.15	-0.62
5. *P. antarctica* (Aus.)	-2.82	3.11	0.96	-0.27	-0.09	-1.73	0.99	-0.40
6. *P. glabrata* (NW)	-2.13	0.71	1.69	2.66	0.87	0.51	-0.12	0.19
7. *P. cladarophylla*	1.53	0.94	4.03	0.61	-1.85	-0.54	0.19	2.13
8. *P. cunninghamii*	2.31	0.24	-1.86	-0.58	0.50	-1.03	-1.13	-0.49
9. *P. drummondii*	-1.75	0.97	-5.83	1.63	-0.70	-0.37	-1.11	1.80
10. *P. bellidioides*	-1.60	3.63	-3.12	-1.50	0.55	0.54	0.54	-0.03
11. *P. turrifera*	6.70	0.08	-1.24	1.96	-0.11	0.66	1.86	-0.34
12. *P. hispida*	-1.02	-1.13	-0.02	-2.87	0.95	2.22	0.97	0.60
13. *P. gaudichaudii*	-2.01	-5.87	-0.52	-0.35	-1.36	-0.97	0.64	-0.18